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FORMULATION OF A NATURAL INTRAORAL DISPERSIBLE FILM (IDF) FOR INTRAORAL DELIVERY OF VARIOUS NATURAL DRUGS USING EDIBLE RICE PAPER FILM AS THE CARRIER VEHICLE

Mukasa Eliphaz,

A dissertation submitted to the Faculty of Health Sciences at the University of the Witwatersrand in the fulfilment of the requirements of a Master's degree in Pharmacy

Johannesburg, 2016

DECLARATION

I, Eliphaz, Mukasa declare that this dissertation is my own unaided work. It is being submitted for the Degree of Master of Pharmacy at the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University

_____ _____

Signature Date

RESEARCH OUTPUT

1.1 PUBLICATIONS

- Eliphaz Mukasa, Paul M. Danckwerts, Yahya Choonara, and Thashree Marimuthu. Intraoral dispersible rice paper films as a natural drug delivery system. Submitted to the supervisor later to be submitted to the *International Journal of Pharmaceutics* (Abstract in Appendix A).
- A patent is filed as "Formulation of a natural intraoral dispersible film (IDF) for intraoral delivery of various natural drugs using edible rice paper film as the carrier vehicle." Mukasa Eliphaz, Paul Michael Danckwerts initiated the SA Provisional Patent Application with Wits Enterprises. The Innovation Edge filed on October 28 2016 (Espacenet Results in Appendix K).

1.2 CONFERENCE PROCEEDINGS

- Eliphaz Mukasa, Paul M. Danckwerts. Formulation of a natural intraoral dispersible film (IDF) for intraoral delivery of various natural drugs using edible rice paper film as the carrier vehicle. Poster presented at the 7th Cross-faculty postgraduate symposium, 1-2 March 2016, University of the Witwatersrand, Johannesburg, South Africa. (Abstract in Appendix C).

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  (Abstract in Appendix K).

ABSTRACT

Background and the purpose of study. At present, pharmaceutical researchers are focusing on instantaneous intraoral dispersible technologies as novel drug delivery systems because; they have outstanding advantages over the traditional oral and parenteral routes of drug administration. Some essential natural drugs have low oral bioavailability due to extensive first pass metabolism and pre systemic degradation in the gastrointestinal tract.

Aim. Now, a cheap rice paper Intraoral Dispersible Film (IDF) has been developed

Objectives. In this study, formulation was optimized using the experimental factorial design. The IDFs were loaded with model, natural, anti-cancer drugs, Resveratrol and Curcumin with low oral bioavailability.

Methods. They were evaluated for thickness, folding endurance, swelling behaviour, among others. These related to their drug release properties. Permeation was evaluated using the pig mucosal membrane mounted on a Franz diffusion cell. Taste testing was done to determine acceptability using a taste panel.

Results and Discussions. Sixteen formulations showed variations in their profiles. Formulation 16 proved optimal. The dissolution rate at steady state concentrations of Resveratrol was 29mg per second and the Permeability coefficient was 389 mg/sec.cm^2. That of Curcumin was 0.25mg per second and the Permeability coefficient was 42.71 mg/sec.cm^2 Resveratrol permeability rate was 0.42 mg/sec. and that of Curcumin was 0.14 mg/sec. Resveratrol Flux was 0.21 mg/sec./cm^2. Curcumin Flux was 0.14 mg/sec. / cm^2. Drug entrapment was 80% for both molecules. The 20 mg of Resveratrol and Curcumin would then dissolve in 47.6 sec. and 71.4 sec. respectively. In this study, after permeation, a concentration of 6.73mg/ml of Resveratrol and 0.061mg/ml of Curcumin were detected after 2 hours of the experiment on administering only 20 mg of each of the drugs suggesting that Curcumin is 100 times less permeable than Resveratrol. **The release profile is a burst release**. On contrast, Curcumin oral dose of 2 g/kg to rats yielded 1.35±0.23 µg/ml in 0.83 hours and in humans, the same dose either undetectable or extremely low (0.006±0.005 µg/ml after 1 hour. Two separate mono-glucuronide metabolites yielded C_{max} ~7.5 µM following a single 5.0 g oral dosage of Resveratrol.

Conclusion. The key finding is, *ex vivo* release profiles of the optimized formulation revealed first order release and later zero order. Therefore, it is evident that rice paper IDF could efficiently deliver natural drugs into the systemic circulation. However, later, further studies are needed to prove increased bioavailability to be performed in human subjects

***Keywords*:** Curcumin; Resveratrol; Instantaneous Intraoral Dispersible films; Edible Rice paper films; *ex vivo* release profile.

SUMMARY

Delivery of drugs using the intraoral route of administration has interested researchers for the last three decades. The intraoral route have been advocated by many authors on the subject of drug delivery systems to be efficient for drugs, which undergo extensive hepatic first pass metabolism or presystemic metabolism in the Gastro Intestinal Tract (GIT). The various advantages of this drug delivery system have made it a novel route of drug administration. These advantages include, that the delivery system is easy to access as it is affordable, easy to apply by the patients themselves, and to remove the drug delivery device. It has a rapid onset of action because of the existence of a large surface area of the oral veins hence a high blood flow and high permeability of the oral cavity membranes.

Drug delivery systems that encourage patient compliance in a most practical way are still needed where safety, efficacy, affordability, availability, are considered as priority to address patient needs. The existing environment of meagre resources for the majority of the world's population calls for new approaches to improve their quality of life. In this study, a safe and effective rice paper Intraoral Dispersible Film (IDF) that is even suitable for both paediatric and geriatric, as well as non-compliant patients like the psychiatrics and the decapitated like the epileptics has been developed.

The reason Resveratrol and Curcumin were chosen as the model drugs is that both these two molecules have low oral bioavailability and at the present time, cancer is on the rise worldwide and the existing interventions do not yield satisfactory therapeutic outcomes. (Parkin et al., 2001). Chemotherapy is associated with many unwanted side effects which include; Nausea, Emesis, Itching at injection site, Shaking all over, Change in the way things taste, Constipation, Diarrhea, Pins and needles in limbs, Weight loss, pain, and many others (Coates et al., 1983). The prognosis is still poor for all existing interventions that research into novel deliveries of essential user-friendly phytochemicals is still called for to bridge the gap. Rice was chosen as a polymer because it is cheap, affordable, and abundant.

Only natural ingredients have been used to formulate the rice paper films as most people prefer a natural form of drugs to orthodox medicines. The rice paper IDF has been studied during the formulation development of the product and has been established to be compatible with simulated saliva and the buccal environment Porcine buccal mucosal membrane was used during the drug permeation experiments. Disintegration and

dissolution studies were done. These studies helped us to establish the fact that rice paper could be an efficient natural drug delivery system.

After analysing the collected data using an Excel program called Essential Experimental Design or Factorial design (Steppan et al., 1998) version 2.2016, and the Taste testing (See surface pH page 121 and Taste testing page 243), we optimised the formulation. The statistical analysis showed a p-value of less than 0.05 for the rice starch, and plasticizer concentration, and tensile strength meaning that varying these factors could significantly affect disintegration, Tensile strength, and Folding Endurance which would consequently affect the release characteristics of the film. In this study, after permeation, a concentration of 6.73mg/ml of Resveratrol and 0.061mg/ml of Curcumin were detected after 2 hours of the experiment on administering only 20 mg of each of the drugs (See Table 5.1) page 185 (See Table 5.2) page 186 and (See Table 5.5:) page 192. *In vivo* dissolution / Disintegration results of the rice paper films using human volunteers indicated that Resveratrol film dissolved / disintegrated in a relatively short time of 24 seconds and Curcumin film in 37 seconds (SeeTable 5.12:).

Basing on experimental results, a conclusion could now be drawn to support the hypothesis that rice paper could be a suitable natural systemic drug delivery system for some essential natural drugs.

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DEDICATION

This work is dedicated to my late mothers Maama Gertrude Namakula, Maama Edith Namakula, Maama Norah Nakijoba Maama Kekirema Nassozi, and Maama Edith Isalot. My late father Taata Kangave Nicholas Sebbowa. Kkojja Edward Mukiibi. Kojja Sserunjogi of Katooke My late grandfather Eliphaz Bitalo Kakutiya, grandmothers Maria, and Kaana Nannono. Kkojja Mutyaaba, Taata Salongo Nsereko at Kamwookya and all the children. Senga Kitigi, Senga Nalumiinsa and Senga Maaso of Kalule. My beloved children, Nalubwaama, Daniel, Norah Kitigi, Jeremiah, and Hope Nanteza. David Kigozi, Anold, Stella Moreene and Nalubwaama, Isaac and Ester Finuusa. Maria, Solome, Jackline Katuusiime, my sisters Nabulime, Nalumansi, and Nanteza Stella, my late sister Jesca Nantongo,Susan Mutuuza, my late brothers Godfrey Luwemba, John Nkozi and Christopher Kuuku, James Mugezi, Lumiinsa, Sendyosse, Rovince Nandawula and the children at Kansaanga. Nakato. My brothers Kaggwa Edward and Bbumba Emanuel, Partric Lumu, Nicholas Ntege, and every person who has been a part of my life. Without your influence, I would not be the person I am now.

ANIMAL ETHICS DECLARATION

I hereby confirm that the following study entitled “FORMULATION OF A NATURAL INTRAORAL DISPERSIBLE FILM (IDF) FOR INTRAORAL DELIVERY OF VARIOUS NATURAL DRUGS USING EDIBLE RICE PAPER FILM AS THE CARRIER VEHICLE” has received the Exemption from full Animal Ethics study approved by the Ethics Screening Committee at the university of the Witwatersrand with approval and clearance letter attached (Appendix D).

HUMAN ETHICS DECLARATION

I hereby confirm that the following study entitled “FORMULATION OF A NATURAL INTRAORAL DISPERSIBLE FILM (IDF) FOR INTRAORAL DELIVERY OF VARIOUS NATURAL DRUGS USING EDIBLE RICE PAPER FILM AS THE CARRIER VEHICLE” has received the approval from the Human Ethics Screening Committee at the university of the Witwatersrand with approval and clearance certificate number M140920 attached (Appendix F). This was together with the Distress protocol Certificate (Appendix E).

PREFACE

The author of this dissertation has written it with great compassion to patients and hopes that the work of this research will materialize to the benefit of the patients. It is his intention that the fruits of the study will not only be the award of a master's degree to the author but the inspirations of these pages have their effects measured by an improvement on the patients' health. The work of this study has been written in chapters to facilitate future publication and improvement as well as easy reading.

All effort has been made to make science readers enjoy and appreciate the purpose of this research. For this reason, the table of abbreviations has been included to make the terminology used understandable. The electronic document has been bookmarked that after highlighting the index and a cursor placed on the heading intended to be reviewed, by pressing the enter button, the computer should direct you straight to that heading in the text.

Cross-references have been included to link the different sections of the dissertation together. This would make it easy to navigate through the document. Constructive criticism to improve this research has also been provided for by including the mailing addresses of the authors to whom such communication should be directed for immediate attention. It is hoped that active participation of all parties will lead to formulation of a natural Intraoral Dispersible Film (IDF) for the intraoral delivery of various essential natural drugs using edible rice paper film as the carrier vehicle.

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LIST OF ABBREVIATIONS

HPLC - High Performance Liquid Chromatography

(NRTD) - Nicotine Replacement Transdermal Delivery

AAPS - American Association of Pharmaceutical Scientists

ACN - Acetonitrile

API - Active Pharmaceutical Ingredient

ASTM - American Society for Testing and Materials

AVG - Average

BE - Bioequivalence

DIN EN ISO - Deutsches Institut fu¨r Normung, Europa ische

DSC - Differential Scanning Calorimetry

EMA - Stands for European Medicines Agency

SEM - Scanning Electron Microscope

FDA- Stands for Food and Drug Administration of the United States of America

FDDF- Fast Dissolving Dosage Forms

FDF-Formulation of Fast Dissolving Films

FIP - Stands for International Pharmaceutical Federation;

FT-IR-Fourier transforms infrared spectroscopy

GIT- Gastro Intestinal Tract

HPLC- High performance liquid chromatography

ICH- Stands for International Conference on Harmonization of Technical

IDF - Intraoral Dispersible Film

IDT - Intraorally Disintegrating Tablet

NDA - National Drug Authority

OD - Oral Dispersible Films

ODT - Oral Dispersible Tablets

OTCs - Over The Counter medicines

Ph. Eur. - European Pharmacopoeia

Requirements for Registration of Pharmaceuticals for Human Use

TS - Tensile strength

USP - United States Pharmacopoeia;

XRPD: X-Ray Powder Diffraction

® - Copyright

CV % - coefficient of Variation percent

RT – Retention time

LOQ – Lower limit if quantification

Chapter 1. Background, Rationale and Motivation for this Study

1.1 Introduction

In a world where most drugs are extremely expensive and not available to a large portion of the population (especially the African population), there is a need for more natural forms of drugs. There are also more and more patients turning to natural forms of therapy. Unfortunately, not all drugs in their natural forms are bioavailable when taken orally. Therefore, the intraoral route has become an important route of drug administration for these natural drugs. The oral mucosa is rich with blood vessels which are permeable to many drugs and this makes this route of administration an important alternative to the traditional oral and parenteral routes for systemic drug delivery (Kaufman, 2013). When these therapeutic agents are absorbed from the oral mucosa, they avoid premature drug degradation, which occurs as a result of enzyme digestion and a low pH of the GIT. Loss of active drug due to first-pass hepatic metabolism is by passed and rapid therapeutic plasma concentration of the drug could be achieved.

Edible rice paper has been used for centuries in the food industry to coat various items like sweets and cakes. It is a flat thin sheet prepared by a solvent casting technique and made from rice flour and water (Dalal, 2014). Rice paper as a starch swells and dissolves quickly in saliva and could be a suitable pharmaceutical vehicle for delivering drugs through the oral cavity, into the blood circulation through the existing intraoral veins.

Some Phytochemicals like Curcumin and Resveratrol are known to have low bioavailability when administered orally (Anand et al., 2007). A rapid release and absorption to achieve therapeutic plasma concentrations in the minimum possible time is desired for these drugs to be effective and if they took long in the buccal cavity like mucoadhesive patches, this could lead to accumulation of the drug in saliva and swallowing the saliva whole with the drugs renders them less effective. In addition to rice flour, and water rice paper sometimes contains tapioca starch, which is a natural food, and therefore not toxic to the body (Dalal, 2014).

Oral forms of some natural drugs such as curcumin, and resveratrol have been introduced into the market as natural products but their oral bioavailability is very low and high doses are required to reach a therapeutic blood level (Anand et al., 2007).These are discussed in a greater detail in the literature review chapter 2 page 19 – 30. This has limited their use to

food supplements as most of them are powerful antioxidants (Anand et al., 2007); (Doheny, 2008); (Mishra and Amin, 2011).

These natural drugs are therefore, good candidates to be formulated into natural intraoral dispersible films (IDFs). Various intraoral dosage forms have appeared on the market. These include quick dissolving tablets, gels, ointments, and of late IDFs (Mohamed et al., 2011). IDFs may be more preferable to all other dosage forms. They are preferred because of their flexibility and comfort in the mouth. In addition, they are better than oral gels which have a relatively short residence time because gels are easily washed away and removed by saliva. The IDFs are also more accurate in terms of dosing of drugs compared to gels and ointments and are also less costly (Mohamed et al., 2011).

Resveratrol is a natural drug extracted from red grape skin and has been used to treat the different forms of angina pectoris, and mild to moderate hypertension (Mishra and Amin, 2011). It generally has less adverse effects but unfortunately, Resveratrol is inactivated by the stomach enzymes (Doheny, 2008). It has anti-oxidant properties, anti-inflammatory, is an antiviral drug, and is active against some cancers, has anti - diabetic properties, and is believed to be effective in some brain disorders as well as Alzheimer's disease (Mishra and Amin, 2011).

Curcumin is the yellow pigment in turmeric and curry spice. Curcumin is a safe drug. When given even at doses of 12 g/day, it still shows no toxic signs in humans (Anand et al., 2007). However, it has poor oral bioavailability because of poor absorption, rapid metabolism, and fast systemic elimination (Anand et al., 2007). Curcumin is effective against various human diseases. It is an anti-inflammatory, antioxidant, effective against various cancers, heart diseases, diabetes, arthritis, as well as neurological and Crohn`s disease (Anand et al., 2007).

Bioavailability is defined as "The ratio between the drugs administered to the drug found in the systemic circulation" (Löbenberg and Amidon, 2000). Physical /mechanical and organoleptic tests 17 of them, especially the dissolution and permeation through the pig buccal membrane mounted on the Franz Diffusion cell (See page 156) have been performed to prove that rice paper IDFs could efficiently deliver the drugs intraorally across the barrier membrane of the oral cavity into the interstitial space of the jugular veins that drain directly into the heart and hence enter the systemic circulation. Saturation of protein binding and entry of the drugs into the human cells is still the subject of future research both pre – clinical, (Full animal study), and clinical trials in patients are yet to be done to prove enhanced bioavailability. At this stage, this is beyond the limits of this study.

Increasing the bioavailability of Resveratrol and Curcumin through clinical trials by employing the intraoral rice paper natural drug delivery system is more likely in the near future to bring these and some other essential natural drugs to the forefront of therapeutic agents for treating disease in humans (Anand et al., 2007). In this study therefore, Resveratrol and Curcumin were employed as model drugs. At this stage, we cannot claim to improve bioavailability until further studies with blood but at least we can say the that by developing the drug delivery system, we intend to address the problem of poor oral bioavailability and the affordability issues by developing an IDF of rice paper impregnated with each of the model natural drug. The affordability issue in line with most essential drug programs worldwide is the very reason why rice starch was chosen among others as the polymer in the formulation of the delivery system. To make the drug delivery system affordable, only cheap and abundant ingredients like rice starch, honey, mint, Stevia, cocoa, and others have been used.

1.2 Possible therapeutic applications

Potential outcomes are, that quality, and cheap drugs utilizing rice paper will be produced and availed to patients with cancer and cardiovascular conditions. Patient compliance will be enhanced, as the rice paper films are portable, have a discrete administration and can be self-administered (See advantage sand disadvantages on page 36). A safe intraoral drug delivery system suitable for paediatric and geriatric use with improved bioavailability of various natural drugs using edible rice paper would be developed. With such a delivery system available, paediatric and geriatric preparations, which are rare, may now also possibly be available. The cost of therapy will be reduced as the rice paper films are made from abundant cheap ingredients. The potential benefit is that a drug carrier that can be used to administer some essential natural drugs that exhibit very low oral bioavailability could be developed. Research findings will be presented at national and international conferences creating more awareness among scientists and healthcare professionals about the intraoral drug delivery systems.

1.3 Novelty

A cheap and abundant form of the rice paper intraoral dispersible film as a natural drug delivery system (IDF). (See Figure 6.2 : Espacenet Worldwide patent database search for the title page 247) can now be used intraorally as a pharmaceutical vehicle for some natural drugs, which are normally associated with poor oral bioavailability. Rice paper IDF is the delivery vehicle itself and not a pharmaceutical excipient like gum acacia or tragacanth, which are additives as thickeners where the vehicle is water (No water is needed in using the IDF). Rice starch could be used in tableting as a lubricant for the dies, it is an excipient when the bulk forming agent in the tablet is dextrose In this form as stated in the Pharmaceutical handbook, it is not natural having undergone a purification synthetic process. Rice paper is natural and edible being used as a pharmaceutical vehicle is a new innovation in IDF drug delivery systems. Rice paper dissolving very quickly in mucosal tissue rapidly delivers the drugs for systemic absorption and provides a valid alternative to the traditional oral and parenteral therapy. Local oral infections could also be treated using the IDF.

1.4 Aim and objectives of this study

This study aims at developing an affordable IDF as a drug delivery system. This could enhance the bioavailabity of natural drugs, which are normally associated with poor oral bioavailability due to hepatic metabolism, and enzyme digestion in the GIT.

The objectives of this study are;

To develop a feasible method for the formulation of an edible rice paper IDF which has been done by developing several variants of the IDF rice paper employing a Factorial Design to determine the effect of different physicochemical variables on the ideal properties of the IDF.

To investigate the addition of a natural sweetener like honey and Stevia by means of setting up a taste panel to score the different sweeteners used in the IDF as well as its palatability once loaded with the natural model drugs.

To determine the physicochemical characteristics of the IDF by means of several tests including Texture analysis and to determine whether the drugs would interact with the vehicle by means of FTIR and DSC.

To develop an effective extraction and HPLC assay technique for the model drugs and to assess the *in vitro* release of each model drug into simulated saliva.

To assess the *ex vivo* drug release characteristics of the optimum IDF through excised oral pig mucosa by doing permeation studies using a Franz diffusion cell.

1.5 Overview of dissertation

The dissertation was prepared as follows for attainment of the aforementioned aim and objectives:

Chapter 1 Contains an introduction into the topic of the Intraoral (IDF) drug delivery systems, the rationale for the research, aim, and objectives.

Chapter 2 Concisely provides a descriptive review of the current global situation of research into IDF systems. This section elaborates on the factors that need to be taken into account, and the different methods utilised in order to attain fast dissolving films. Within this chapter, recent innovations are also described. The gap is identified to justify the need for the research.

Chapter 3 Describes the development, design and optimisation of the IDF drug delivery system loaded with natural drugs. The model candidate formulation, possessing the advantages of simple and effective manufacture, and favourable *in vitro* release behaviour, are identified utilising a model-independent approach for further investigation and optimisation. A Box-Behnken or Factorial experimental design was employed to synthesise several variants of the candidate formulations to determine the best formulation for the delivery of the model drug by means of measuring physicochemical characteristics like disintegration, folding endurance, tensile strength, and variation of thickness. The optimum formulation was identified by instituting the principles of Response Surface Methodology.

Chapter 4 Focuses on the development of a novel formulation loaded with the model natural drugs developed in Chapter 3. A Box-Behnken Design was used and resulted in 27 statistically derived formulations from which 16 for each model drug were chosen. Thirty two (32) formulations were prepared. This was done in order to identify the optimal polymeric concentrations. The Mean Disintegration time (MDT) was utilized as a formulation constraint. Regression analysis of the response surface plots was as well utilized in order to obtain the optimal formulation.

Chapter 5 Discusses the *ex vivo* animal testing, the Human taste testing, HPLC extraction methods of the model drugs, and HPLC analysis of Resveratrol and Curcumin loaded into the rice paper, trans-mucosal, intraoral drug delivery system, or rice paper (IDF) respectively as a pharmaceutical vehicle.

Chapter 6 Concludes the dissertation and ties together the significant issues addressed regarding the formulation of a Natural rice paper intraoral drug delivery system, with recommendations for future investigations.

Chapter 2. The Recent Developments on Instantaneous Dissolving Intraoral Films as Novel Drug Delivery Systems, Their Design and Evaluation

2.1 Introduction

Traditionally, the oral route of drug administration has been the most used and acceptable by patients. However, the Intraoral Dispersible Films are a relatively new dosage form of drug administration, the size of which is about that of a postage stamp (Hariharan and Bogue, 2009). These strips are thin polymeric films formulated to disintegrate fast when placed onto the tongue (Hariharan and Bogue, 2009). Drug delivery is defined as a method of administering a pharmaceutical compound to humans or to animals to attain a therapeutic goal. It is used synonymously with a pharmaceutical vehicle or drug carrier. However, in recent terms, it refers to technologies used to modify drug release profile in terms of absorption, distribution, and elimination to improve product bioavailability, effectiveness, and safety profile, in addition to cost effectiveness, patient convenience, and compliance.

Quick Intraorally dispersible dosage forms are now popular drug delivery systems due to their unique properties (Hariharan and Bogue, 2009). These quickly disintegrate in the mouth and can be administered without water, making them particularly suitable for patients who are uncooperative like the psychiatric, disabled like the epileptics unable to swallow, as well as, non-compliant patients like paediatrics and those advanced in age, the geriatric patients. As they quickly disintegrate or dissolve, they leave minimal or no residue in the mouth after their administration (Barnhart and Vondrak, 2008); (Garsuch, 2009); (Hariharan and Bogue, 2009). The present study aims at formulating a natural (IDF) for intraoral delivery of various natural drugs using edible rice paper film as the carrier vehicle. IDFs are classified according to their disintegration kinetics as quick dissolving, slow dissolving, or non-dissolving. They release the drug in a short period of seconds, 1 - 10 minutes and greater than 10 minutes respectively. (Siddiqui et al., 2011). They are delivered into the mouth to exert a local effect or for systemic absorption via the intraoral route. Additional advantages of these IDFs include; no need of swallowing, they are administered without water, and they have a quick onset of action as they are absorbed fast (Bhyan et al., 2011); (Bhura et al., 2012)

They are portable, easy to administer by the patients themselves with accurate dosing, affordable, with improved patient compliance as a convenient dosage form, and the oral cavity provides a larger surface area for their absorption (Fulzele et al., 2002). The oral

mucosa is rich in blood vessels and these veins are different from those in the GIT because they do not flow to the portal hepatic system that goes to the liver and therefore bypass the liver saving the drugs from its undesirable destructive metabolism (Hariharan and Bogue, 2009).

The drugs administered through this intraoral route around the upper third of the oral cavity, the upper jaw, and the hard palate directly enter the blood circulation via the jugular vein hence avoid and bypass the liver (Malke et al., 2007). Another benefit is that the intraoral route does not irritate the stomach since it avoids it and its hostile low pH (Mishra and Amin, 2007). Additional advantages of the intraoral route are; enhanced bioavailability, rapid absorption, and onset of actions therefore less amounts of the drug is needed and consequently fewer side effects and ability to swallow is not needed (Mahajan et al., 2011). (See Figure 2.1) below illustrates the drug flow via the intraoral route to the systemic circulation (Mahajan et al., 2011).

There are multilayered squamous epithelium barrier of the oral mucosa whose cells are bound together by small desmosomes and tight junctions with about 20 mm between its adjacent cells. This structure may act as a barrier to drugs into the systemic circulation but this is overcome with careful formulation of the IDF (Siddiqui et al., 2011). The various pathways by which drugs can permeate through the oral mucosa is presented (See Figure 2.3).

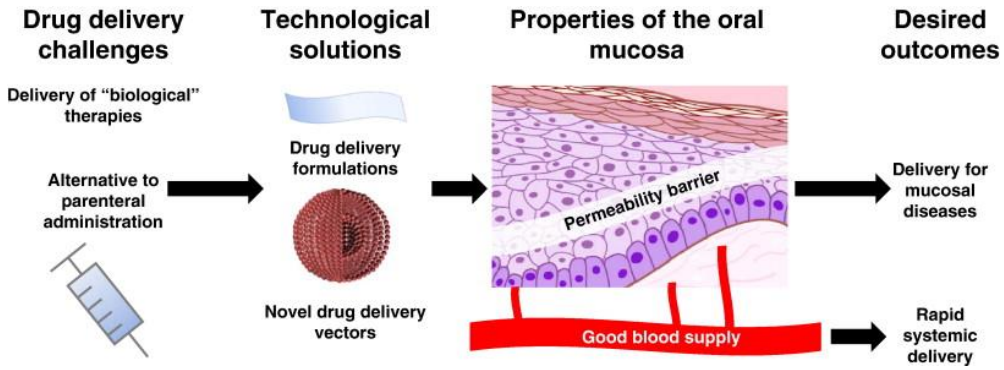


Figure 2.1: The flow of the drugs administered into the mouth via the jugular vein for systemic absorption (Hearnden et al., 2012).

The anatomical structure of the oral cavity is illustrated in Figure 2.2 below:

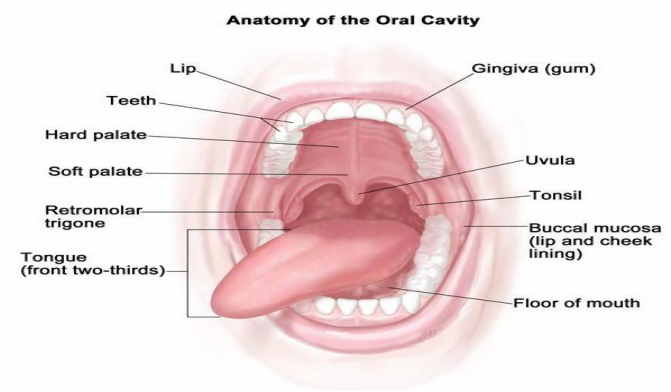


Figure 2.2: Anatomy of the oral cavity
www.picsearch.com/imageDetail.cgi?iOralcavity&querySafe=unsafe(Accessed on 01/02/15).

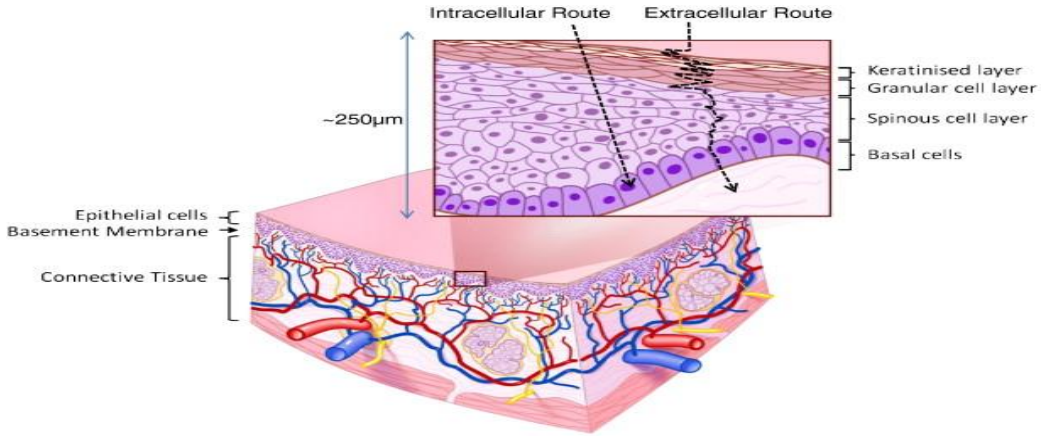


Figure 2.3: The structure of the oral mucosa
www.picsearch.com/imageDetail.cgi?Oralcavity&querySafe=unsafe (Accessed on 01/02/15).
The arrows in figure 2.3 indicate the various pathways in which the drugs can permeate through the oral mucosa.

Drugs that have an effect on keratinocyte cell surface receptors have an action on the connective tissue as well (Hearnden et al., 2012). Therefore, if the IDFs are for systemic delivery, they should be able to cross the epithelium barrier via the intracellular route without being internalised or trapped by the cells of the epithelium themselves (Hearnden et al., 2012). A permeation study was done to ensure this property. Drugs and therapeutic agents must overcome the oral mucosa barriers for them to be systemically absorbed. This can only be realised through proper formulation, which is achieved by this study.

2.2 Proposed drug formulation matrix interactions

In the figures that follow, the polymer complexes and the full geometry optimization are illustrated. (See Figure 2.4 to Figure 2.12). Figures 2.4 to 2.12 show the effective hydrogen bonds, and van der Waals forces which are involved in the formation of the rice starch complexes. These hydrogen bonds are easily broken on solution in saliva. The existence of these bonds could be proved by an FTIR scan which can reveal the functional groups present in the films (See Figure 4.14). IR spectroscopy is a reliable technique for hydrogen bond characterization as the molecular vibrations for O-H stretching can be used to confirm the presence of water functional groups and to ascertain the degree of inert and intramolecular interactions. The very low energy binding that exist give rise to the good IDFs drug release attributes. The morphology of the rice paper film supports a good carrier system with a lot of harboring sites.

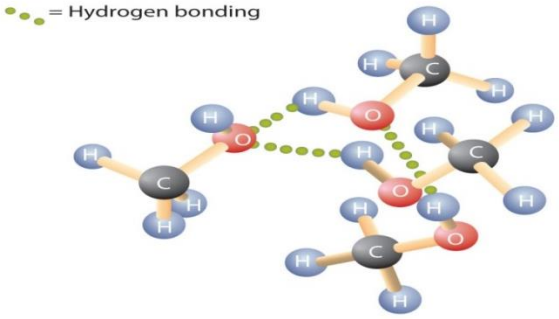


Figure 2.4: The three-dimensional hydrogen bonding in starch with Resveratrol www.google.co.za/search?q=images+of hydrogenbonding+between+starch+and+Resveratrol (Accessed on 01/02/15).

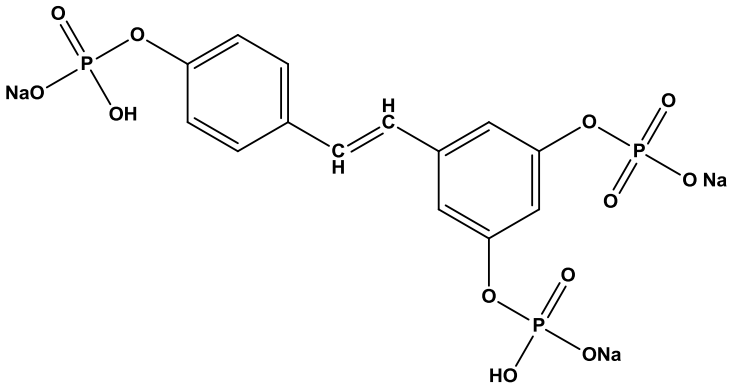


Figure 2.5: Polymerization in the starch matrix www.google.co.za/search?q=images+polymerization in the starch (Accessed on 01/02/15)..

Unsaturation will occur at the oxygen double bond because of the existence of a lone pair and polymerization (Chain formation) will take place (See Figure 2.6) and (See Figure 2.7).

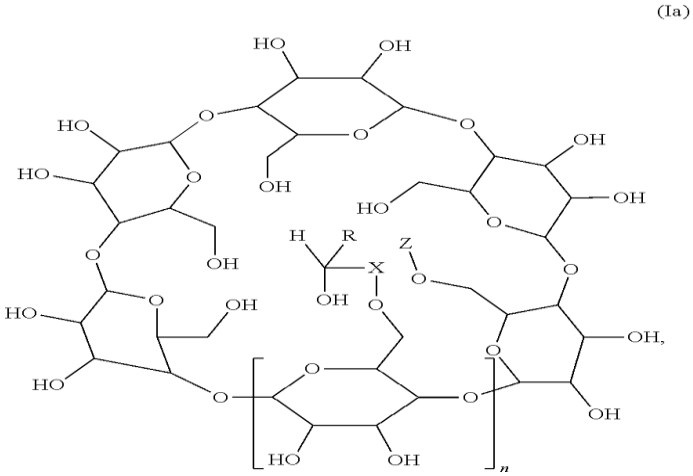


Figure 2.6 Possible weak Van der Waal forces existing between Resveratrol and starch www.google.co.za/search?q=images+ of bonding++between +starch+and+Resveratrol (Accessed on 01/02/15)..

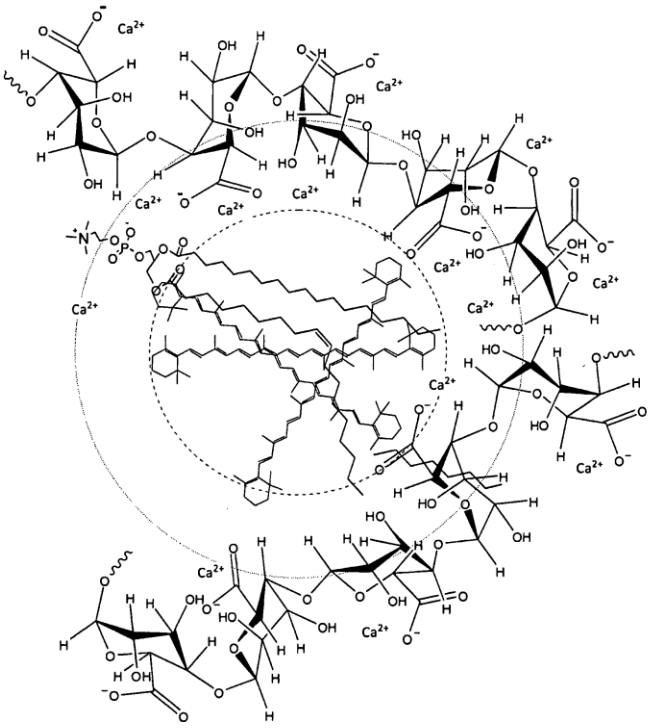


Figure 2.7 Curcumin starch complexion using hydrogen bonding.

www.google.co.za/search?q=images+ofbonding++between+starch+and+curcumin (Accessed on 01/02/15).

Weak hydrogen bonds weaken on solution in saliva, break and the free drug is easily released. If FTIR studies show no effective interactions between the model drugs and therice starch , then our rice paper film could only be a drug carrier as the title of the study states.

2.3 Fast dissolving dosage forms (FDDF)

2.3.1 Drug absorption through the oral mucosa

The upper third of the upper jaw bone of the oral cavity, in the extracellular space, is lined with epithelial cells of phospholipids (Siddiqui et al., 2011). This layer of membrane forms a protective barrier to some drugs intended to pass through the oral mucosa. Fortunately, some lipophilic molecules and certain natural drugs can permeate through this phospholipids membrane. As these drugs readily diffuse through this layer of phospholipids, they can easily reach the systemic circulation via the oral cavity (Galey et al., 1976).

The mechanism by which these drugs cross the cell membranes of the oral mucosa is by passive diffusion against a concentration gradient, and random molecular movements of the existing thermal energy. The rate of transfer depends on concentration differences on each side of the membrane (Malke et al., 2007).

The solubility of the recipients being carried in the epithelial membranes also plays an important role. On the other hand, active transport and pinocytosis by the aqueous cellular pores play a small role to transport the drugs across the oral mucosal membranes to the circulatory system. Therefore free un-ionized or neutral drug release from the vehicle is important. The IDF is also sensitive to moisture (Mahajan et al., 2011).

However, molecular size and degree of ionization are strongly related to the ability of these drugs to pass through the oral mucosa and only to smaller extent lipid solubility and the concentration gradient (Reddy et al., 2009). Drugs of molecular size less than 250 Daltons readily cross the oral mucosa membrane. On the other hand, when molecular size increases, the permeability steadily decreases (Brinker and Mukherjee, 1981). Un-ionized or neutral drug molecules are absorbed readily but highly polar molecules are insoluble and unable to penetrate the mucosal cellular lipid membranes. Therefore, particle size and ionization potential of the film ingredients must be considered carefully during the formulation stages (Galey et al., 1976).

The oral delivery of natural drugs via the GIT into systemic circulation poses several disadvantages which could include acid-induced hydrolysis in the stomach, enzymatic degradation in the GIT, bacterial fermentation in the colonic region as pre-systemic metabolism, and first pass hepatic metabolism (Bala et al., 2013). These impediments put together, significantly lower oral bioavailability of these natural drugs. Insolubility, hydrophobic nature, and the molecular structure of some drugs may also reduce their absorption from the GIT. This is illustrated in (See Figure 2.8) below;

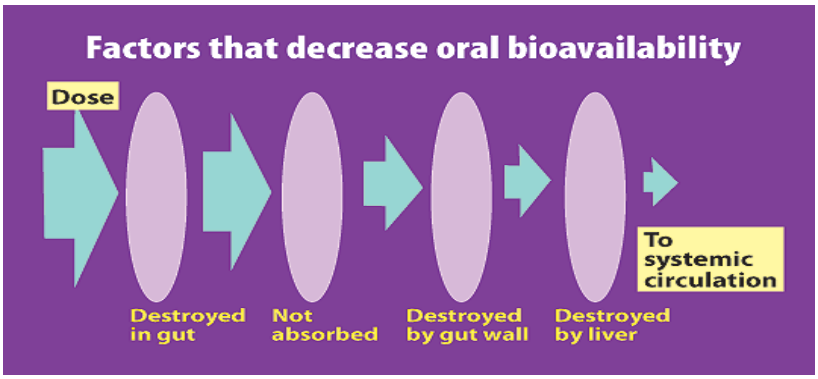


Figure 2.8: The main factors that lower the oral bioavailability and effectiveness of some essential natural dugs.
www.lifeenhancement.com/image/LEM1301Factors536.gif (Accessed on 01/02/15).

2.4 Technologies used for the small and large scale production of IDFs

Relatively rapid disintegration or constituents partly dissolving in the saliva of the oral cavity characterize the technologies used in formulating the IDFs (Bhyan et al., 2011). Chewing and swallowing are neither necessary nor desired to achieve rapid breakdown of the films (Fulzele et al., 2002). Adjuncts such as plasticisers, flavours, sweeteners are added as excipients to facilitate soft texture, flexibility as well as assisting in taste masking (Nagar et al., 2011). Particle size reduction techniques like Nanoparticle and Sol – Gel methods (See Figure 2.16) are employed in film forming (Brinker and Mukherjee, 1981). The reason behind this is to have the excipients present in their finest particle form possible to reduce the adhesion forces and to facilitate rapid drug release. The type of taste-masking technique to be used for a particular formulation is deduced by the physicochemical properties and physical form of the active pharmaceutical ingredient (Hariharan and Bogue, 2009).

The process of masking the taste must meet one opposing requirement, which is significant dissolution in the oral cavity. The technology developed in processes of casting the film,

ensures that both the mechanical integrity and the physical properties of the dosage form are maintained before the product gets into contact with the saliva (Nagar et al., 2011). Immediately the film gets in contact with the oral cavity, it absorbs the saliva, swells, and becomes soft and disintegrates within seconds. The licking movement between the upper jaw and tongue exerts pressure and the film disintegrates quickly. Heated rollers are employed in large-scale manufacture of IDFs in the industry.

2.5 Recent delivery systems for curcumin and resveratrol

2.5.1 Recent delivery systems for Curcumin

Since ancient times, curcumin has been used in Asian countries against human ailments. Modern science has now delineated its pharmaceutical uses basing on the molecular basis. Several studies in the past decade have proved the safety and efficacy of this polyphenol compound and have provided a solid basis for evaluating its efficacy in human clinical trials (Prasad et al., 2014). Despite its efficacy and safety, limited curcumin bioavailability continues to be highlighted as a major problem. However, in attempts to improve the bioavailability of curcumin, several strategies have been explored such as modulation of route and medium of curcumin administration, blocking of metabolic pathways by concomitant administration with other agents, conjugation, and structural modifications of curcumin (Agarwal et al., 2013).

2.5.1.1 Unformulated curcumin

The pharmacological studies showed that curcumin is safe and effective. Therefore, this makes it a potential compound for treatment and prevention of a wide range of human diseases. Despite these findings, more data continue to prove that curcumin has low bioavailability and poor solubility in aqueous solution (Siebenand, 2010). For the first time, Wahlstrom and Blennow in 1978 reported that, after oral administration of 1 g/kg of curcumin in Sprague-Dawley rats, negligible amounts of curcumin in blood plasma of rats was observed. This could have been due to its poor absorption from the GIT. Later, several studies were conducted on bioavailability of curcumin and found that certain amount of curcumin was bioavailable in serum of animals. In another study, when curcumin was given orally at a dose of 2 g/kg to rats, a maximum serum concentration of 1.35±0.23 μg/ml was observed at time 0.83 hours, whereas in humans the same dose of curcumin resulted in either undetectable or extremely low (0.006±0.005 μg/ml at 1 hour) serum levels (Siebenand, 2010).

2.5.1.2 Nanocurcumin

To increase the bioavailability of curcumin, different formulations have been prepared. Among them, nanoglobules based nanoemulsion formulations were prepared to evaluate the potential for the solubility enhancement of curcumin. During *ex vivo* study, the release of curcumin from nanoemulsion was found much higher than curcumin suspension. This indicated the enhancement of solubility of curcumin in aqueous solution (Kumar et al., 2012). Another study involved the encapsulation of curcumin into the hydrogel nanoparticles yielded homogenous curcumin dispersion in aqueous solution compared to the free form of curcumin. Also, the *in vitro* release profile showed up to 95% release of curcumin from the developed nano-microparticulate systems (Guzman-Villanueva et al., 2013).

2.5.1.3 Polylactic-co-glycolic acid (PLGA)

To improve the pharmacokinetics of curcumin and enhancing its bioavailability, other effective formulation of PLGA encapsulated curcumin were prepared. *In vitro* study showed that PLGA-curcumin has very rapid and more efficient cellular uptake than curcumin (Guzman-Villanueva et al., 2013). Intravenous administration of either curcumin or PLGA-curcumin (2.5 mg/kg), exhibited almost twice as high serum concentration of PLGA-curcumin than curcumin alone (Guzman-Villanueva et al., 2013) Another formulation of PLGA and PLGA-polyethylene glycol (PEG), (PLGA-PEG) blend nanoparticles containing curcumin were prepared. The PLGA and PLGA-PEG nanoparticles increased the curcumin mean half-life in approximately 4 and 6 hours, respectively, and the C(max) of curcumin increased 2.9- and 7.4-fold, respectively (Khalil et al., 2013). Compared to the curcumin aqueous suspension, the PLGA and PLGA-PEG nanoparticles increased the curcumin bioavailability by 15.6- and 55.4-fold, respectively. Thus these formulations were identified to be potential carriers for the oral delivery of curcumin (Khalil et al., 2013).

2.5.1.4 Liposomal encapsulation

Another formulation designed to improve the bioavailability of curcumin is liposomal curcumin. Liposomes have been demonstrated to be effective drug carriers because of their ability to solubilize hydrophobic compounds and to alter their pharmacokinetic properties (Takahashi et al., 2009). Oral administration of liposome-encapsulated curcumin (LEC) in rats showed high bioavailability of curcumin. In addition, a faster rate and better absorption of curcumin was observed as compared to the non- encapsulated forms. Oral LEC gave higher C(max) and shorter T(max) values, as well as a higher value for the AUC, at all-time points (Takahashi et al., 2009).

2.5.1.5 Cyclodextrin (CD)

Cyclic oligosaccharides have been also used to improve curcumin's delivery and bioavailability via its encapsulation with CD. It has been found that CD encapsulated curcumin (CDC) had a greater cellular uptake and longer half-life in the cancer cells compared with free curcumin indicating CDC has superior attributes compared with free curcumin for cellular uptake (Yadav et al., 2010).

2.5.1.6 Piperine

The natural compounds piperine has been used to increase the bioavailability of curcumin. It is a major component of black pepper, known as an inhibitor of hepatic and intestinal glucuronidation (Shoba et al., 1998).

2.5.1.7 Biological Activities of Formulated Curcumin

Accumulating data is evident that most, if not all, formulated curcumin have better bioavailability and biological activities than unformulated curcumin. Nanosuspension of curcumin also induces more cytotoxicity in Hela and MCF-7 cells than curcumin (Gou et al., 2011). The list of effort to enhance the bioavailability of curcumin cannot be exhausted (See Table 2.1) below;

Table 2.1 Current formulations of curcumin for enhanced bioavailability

Formulation	Ref.	Formulation	Ref.
Nanosuspension	(Gao et al., 2011)	PEGylated curcumin analogs	(Pandey et al., 2011)
Aqueous formulation	(Gao et al., 2010)	Curcumin-loaded MPEG-PCL	(Gao et al., 2011)
Nanoemulsion	(Zhongfa et al., 2012)	Aqueous PLGA nanoparticulate	(Nair et al., 2012)
pH-sensitive nanoparticles	(Dandekar et al., 2010)	Curcumin loaded cellulosenanoparticles	(Mohan Yallapu et al., 2012)
Microemulsion-based ion-sensitive	(Wang et al., 2012)	PLGA microparticle of curcumin	(Shahani and Panyam, 2011)
Curcumin-loaded nanoparticles	(Peng et al., 2014)	Curcumin-loaded PLGA nanospheres	(Mukerjee and Vishwanatha, 2009)

Formulation	Ref.	Formulation	Ref.
Curcumin-loaded carbon nanotubes	(Li et al., 2014)	Curcumin MPEG-PCL micelles	(Gong et al., 2013)
Curcumin co-solvent formulation	(John et al., 2013)	Curcumin PLGA-b-PEG-TPP	(Marrache and Dhar, 2012)
Silica-coated flexible liposomes	(Li et al., 2012)	Curcumin PCL-PEG-PCL	(Feng et al., 2012)
Artemisinin liposomal formulations	(Isacchi et al., 2012)	Curcumin methoxy PEG-zein micelles	(Pandelidou et al., 2011)
Liposomal curcumin	(Agarwal et al., 2013)	Curcumin-loaded alginate foams	(Hegge et al., 2011)
TMC-coated liposomes	(Chen et al., 2012)	Thermosensitive poloxamer hydrogel	(Chen et al., 2013)
Liposomes-propylene glycol liposomes	(Zhang et al., 2012)	Curcumin-loaded polymeric micelles	(Liu et al., 2013)
γ-Cyclodextrin liposomal nanoparticles	(Dhule et al., 2012)	CSO-SA	(Wang et al., 2012)
Cationic liposome-PEG-PEI complex	(Lin et al., 2012)	Curcumin-loaded amphiphilic peptide	(Park et al., 2012)
Curcumin-decorated nanoliposomes	(Mourtas et al., 2011)	Cyclodextrin-curcumin	(Yadav et al., 2010)
Liposomes of DMPC and cholesterol	(Thangapazham et al., 2008)	Dipeptide nanoparticles	(Alam et al., 2012)
Lipid-based oral formulations	(Pawar et al., 2012)	Phosphatidylcholine encapsulation	(Aditya et al., 2012)
Curcumin-loaded solid lipid nanoparticles	(Sun et al., 2013)	Graphene-based curcumin nanosystems	(Liu et al., 2013)
Curcumin nanoglobules/nanoemulsion	(Kumar et al., 2012)	Curcumin PHEMA-NPs	(Kumar et al., 2014)
Curcumin-loaded cationic liposome	(Saengkrit et al., 2014)	Silica nanoparticles	(Gangwar et al., 2013)
PGL nanocapsulated curcumin	(Ghosh et al., 2012)	Pluronic-curcumin formulation	(Singh et al., 2013)

Formulation	Ref.	Formulation	Ref.
Curcumin TRC-NPs (Rejinold et al., 2011)		EMC-curcumin (Suwannateep et al., 2013)	

2.5.1.8 Absorption of Curcumin in Blood, Liver, Brain, Kidney, and Other Organs

Uptake and distribution of curcumin in body tissues is obviously important for its biological activity. Most of curcumin get metabolized in liver and intestine however, a small quantity is still remains detectable in the organs (Prasad et al., 2014).

Evidence from literatures indicated its increased bioavailability and efficacy in different experimental models with these strategies. In spite of these, improvements in curcumin bioavailability enhancement and efficacy have not gained significant attention in human. Therefore, further exploration in attempts to enhance the bioavailability, medicinal value, and application of this interesting molecule from Mother Nature is still called for.

2.5.2 Recent delivery systems for Resveratrol

The poor bioavailability of resveratrol in humans has been a major concern for translating basic science findings into clinical utility (Smoliga and Blanchard, 2014).
A number of theoretical solutions have been developed to improve the bioavailability of resveratrol, including consumption with various foods, micronized powders, combining it with additional phytochemicals, controlled release devices, and nanotechnological formulations. While laboratory models indicate these approaches all have potential to improve bioavailability of resveratrol and optimize its clinical utility, there is surprisingly very little data regarding the bioavailability of resveratrol in humans(Smoliga and Blanchard, 2014).

If bioavailability is indeed a limitation in the clinical utility of resveratrol, there is a need to further explore methods to optimize bioavailability in humans.
While many human clinical trial results are promising, there are many challenges to overcome in developing resveratrol into effective therapeutic agents (Smoliga et al., 2012). From the most consistent data presented in the literature, one of the major issues surrounding resveratrol's future may be achieving adequate bioavailability at tolerable doses, a common issue in translating promising findings from cell culture and animal models into

clinical efficacious drugs. There are a number of mechanisms to explore which can be employed to potentially enhance the delivery of resveratrol to achieve therapeutic threshold. Despite a wealth of animal data, only a limited number of these have been attempted in humans.

Regardless of which type of exposure is most important that, sufficient quantities of resveratrol must first be absorbed into the bloodstream and be delivered to the target tissues.

Resveratrol is absorbed at a relatively high rate through the small intestine (Walle et al., 2006). The small and non-polar character of *trans*-resveratrol may allow for its absorption across the membranes by passive diffusion (Walle et al., 2006) yet there is compelling evidence that resveratrol is chiefly transported across the intestinal epithelium cells via ATP-dependent binding cassette (ABC) transporters (Planas et al., 2012). Inside the enterocytes of the small intestine and hepatocytes of the liver, the glucuronide and sulphate conjugation of *trans*-resveratrol to the major metabolites is extensive (Boocock et al., 2007); (Kuhnle et al., 2000, Miksits et al., 2005); (Walle et al., 2004). This conjugation to sulphates and glucuronide increases resveratrol's aqueous solubility, and reduces flux across membranes preventing non-polar molecules from interacting with essential macromolecules and allows for Resveratrol excretion by the kidneys via urine (Boocock et al., 2007); (Walle et al., 2004).

Human clinical trials have focused on single or multiple oral dosages of resveratrol capsules and tablets, and the majority of bioavailability data in humans reflect this delivery method. These oral dosages have been administered through various dose regimens, sizes, and physiological formulations (Almeida et al., 2009); (Amiot et al., 2013); (Boocock et al., 2007); (Elliott et al., 2009); Further, the photo stability of the resveratrol itself must also be considered when developing formulations (Francioso et al., 2014). Strategies to increase bioavailability from oral delivery of resveratrol are generally focused on increasing the rate of resveratrol absorption into the enterocytes and decreasing intracellular metabolism (Amiot et al., 2013); (Das et al., 2008); (Jose et al., 2014); (Amri et al., 2012).

2.5.2.1 Bioavailability in Humans Following Standard Oral Dosing.

The pharmacokinetics of resveratrol between human studies revealed that the greatest C_{max} and AUC of free *trans*-resveratrol have been observed following the largest dosages, generally 5 g per day (Brown et al., 2010); (Boocock et al., 2007); (Howells et al., 2011). These doses demonstrate that a C_{max} of approximately 4 µM is attainable in human plasma,

and this may be sufficient to achieve some of the physiological benefits demonstrated in laboratory models from *trans*-resveratrol alone (Thompson et al., 2014); (Boocock et al., 2007); (Kenealey et al., 2011). Theoretically, greater dosages could be used to maximize resveratrol exposure, but larger dosages of resveratrol may also be associated with poor tolerance (Brown et al., 2010) ; (Howells et al., 2011). Thus, considerable effort has been put forth to increase the ratio between free *trans*-resveratrol concentration and dosage administered.

One simple approach to enhance bioavailability has been combining various food and beverage consumption with resveratrol administration, with the notion that the combination of resveratrol with multiple polyphenols is ultimately responsible for the "French Paradox". Goldberg *et al.* (Goldberg et al., 2003) found similar plasma AUC for total resveratrol (parent compound combined with metabolites) following administration of 25 mg resveratrol with grape juice, V8 juice, or red wine, though resveratrol clearance was slowest following grape juice.

Despite much interest on the synergistic effects of resveratrol with red wine polyphenols, no further published works have explored the combination of resveratrol supplementation with other beverages. There is only one study exploring the influence of food intake on resveratrol, whereby LaPorte *et al.* (la Porte et al., 2010) reported a standard breakfast with 2000 mg resveratrol supplementation yielded a significantly greater C_{max} and AUC than that obtained following a high fat breakfast. Unfortunately, neither of these studies included a control condition to determine whether food or beverages enhanced or impaired bioavailability compared to resveratrol itself.

Increases in solubility, such as that of SRT501, are widely known to influence absorption from increasing the amount of drug in free form, but there may be a limit to this effect. Das *et al.* (Das et al., 2008) improved aqueous solubility nearly 60,000 fold using hydroxypropyl-β-cyclodextrin, and this formulation, which increases rate of absorption, had a significant impact on the maximal plasma concentration, yet negligible impact on the bioavailability of resveratrol in rats.

2.5.2.2 Synergetic/Additive Interactions

Varieties of other molecules have been demonstrated to have synergistic and additive effects when combined with resveratrol for *in vitro* models, and such interactions may potentially enhance bioavailability. Piperine, a polyphenol found in black pepper, has been shown to substantially increase serum C_{max} and AUC of resveratrol in rats (Johnson et al., 2011). In coadministration in an oral gavage of 100 mg/kg and 10 mg/kg piperine, Johnson *et al.* showed a 1000% increase of peak plasma levels for resveratrol while delaying a major glucuronide resveratrol metabolite (Johnson et al., 2011). Consequent research in cellular (Tak et al., 2012) and animal (Huang et al., 2013) models has indeed shown piperine to potentiate the effects of resveratrol. However, only one human study has explored this combination, and found that piperine may enhance resveratrol's effects on cerebral blood flow, yet does not increase its bioavailability (Wightman et al., 2014).

2.5.2.3 Resveratrol Precursors/Pro-Drugs

Another approach to maximize the bioavailability of free *trans*-resveratrol is to develop resveratrol prodrugs. Assuming that maximizing free *trans*-resveratrol is the primary goal, the theoretical ideal is that a resveratrol prodrug would allow resveratrol to be generated through enzymatic reactions *in vivo* to allow for a physiological relevant concentration without toxicity (Biasutto and Zoratti, 2014). For a prodrug to be effective, at least one of the following must occur: (1) metabolism of the prodrug to generate high plasma concentrations of resveratrol; (2) entry of the prodrug into tissues of interest, which can then metabolize the prodrug into resveratrol to maximize tissue concentration; (3) acceptable pharmacokinetics of the prodrug itself. There has been a single rodent study reporting bioavailability of a resveratrol prodrug, and with promising results. Regardless, the prodrug approach has not yet been attempted in humans.

2.5.2.4 Alternative to Standard Oral Dosages

As previously mentioned, oral delivery of resveratrol has received the majority of clinical attention, but it is unclear if the many reported potential micronized and encapsulated formulations will be able to overcome issues faced by the widely explored micronized resveratrol formulation SRT501 (Amri et al., 2012); (Elliott et al., 2009); (Howells et al., 2011); (Popat et al., 2013). With this in mind, few trials have investigated potential delivery routes beyond traditional oral administration. These methods bypass the constraints to concentration of the gastrointestinal tract and first pass hepatic metabolism, but have their own challenges to overcome. Perhaps the most obvious way to maximize absorption of

resveratrol is to bypass the gastrointestinal tract completely and deliver resveratrol directly into the bloodstream.

While intravenous injection may provide greater plasma concentration of free *trans*-resveratrol than typical oral administration, it is not clinically practical if chronic self-administration is desired. However, intravenous administration may be useful to rapidly deliver a large bolus of resveratrol. In rats, resveratrol metabolites were detected within one minute after intravenous injection via the tail vein, and glucuronide conjugates achieved a greater concentration than parent compound within three minutes (Juan et al., 2010). This rapid delivery may produce quick physiological responses, which can lead to valuable clinical applications. Indeed, intravenous resveratrol administration has been demonstrated to acutely increase renal blood flow in rats (Gordish and Beierwaltes, 2014) and acutely influence outcomes in various animal models of ischemia (Karaoglan et al., 2008); (Shin et al., 2010). Although intravenous administration of resveratrol in animal models has demonstrated great potential, there are no reports of intravenous administration in humans throughout the scientific literature. Nonetheless, there is no known reason for intravenous administration of resveratrol to be contraindicated in humans, and this is an area for further exploration.

A non-invasive means of resveratrol delivery, which may circumvent the constraints of the gastrointestinal tract and first pass hepatic metabolism, is oral transmucosal dosing(Amri et al., 2012) (Ansari et al., 2011) (Blanchard et al., 2014). There are multiple known limitations for the method, such as a limited dose size and difficulties achieving absorption (Madhav et al., 2009); (Streisand et al., 1998); (Zhang et al., 2002).

One potential method to maximize free *trans*-resveratrol concentration near the tissue of interest is through implantable devices, and this approach has been demonstrated to be promising in animal models. While currently approved drug eluting stents have made major improvements in restenosis over bare metal stents, they have also been shown to prevent vascular wound healing (Hamid and Coltart, 2007). This prevention of healing is understood to be a factor in developing late thrombosis with Taxol and Rapamycin derivative stents (Hamid and Coltart, 2007); (Kleinedler et al., 2012); (Yurdagul et al., 2014). While this application likely increases local bioavailability, its systemic effects remain unknown, and these have not yet been tested in humans. Nonetheless, such localized delivery systems hold potential for clinical utility.

2.5.2.5 Nanotechnological Approaches

A number of recent studies have focused on using nanotechnology to improve the bioavailability of resveratrol and have generally demonstrated improved stability and bioavailability with minimal side effects compared to oral dosing. Nanoformulations can improve resveratrol's solubility and transport across the plasma membrane, and thus enhance its effects within cells (Ansari et al., 2011). There is emerging evidence that nanoformulations of resveratrol can protect resveratrol from metabolism during the digestive process, which ultimately increases tissue absorption in animal models (Sessa et al., 2011). Resveratrol loaded onto lipid-core nanocapsules improved tissue concentration in the brain, liver, and kidney of healthy rats compared to free resveratrol (Sessa et al., 2011).

2.5.2.6 Metabolite Activity-Implications for Bioavailability

Controversy remains as to whether resveratrol itself is the primary molecule responsible for health benefits, or whether the activity of its metabolites contributes significantly to its biological effects (Calamini et al., 2010); (Hoshino et al., 2010). If resveratrol metabolites have a similar or greater magnitude of physiologic activity and tissue distribution compared to resveratrol itself, this could lead to a paradigm shift and dramatically change the future direction of dosing research, with less emphasis needed on parent compound.

Taken together, this data suggest that the metabolites of resveratrol do have biologic significance, either through direct activity or through recycling. There is not yet sufficient information to determine the relevance of metabolites *in vivo*. Regardless, metabolites contribute to the majority of total resveratrol concentration following oral administration, and thus there are logically few options to further increase the metabolite levels *in vivo* (Boocock et al., 2007) (Walle et al., 2004); (Brown et al., 2010). For instance, Boocock (Boocock et al., 2007) reported C_{max} for two separate mono-glucuronide metabolites, for a total glucuronide metabolite concentration ~7.5 µM following a single 5.0 g oral dosage of resveratrol. Thus, even if metabolites play a role in resveratrol's *in vivo* physiological effects, strategies to enhance bioavailability remain highly relevant.

2.5.2.7 Limitations of current resveratrol bioavailability research

There are clearly many possible approaches to increase the bioavailability of *trans*-resveratrol, but there remains much uncertainty. While larger doses appear to be associated with greater bioavailability, there is considerable variability in the results between studies. For instance, Boocock *et al.* (Boocock et al., 2007) reports a C_{max} =538.8 ng/mL (~2.4 µM) following a single dose of 5000 mg resveratrol, but this is far lower than the 1274 ng/mL

(~5.6 µM) reported by LaPorte *et al.* (la Porte et al., 2010) following a much lower dosage (2000 mg with a standard breakfast) (Boocock et al., 2007); (la Porte et al., 2010). It is not be surprising that the results from human clinical trials using different dosage protocols sometimes conflict one another. There remains concerns that the concentrations found *in vivo* in humans are still insufficient to elicit beneficial results.

Bioavailability of resveratrol is generally characterized through quantifying plasma or serum concentrations, but this does not consider resveratrol found in red blood cells (Blache et al., 1997) or that which has been distributed to other tissues. As such, evaluation of resveratrol in plasma represents only a small fraction of that actually found in the blood (Biasutto et al., 2010) and may not be an accurate indicator of resveratrol absorption and exposure. Further, it must be remembered that blood represents only one biological target tissue. While removal via the hepatobiliary and renal systems does account for one aspect of the time-dependent clearance from the blood, resveratrol does accumulate in other tissues where it may have beneficial effects, such as the heart, liver, and skeletal muscle (Andres-Lacueva et al., 2012); (Lin et al., 2012); (Lou et al., 2014); (Wang et al., 2008).

2.5.2.8 Conclusions

For over a decade, it has been realized that resveratrol has poor bioavailability in humans. In the time since, a number of human clinical trials have been performed using a wide range of dosage protocols, and conflicting findings have caused considerable controversy over clinical utility. While there are a number of promising methods to increase bioavailability of resveratrol, as demonstrated in animal models, surprisingly very little work has been performed in this regard for humans. This is especially concerning, given that conclusions about resveratrol's future are being generated from human clinical trials that are currently being performed without a full understanding of the optimal dosage protocols. The ultimate clinical potential of resveratrol cannot be fully realized until proper dosage protocols, which provide optimal bioavailability in humans to ensure sufficient tissue distribution, are established. Until then, poor bioavailability is just one of many factors, which may account for discrepancies between laboratory models and human clinical trials. Interpretation of human bioavailability literature is complicated by major differences between dosing protocols and quantification methods, with a lack of comparative bioavailability studies. Lastly, current bioavailability measurements may not fully represent the total resveratrol pool available in the blood and generally do not quantify tissue distribution. Whenever possible, future research exploring the bioavailability of resveratrol in humans is still called for and should:

(1) Include a control resveratrol condition (e.g., standard oral dosage) that can serve as a reference to determine the effectiveness of novel formulations.
(2) Explore a variety of dosages to determine how bioavailability parameters, including metabolite distribution, are influenced by quantity of resveratrol administered.
(3) Measure concentration of resveratrol and its metabolites in the plasma and whole blood, rather than the plasma alone.
(4) Include tissue samples when ethical and clinically feasible (e.g., bioavailability studies in patients undergoing surgical resection, as part of studies requiring muscle and adipose tissue biopsies, etc..
The intraoral Dispersible Film still has many advantages over these dosage forms, as we shall see later in the study but also leaves a need to do more research.
Though efforts to increase bioavailability can be extended to many other molecules, the discussion in this study is limited to only the module drugs Curcumin and Resveratrol.

2.6 Formulation of market available fast dissolving films (FDF)

Oral filmstrips were first introduced in the market as breath fresheners. These films were put in the mouth where they dissolved instantaneously and released the mint flavour which gave the mouth a fresh breath (Barnhart and Vondrak, 2008); (Hariharan and Bogue, 2009). The IDFs meet with the attributes that today's patient is looking for in a dosage form which includes; better portability, ease and accurate dosing, as well as overall convenience. These films generally dissolve within seconds to release the active agents (Fulzele et al., 2002); (Dixit and Puthli, 2009). A film is defined as a dosage form that contains water dissolving or disintegrating polymer which when placed on the tongue or on any part of the oral mucosa absorbs water, swells, disperses in saliva, and is absorbed to provide rapid local or systemic drug delivery (Hariharan and Bogue, 2009). Drug release can be instant or slow. This is achieved by altering the rate at which the films disintegrate orally.

We can now have a look at existing oral formulations on the market and other formulations in literature that are used to overcome poor oral bioavailability. There are patented approaches that include X-Gel X Gel™, Sol leavers, WafreTabs, Wafertab™, Foam Burst, and MiniCap (Bala et al., 2013). Further on, Zydus by UK Laboratories was patented in the 1970s. Instantaneous oral disintegrating dosage forms are given names like oro-dispersible, melt-in-mouth, fast dissolving, porous tablet, quick dissolving, and orally rapidly disintegrating dosage forms to mention a few. In my opinion, melt-in-mouth would give a wrong impression as these products only dissolve or disintegrate in the mouth. Suitable drug candidates for

IDFs include natural drugs like Resveratrol and Curcumin used in this study as model drugs (Doheny, 2008); (Fantini et al., 2015), and Nicotine Replacement Transdermal Delivery (NRTD) (Gali, 2013).

Anti-ulcer and antihistamines could also be formulated as IDFs. Antipsychotic and sleeping disorder drugs are also potential candidates to consider as prescription IDF products because in most cases their rapid delivery is needed to induce a quick effect or relieve pain. Intraoral Dispersible Films (IDFs) have gained more popularity and attention than other forms in the same class because of their improved patient compliance and ease of administration of the drug delivery system (Liew et al., 2013). Quick Intraorally Disintegrating Tablets (IDTs) are the only dosage form of this nature recognized by the FDA as they are listed in its Orange book. The European Pharmacopoeia calls them “disperse” tablets, which when placed in the mouth, disperse instantaneously before swallowing.

IDFs can be defined as “A solid dosage form containing medicinal substances, which disintegrates rapidly, usually within seconds, when placed on the tongue”. The problem of poor oral bioavailability of drug molecules encountered with traditional oral dosage forms can now be overcome by using pregastric drug delivery formulations like IDFs. In September 2003, Chloraseptic, the sore throat relief thin films were launched by Prestige Brands international in the United States (Barnhart and Vondrak, 2008); (Garsuch and Breitkreutz, 2009); (Hariharan and Bogue, 2009); (Siebenand, 2010).

Zengen Inc. as a new delivery technology in Europe introduced a bilayer medicated oral film. Boots UK, also introduced vitamin oral strips on the market. Pfizer through Warner-Lambert its consumer healthcare subsidiary (Kandybin and Kihn, 2004) put the breath freshening strips Listerine PocketPaks on the market in 2001 (Hearnden et al., 2012). These films mainly contain water-soluble hydrocolloids for example hydrocellulose, pullulan, pectin, and carboxymethyl cellulose, with an active agent.
Other excipients included flavoring agents, plasticizers and preservatives. Some contain Chitosan (Powdered shell of a fish) as a film-forming agent. The solution ability of these films to disintegrate depended on thickness of the hydrocolloids produced. However, thorough compliance by the patient plays a major role in ensuring rapid solubility of the films. The main problem with the films is with drug loading as this is generally limited to about 30 mg whereas this problem can be solved by increasing the thickness of the film (Nagar et al., 2011). However; solubility of the product is compromised and may change it to a slow dissolving product.

Despite the challenges, drug companies have picked interest in the technology of intraoral thin films as it provides fast, accurate dosing with an expected increase in patient compliance, and rare paediatric formulations could be possibly made as IDFs. Cardiovascular, anticancer, antipsychotic antiepileptic and sleeping disorder drugs are the potential IDF formulations (Hamilton, 2008); (Borsadia et al., 2003); (Garsuch, 2009); (Nishimura et al., 2009); (Patel et al., 2010); (Reiner et al., 2010).

2.7 Types of oral films

Depending on disintegration time and design, a distinction between fast-dissolving and sustained-release films, mucoadhesive films or oral patches can be made. However, there is no clear dividing line (Barnhart and Sloboda, 2007). Mucoadhesive films and oral patches are commonly present on the market as buccal sustained release dosage forms. Local or systemic drug therapy can be achieved with all types, but particularly for mucoadhesive films, retention time plays an important role. Systemic therapy may be realized mainly by means of absorption of the API through the oral mucosa. Different application areas are possible. IDFs are usually placed onto the tongue (Bhura et al., 2012). Mucoadhesive films are typically placed onto the cheeks, but the palate or sublingual are feasible as well. Further, based on IDF technology, films for vaginal or rectal applications may be possible. This study is limited to or focuses only on intraoral soluble films / intraoral dispersible films only.

2.8 Advantages and disadvantages of IDFs

IDFs are supposed to be administered on the tongue, which mechanically pushes the film to the upper jaw palate where it is crushed. It disintegrates or dissolves rapidly in a small amount of saliva that is trapped between the tongue and the upper palate (Bhura et al., 2012). Swallowing whole is neither necessary nor desired. For this reason, IDFs are suitable for children and elderly patients. They are useful also for bedridden patients and those suffering from dysphagia, Parkinson's disease, mucositis and vomiting (Barnhart and Vondrak, 2008); (Hariharan and Bogue, 2009); (Nishimura et al., 2009). Owing to the fast wetting, oral films may go to solution in the oral mucosa readily, and they cannot be spat out easily (Garsuch, 2009). Some patients even refuse orally disintegrating tablets because of their fear of choking or inhalation. IDFs overcome swallowing problems (Liang and Chen, 2001).

IDFs need no water to be administered. They are therefore suitable for travellers and for patients who cannot access drinking water easily. As an added advantage, the intake is

discreet. Intraorally disintegrating tablets (IDTs) have gained popularity during the last few years. In order to accelerate their ability to disintegrate quickly, many IDTs are friable. This gives rise to problems during manufacture, handling, including transportation and uniformity of weight during administration. On the other hand, IDFs are flexible but still robust to mechanical forces (Borsadia et al., 2003). Lyophilisation process has been employed in the manufacture of IDTs and transdermal patches but is an expensive technological venture for producing IDFs (Reiner et al., 2010).

Therefore, the solvent casting method, which is less expensive, has been adopted for the small-scale manufacture of IDFs. Compared to liquid formulations such as drops or syrups, IDFs offer more convenient and accurate dosing formulations (Barnhart and Vondrak, 2008). As the drug is released within seconds in the mouth, a quick onset of action could be ensured. First-pass effect in the liver is avoided because the drug is absorbed only in the mouth and this improves the bioavailability of some essential natural drugs (Dixit and Puthli, 2009); (Patel et al., 2010). Buccal absorption could be used beneficially for migraine patients as well (Breitkreutz and Boos, 2007); (Patel et al., 2010). However; some patients may experience drowsiness, owing to the quick onset of action (Mishra and Amin, 2009).

Drug load is limited. Therefore, IDFs are restricted to highly potent low-dose drugs. Moreover, manufacturing typically requires solvents and heat for drying. These factors potentially affect stability of the drug and or other excipients such as sweeteners and flavours (Patel et al., 2010). A general major drawback of orodispersible dosage forms is taste. Taste masking may reduce maximum drug load further. For extremely bitter APIs, taste masking may even be impossible.

2.9 Required characteristics of IDFs

An ideal IDF should be thin and flexible, but stable to guarantee a robust manufacturing and packaging process and ease of handling and administration by the patient (Cilurzo et al., 2008); (Corniello, 2006); (Peh and Wong, 1999). The Intraoral Dispersible Films should be transportable, and not tacky, thus keeping a plain form without rolling up on itself (Cilurzo et al., 2008); (Corniello, 2006). They should provide a good taste and mouth-feel (Corniello, 2006). Disintegration time should be as short as possible. It is not easy to meet all the requirements, because of the reciprocal relationship existing between the poor mechanical properties and the disintegration time of the rice starch as a polymer (Corniello, 2006).

2.10 Manufacturing

2.10.1 Process highlights.

The manufacture of IDFs involves established techniques like, the solvent casting method, and sometimes hot melt extrusion process (Khairnar et al., 2009). Usually, a wide web is produced first, which is cut into the final dosage form afterwards. Consequently, the uniformity of the wide web is the key concern during manufacturing. Oral films have a wide range of dissolution profile depending on the specific sites or region in the mouth. IDFs do not require either water or swallowing (Bhyan et al., 2011). They can enhance compliance of different patient groups and are an attractive new dosage form. Manufacturing of IDFs is very flexible, because some process steps are challenging. The solvent casting technique is the method of choice at present. IDFs require moisture protecting packaging (Bhyan et al., 2011). Basic excipients of IDFs are film-forming polymers and plasticizers. In most cases taste masking is essential. There are no standardized tests for IDFs available yet in the official Pharmacopoeias. Therefore, in this study, we are going to develop our own suitable in house characterisation methods as derived from those employed for standard oral solid dosage formulations.

2.10.2 Solvent casting

A wet coating mass is prepared first. Typical production steps are summarized in (Figure 2.17). Solutions, emulsions and suspensions can also be cast (Barnhart and Vondrak, 2008). The film-forming polymers are dissolved in a solvent, either in pure water or in mixtures of water and organic solvents. The organic solvents could improve solubility of the API and shorten drying time (Breitkreutz and Boos, 2007). Further on, excipients are added, followed by a homogenization step, resulting in a viscous solution. In the last step the API is added, for example, pre-dissolved or pre-dispersed in a liquid (Patel et al., 2010). If suspensions or emulsions are cast, homogeneity has to be assured during the whole casting process. This was achieved by casting the rice paper films in plastic Petri dishes laid flat and left undisturbed in open air on a perfectly levelled table.

High viscosity slows down sedimentation. Particle size can be a critical factor. Particles > 250 µm can accumulate in the fluid flow path and cause scratches on the surface of the film (Barnhart and Vondrak, 2008); (Barnhart and Sloboda, 2007). Continuous stirring and application of a vacuum (Dixit and Puthli, 2009); (Hariharan and Bogue, 2009) to achieve de-aeration of the coating mass. In our case, the Petri dishes were tapped to eliminate the entrapped air bubbles. The coating mass is cast as a wide web onto a single belt or a

release coated substrate called intermediate liner (Barnhart and Vondrak, 2008). The adjusted wet film thickness determines the drug content of the final strip (Dixit and Puthli, 2009).

The wet film is conveyed through an oven for the removal of process solvents and subsequently rolled on itself for intermediate storage and transport jumbo roll (Barnhart and Vondrak, 2008); (Dixit and Puthli, 2009). The jumbo roll is cut into smaller daughter rolls of variable width, which are finally punched or cut into the desired size (See Figure 2.13: Industrial Film Manufacturing process for intraoral dispersible films of varying sizes (Shimoda et al., 2009).). The intermediate liner is removed and the films are packaged individually. Some factors are critical. Segregation or sedimentation should not occur in the coating mass (Barnhart and Vondrak, 2008); (Barnhart and Sloboda, 2007).. The intermediate liner has to be chosen carefully. The film should show a sufficient adhesion to the liner but has to be removable at the end of the process (Hariharan and Bogue, 2009).

During drying, it is important to avoid the so-called ripple effect. Being exposed to hot dry air, the process solvents can evaporate immediately, dry only the surface and leaving the bottom wet and sealed off from further drying because evaporation is not possible. Raising temperature increases further the vapour pressure until the film surface rips. Surface building and rupturing occurs several times, leading to uneven film surfaces (Goel et al., 2008).

The end point of drying has to be controlled carefully (Hariharan and Bogue, 2009); (Boateng et al., 2009a). If organic solvents are used, they have to be removed to acceptable levels (Barnhart and Vondrak, 2008). Residual water content is necessary to obtain flexible films (Dixit and Puthli, 2009). Nevertheless, high water content can lead to tacky films particularly if the drug was partly dissolved in the coating mass. Crystal growth during the drying process and storage can affect content uniformity (Gaisford et al., 2009); (Garsuch, 2009). Possible variations of the basic process are semi-solvent casting or introducing gas bubbles to shorten disintegration time (Arya et al., 2010).

Manufacturing of films is very flexible, because various strengths can be cut out of one jumbo roll using different film sizes (See Figure 2.13). Usually content uniformity is in the magnitude of 1 - 2% (Barnhart and Vondrak, 2008). Therefore, solvent casting is commonly used for the small-scale manufacturing of IDFs. Nevertheless, the drying step is energy consuming. Problems may occur with residual solvents and instability of the API or flavours in the final products (Patel et al., 2010).

2.10.3 Principle of Sol – gel to synthesize the films

The principle is based on reducing the solid particles to as small particle size as possible but maintaining the integrity of the molecule, generally below 250µm. micronising the molecules reduces the adhesion forces between them such that it becomes possible to make a very thin sheet out of these molecules. We were therefore able to prepare the rice paper this way. Figure 2.9 below shows the Principle of Sol – gel to synthesize the films.

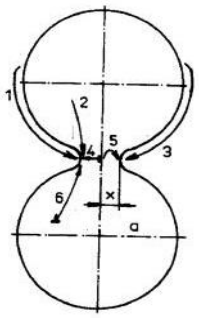


Figure 2.9: The sol – gel principle as employed to produce the intra oral dispersible films on a small scale (Brinker and Mukherjee, 1981).

2.10.4 Sol-Gel Methods

The Sol-Gel Methods (See Figure 2.9)Figures 2.9 – 2.12 are defined in the following paragraphs and involves the following steps: ‘Sol’ stands for; a stable suspension of colloidal solid particles or polymers suspended in a liquid and ‘Gel’ stands for a porous, three-dimensional, continuous solid network that is surrounding a continuous liquid phase (Brinker and Mukherjee, 1981); (Brinker and Scherer, 1990). In the first step the colloidal particulate gels, that is, it forms an agglomeration of dense colloidal particles. Secondly, it forms polymeric gels, this means, an agglomeration of polymeric particles made from sub colloidal units, and lastly, agglomeration. In this step, covalent bonds, van der Walls forces, and hydrogen bonds come into play to form a polymeric chain entanglement, which we call films (Hench, 1990; West, 1990). This is illustrated in the process flow diagram represented by figure 2.15 below:
The schematic flow of the sol - gel process is shown below;

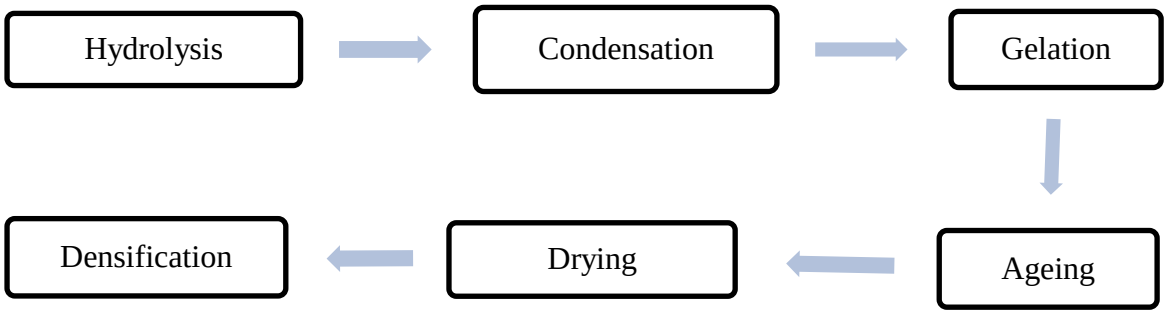


Figure 2.10: The sol-gel process.

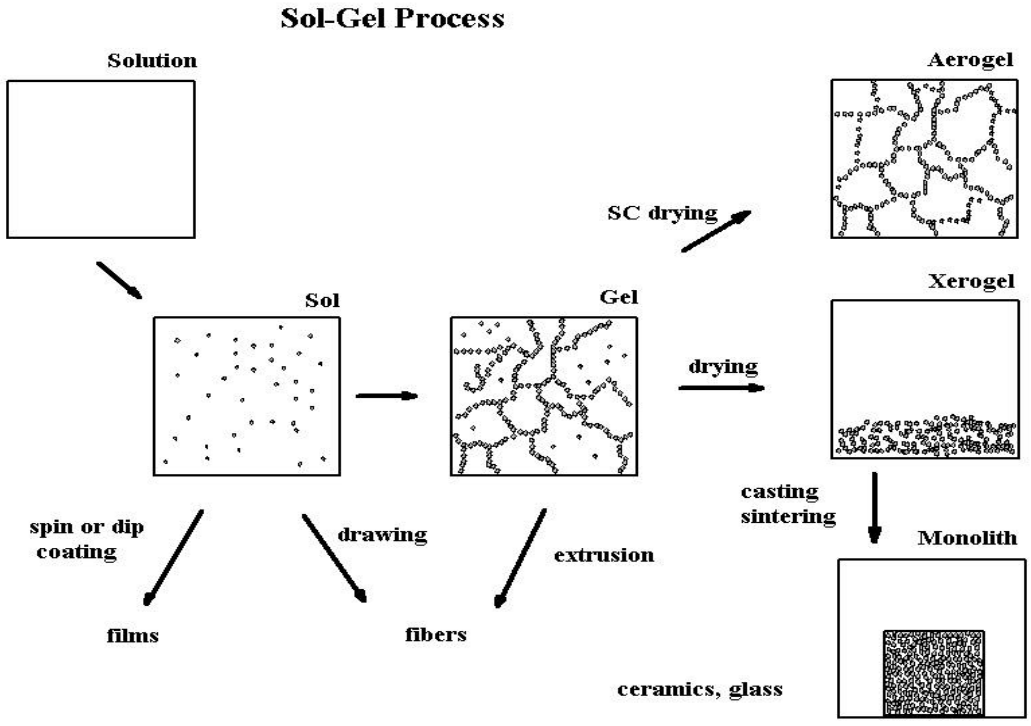


Figure 2.11: Illustrates the Sol – gel technology as employed to produce the intra oral Dispersible films on a small scale (Brinker and Mukherjee, 1981).

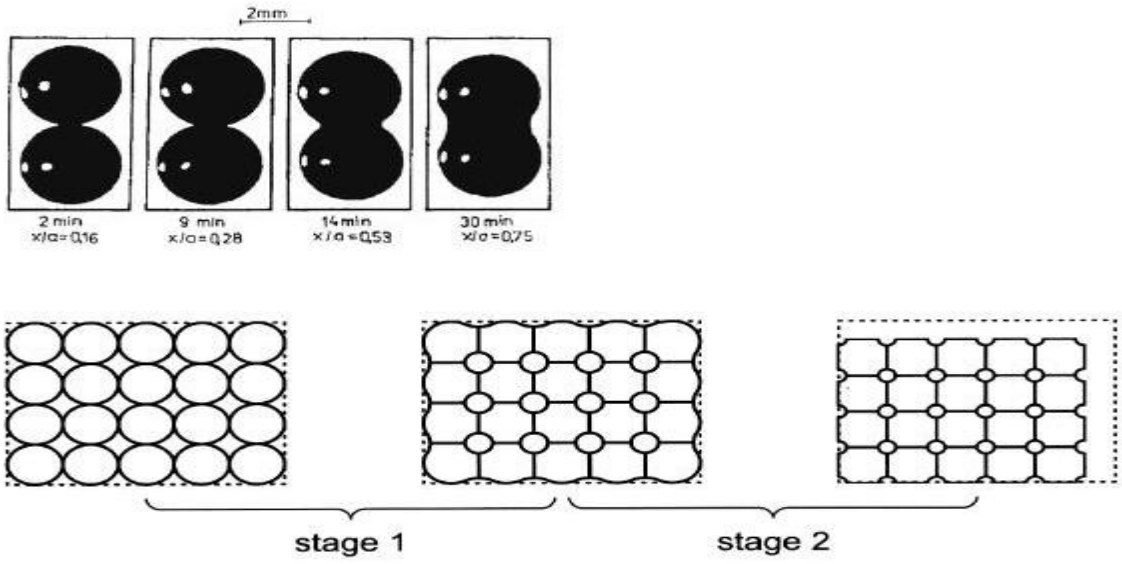


Figure 2.12: Stages in sol – gel technology as employed to produce the intra oral Dispersible films on a small scale (Brinker and Mukherjee, 1981).

2.11 Hot-melt extrusion process

Hot-melt extrusion is a solvent-free production process for IDFs. The API is blended with excipients in solid-state. The blend is first heated and pressed between the nozzle slots to form a web, which is later cooled and cut into the required film size (Cilurzo et al., 2008); (Ghebre-Selassie and Martin, 2003). Recently, another method was introduced. This uses spherical dies and cooled rollers to form the thin web after the hot-melt extrusion. (Koster and Thommes, 2010). Extrusion surely has some advantages over solvent casting, such as lack of solvents and drying. However, the melting process may affect API, flavour or polymer stability (Barnhart and Vondrak, 2008); (Garsuch and Breitkreutz, 2010); (Patel et al., 2010). Yet, the main problem seems to be the lack of suitable polymers (Patel et al., 2010).

Cilurzo et al. compared hot-melt extrusion with solvent casting with maltodextrin as the main polymer. To obtain mechanically stable and non-tacky films by means of hot-melt extrusion, microcrystalline cellulose had to be added, which affected disintegration time and mouth-feel substantially (Cilurzo et al., 2008). Hot-melt extrusion may be suitable for the manufacturing of sustained release films or patches, but achieving the required small thickness and short disintegration time for IDFs still seems to be impossible with the current techniques.

2.12 Others

For the rolling method, a paste-like or highly viscous material is rolled onto a plain carrier. To obtain a paste-like texture a solvent is necessary, which has to be removed in a following drying step (Arya et al., 2010). Yang et al. described a method using three rollers. The coating mass is metered on the first roll, which determines the coating thickness. The mass is transferred to a second roller, which conveys the mass on an immediate liner transported by a third roll (Yang et al., 2008). Spraying of a drug-loaded solution or suspension onto a plane carrier could be another alternative for film forming. A multilayer film is described in the patent literature. A solvent casting method produces one layer, for example,; the second layer is sprayed onto the first as suspension or by electrically charging a powder mixture including the API (Davidson and Kehoe, 2004); (Yu et al., 2010).

Electrostatic spinning is gaining popularity for pharmaceutical applications. Thin polymer fibers are produced by applying a high electric field on a drug-loaded polymer solution. A solid dispersion with improved solubility of the poorly soluble drugs is obtained (Shimoda et al., 2009); (Nagy et al., 2010). When high voltage is applied to a liquid droplet an electrostatic repulsion results which overcomes the surface tension. This stretches the droplet and at some critical point, a liquid jet is formed at the surface, which is drawn by electrostatic forces between two electrodes. The solvent evaporates rapidly. The resulting nanoscale fibres form a non-woven web on a collector (Shimoda et al., 2009); (Nagy et al., 2010); (Yu et al., 2010). They produced electrospun API-loaded webs for fast release and compared them with cast films. Owing to the high surface area, dissolution time was improved. The industrial manufacturing process is illustrated in (See Figure 2.13).

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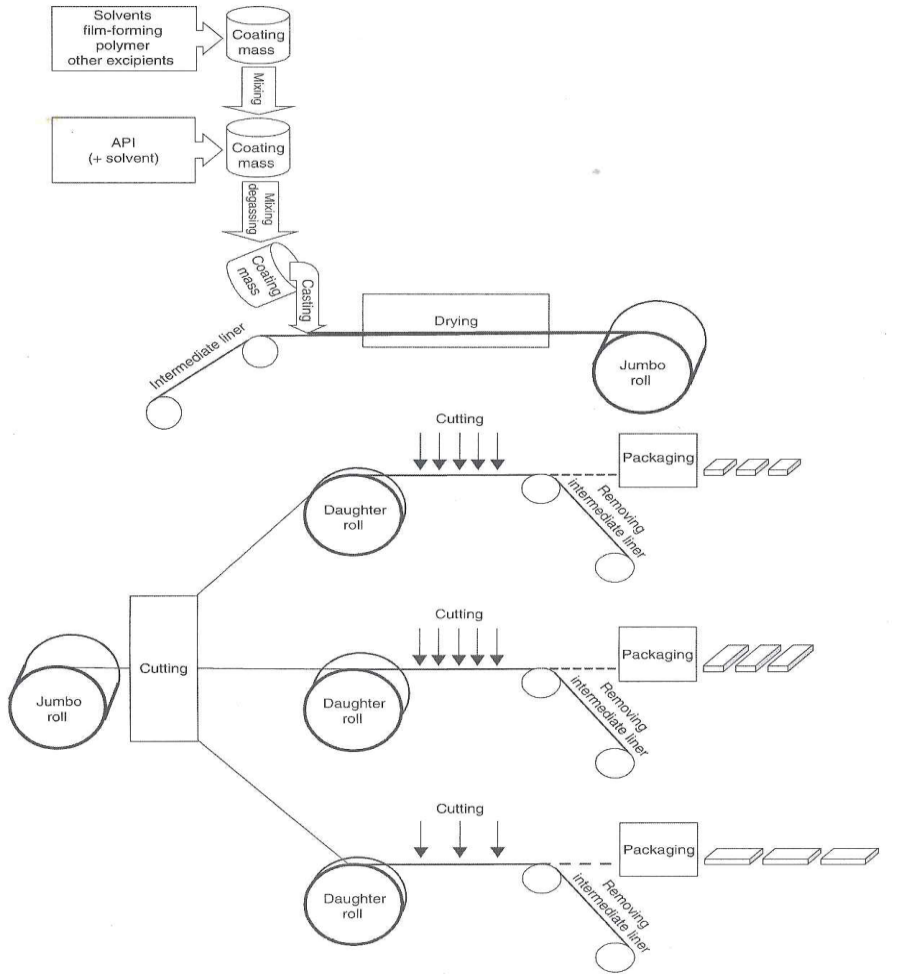


Figure 1. Manufacturing of orodispersible films of varying size.
API: Active pharmaceutical ingredient.

Figure 2.13: Industrial Film Manufacturing process for intraoral dispersible films of varying sizes (Shimoda et al., 2009)**.**

2.13 Packaging of rice paper films

As IDFs are sensitive to moisture, unpacked storage should be avoided (Dixit and Puthli, 2009). Usually ODFs are sealed individually. Solvents, film-forming, polymer, other excipients, API, Coating mass, Mixing, degassing, Casting, Drying, Packaging, Intermediate liner Removing, intermediate liner Cutting, Cutting Jumbo, roll Jumbo roll Daughter roll, Daughter roll, Daughter roll Cutting , must all be carried out in a temperature and Humidity controlled environment. Packaging materials that provide a moisture barrier are required. Visual inspection by automatic systems is required as in-line in-process control; unit weight variation could be measured off-line (Hariharan and Bogue, 2009). Multi-dose and single-dose packaging is possible, but single packages should be preferred to avoid accidental overdosing by films sticking together.

A roll dispenser with a continuous film, which can be cut individually into desired sizes (with equal individual dosing), provides new opportunities for personalized medicine (Malke et al., 2010); (Allen et al., 1989). Advanced packaging technologies such as the Rapidcard (Lehrke et al., 2005), are equivalent to the size of a credit card. This has three films rolled on it. These rolls are suitable to be used as take away medicine (Lehrke et al., 2005). Some examples for IDFs and different packaging technologies are given in (See Figure 2.14). Child-resistant and senior-friendly packaging should be taken into account. To meet industry regulations, necessary information can be printed directly onto the film before packaging (Frey, 2006).

In the pharmaceutical industry, it is vital that the package selected adequately preserve the integrity of the product for its entire shelf life. This is contrasted with the cost of the final product and its affordability by the patient. Expensive packaging should be avoided and special care must be taken during formulation, manufacturing, and storage to protect the films in their fast dissolving dosage form. The film has to withstand all handling stages of Manufacture, Transportation, Storage, and finally robust enough to be appreciated by the patient. A variety of packaging options are available for films but Single packaging is the most preferred.

Strips of 24s would probably be a convenient dosage schedule for our IDFs. This would also cater for hygiene and safety purposes of the product. Aluminum foil would be the most ideal and appropriate packaging in glass jars or colligated paper boxes of a specific ply as a

secondary packaging which will specifically be designed for the films. Every dose could be taken out individually to avoid sticking together and the event of accidental over dosage.

In general, the material selected must have the following characteristics:

- It must protect the preparation from environmental conditions.
- It must be recommended by the Medicines Control Council (MCC).
- It must meet with applicable tamper resistant requirement.
- It must be non-toxic.
- It must not react with the product.
- It must not impart to the product tastes or odors
- It must not make the product expensive

2.13.1 Foil, paper or plastic pouches

The flexible pouch is a packaging concept capable of providing not only a package that is temper- resistance, but also by the proper selection of material, a package with a high degree of environmental protection. A flexible pouch is usually formed during the production of the product. This could be a primary packaging for the IDF as well. This study does not go further to explore packing and labeling. It is restricted in scope to only wrapping the films into the aluminum foil as the primary moisture barrier. Packing will later be studied at the manufacturing and registration stages of the product development.



Figure 2.14: The Blister card (Lehrke et al., 2005).

2.14 Reported formulation materials employed for the fabrication of FDFs

A typical IDF contains; “Active pharmaceutical ingredient.API: 1 to 30%, Water-soluble polymers 40 to 50%, Suitable plasticizers 0 to 20%, Other excipients such as flavours, fillers, colours, and so on, 0 to 40%”(Frey, 2006). As IDFs are an appropriate dosage form for children and the elderly, excipients should be carefully selected regarding safety to protect them from hazards (Breitkreutz and Boos, 2007).

2.14.1 Active pharmaceutical ingredient

The API can be incorporated into the films as particles or molecularly dispersed/dissolved. Particularly for dispersed APIs, particle size, particle size distribution and polymorphism become a critical quality attributes. It is well known that these factors may affect solubility, rate of dissolution and ultimately bioavailability. As drug load is limited, high potency low-dose drugs are preferred (Hariharan and Bogue, 2009). Maximum drug load depends on the solubility of the API and/or its compatibility with the excipients (Chen et al., 2008).

A critical drug load can result in recrystallization or excessive influence on mechanical or disintegration properties of the films (Cilurzo et al., 2008); (Gaisford et al., 2009); (Garsuch and Breitkreutz, 2009). Typically, drug load is limited to a maximum of 25 mg. Gas-X films from Novartis Consumer Health, Basel; contain a surprisingly high loading of 62.5 mg of simethicone. Other challenges may arise from bad API taste and limited API stability resulting from manufacturing conditions.

2.14.2 Film-forming polymers

Film-forming polymers are essential excipients for IDFs. Numerous polymers have been proposed in literature. Yet, the selection remains challenging. Although the films should dissolve as fast as possible in the oral cavity, mechanical properties have to remain sufficient for handling, packaging and storage (Corniello, 2006). Polymer properties depend on their molecular mass. As a general rule, low-molecular-mass polymers dissolve quicker, whereas a higher molecular mass results in better mechanical properties (Arya et al., 2010); (Chen et al., 2008); (Corniello, 2006).

Mishra and Amin compared various grades of hypromellose with increasing molecular mass (E3 < E5 < E15). They concluded that E3 quality is superior to E5 and E15 grades for the

manufacturing of cetirizine hydrochloride IDFs (Mishra and Amin, 2009). Dinge and Nagarsenker preferred E5 over E3 and E15 for the formulation of triclosan IDFs (Dinge and Nagarsenker, 2008).

Cilurzo researched on the impact of maltodextrin masses on film properties (Cilurzo et al., 2010). Films made of maltodextrin with a high molecular mass were stiffer than films made of maltodextrin with a lower molecular mass. They showed a higher tensile strength, a higher elastic modulus and a lower elongation at break. Further, they were less sticky than the films made of maltodextrin with a lower molecular mass (Cilurzo et al., 2010).

The viscosity of the coating mass increases with increasing polymer molecular mass (Corniello, 2006). Viscosity must be high enough to prevent sedimentation of the drug in the coating mass but not too high to avoid problems during mixing or during coating by poor spreadability (Hariharan and Bogue, 2009). To achieve the desired film properties, often combinations of different polymers or different molecular masses of the same polymer are used (Corniello, 2006). Cellulose derivates, polyvinyl alcohols and pullulan are commonly used. Low viscosity grades of hypromellose, hyprollose or carmellose are typically used cellulose derivates (Boateng et al., 2010).

As pullulan, is a linear polysaccharide with a-1, 6-linked maltotriose, it is quite expensive and efforts have been made to replace it in parts by using starches (Dixit and Puthli, 2009).
Further suggested polymers are, macrogols, sodium alginate, gelatine and pectin. As pectin dissolves slowly, it is more suitable for sustained-release films (Dahiya et al., 2009). Sharma et al. combined hypromellose with a “cationic copolymer based on dimethylaminoethyl methacrylate, butyl methacrylate and methyl methacrylate” (Eudragit E PO) to prepare valdecoxibe IDFs (Sharma et al., 2007). Ali and Quadir prepared ODFs using combinations of various high molecular- mass povidones and synthetic copolymers of macrogol-polyvinyl alcohol (Ali and Quadir, 2007).

As the discussion on probable polymers for our IDF continues, it may be noted here that Starches could be possible polymers for the IDF. Rice starch has particularly been chosen as the polymer for our rice paper film on grounds of low cost, or affordability, edible, compatibility, non-toxic and abundance, or availability.

Kulkarni compared different film-forming polymers and combinations thereof. Pullulan, polyvinyl alcohol, povidone, gelatine, a copolymer of ethyl acrylate, Chitosan is restricted only to those that tolerate fish protein. Methyl methacrylate and a low content of methacrylic

acid ester with quaternary ammonium groups (EudragitRL 100), various grades of hypromellose and combinations with guar gum, xanthan gum or carrageenan were investigated regarding their film-forming capacity, appearance and disintegration time (Kulkarni et al., 2010).. Medicinal carbon films were derived from carmellose, hypromellose or sodium alginate (Sakuda et al., 2010).

Films containing caffeine were made from several types of carmellose hypromellose, sodium alginate and a synthetic copolymer of macrogol-polyvinyl alcohol.
Hypromellose films turned out to be the most appropriate because of the fast dissolution and the homogenous API distribution within the films (Garsuch and Breitkreutz, 2010);. Cilurzo et al demonstrated that maltodextrins are suitable for manufacturing IDFs by both solvent casting and hotmelt extrusion (Cilurzo et al., 2011).

A combination of maltodextrin and hypromellose was optimized by Patel et al. for ondansetron ODFs prepared by solvent castin (Patel et al., 2009). As several IDF formulations are patent protected by pharmaceutical drug manufacturers, Roquette offers LycoatNG73, a patent-free granular hydroxypropyl starch polymer specifically designed for IDFs. El-Setouhy and El-Malak compared it with hypromellose, hyetellose and polyvinyl alcohol (El-Setouhy and El-Malak, 2010) For the development of sustained-release films or mucoadhesive films, polymers such as polycarbophil or polyacrylic acid could be added to the formulation (Asane et al., 2008); (Garsuch and Breitkreutz, 2010); (Repka et al., 2003).

2.14.3 Plasticizers

The addition of a plasticizer is often necessary to obtain flexible, non-brittle IDFs. Glycerol, propylene glycol, sorbitol, low-molecular-mass macrogols, phthalates, citrates or combinations thereof are commonly used (Mishra and Amin, 2009); (El-Setouhy and El-Malak, 2010); (Garsuch and Breitkreutz, 2009); (Mashru et al., 2005); (Patel et al., 2010). As IDFs still have relatively high moisture content after drying, water itself acts as plasticizer.(Boateng et al., 2010). Plasticizers interact with the film-forming polymers by lowering their glass transition temperature and thereby improving plasticity and elasticity of the resulting films (Boateng et al., 2010); (Patel et al., 2010). Most of them have more effects that have to be considered in addition, for example, sorbitol is also used as sweetener and a plasticiser (Li and Huneault, 2011); (Vieira et al., 2011).

Plasticizers may affect solubility of the API and drug absorption (Patel et al., 2010). High concentrations of plasticizers may cause an impaired moisture resistance, resulting in

stability problems or tacky films (Cilurzo et al., 2008); (Dahiya et al., 2009). Macrogol 400, an acid ester was found inappropriate for plasticizing maltodextrin films because of the lack of miscibility. Increasing the content of glycerol or propylene glycol in maltodextrin IDFs decreased the elastic modulus and increased the elongation at break. Concentrations higher than 18% w/w caused blooming phenomena and stickiness. Cilurzo conducted a study to compare the taste of the films prepared with either glycerine or propylene glycol.

The taste of IDFs where glycerol was used as a plasticizer was preferred over the taste of the IDFs where propylene glycol was used as plasticizer (Cilurzo et al., 2008). Mashru used a 33 full factorial design to optimize the formulations of IDFs where polyvinyl alcohol was used as a polymer, glycerol as plasticizer, and mannitol as filler (Mashru et al., 2005). Higher percentages of polyvinyl alcohol yielded IDFs with higher tensile strength, lower drug release and an overall higher scoring. The addition of glycerol resulted in lower tensile strength, lower drug release and higher overall scoring. Increasing the amount of mannitol produced films with lower tensile strength, but higher drug release and an overall higher scoring (Mashru et al., 2005). Glycerol, Sorbitol, and Honey have been compared as possible plasticizers for our rice paper films and D-Mannitol was used as filler.

2.14.4 Taste masking

Many APIs have an unpleasant taste therefore, the use of taste masking excipients is often essential. Depending on the physical state of the API in the film (dissolved or dispersed) and its solubility in saliva, different taste-masking techniques have been used. They ranged from the simple addition of flavours, sweeteners and bitter-blockers to particle coating, encapsulation or complexation with ion exchange resins, where the larger particles may cause scrapes during casting and may give an unpleasant gritty mouth-feel (Barnhart and Vondrak, 2008); (Cilurzo et al., 2011); (Corniello, 2006); (Hariharan and Bogue, 2009).

Brown showed that taste and mouth-feel are more important for the acceptability of orally disintegrating dosage forms than short disintegration times (Brown, 2003). However, all drugs that are even partly soluble in saliva will be accessible to taste sensation. Suitable sweeteners include natural molecules such as glucose, maltose or stevioside, artificial sweeteners such as acesulfame-K or saccharin-Na, dipeptide based sweeteners such as aspartame and protein-based sweeteners such as thaumatin (Patel et al., 2010).
This study is restricted to Stevia, a natural sweetener with low sugar content to accommodate Diabetic patients.

The sweetness has to be perceived on the tongue before onset of the bitter taste and during the bitter after taste sensation. Therefore, often combinations of different sweeteners and flavours are incorporated in IDFs. Mishra and Amin found a combination of citric acid and passion fruit flavours to be suitable for complete taste masking of cetirizine hydrochloride IDFs (Mishra and Amin, 2009). Dinge and Nagarsenker incorporated eugenol, aspartame and xylitol in triclosan IDFs (Dinge and Nagarsenker, 2008). Polyhydric alcohols such as xylitol are sweet and can improve mouth-feel further owing to a cooling effect (Dinge and Nagarsenker, 2008); (Gavaskar et al., 2010). Sprinkling flavours or sweeteners on the film surface has been mentioned as another improvement (Corniello, 2006).
For the purpose of our study, I used pure Cocoa and peppermint as flavours.

Other techniques for taste masking include complexation with cyclodextrins or application of insoluble salts of the API. All commonly applied taste-masking techniques can greatly affect maximum drug load (Hariharan and Bogue, 2009). Further, stability, disintegration time and mechanical properties may be influenced by the type and amount of taste-masking agents. Therefore, a taste-masking system has to be developed carefully for each individual drug. In particular, a 'too pleasant', 'candy-like' formulation should be avoided in the therapy of paediatrics (Cram et al., 2009). Overdosing is a potential risk for children, who may see IDFs as sweets or breath-fresheners.
The Taste Testing panelist of volunteers Doctors was set up at Charlotte Maxeke Academic Hospital and a questionnaire was used for this study (See Appendix J).

2.14.5 Others

Further excipients for IDFs include fillers, colours, opacifiers, cooling agents, lubricants or anti-tacking agents, preservatives and stabilizers (Garsuch and Breitkreutz, 2009); (Hariharan and Bogue, 2009); (Patel et al., 2010). Saliva-stimulating agents such as citric acid are reported to enhance salivation and shorten disintegration time (Dixit and Puthli, 2009). To increase mucoadhesion, appropriate polymers can be added (Asane et al., 2008). If absorption through the oral mucosa is desired, penetration enhancer and buffering agents may improve buccal bioavailability (Nicolazzo et al., 2005).

Enzyme inhibitors can prevent drug degradation (Hao and Heng, 2003). For some IDFs solubility enhancers may be necessary (Hao and Heng, 2003). Surfactants can improve spreading of the coating mass on the intermediate liner as well as wetting by saliva in the oral cavity (Arya et al., 2010); (Liang and Chen, 2001). Stabilizers and thickening agents

may be necessary to prevent particles from sedimentation. Natural gums such as xanthan or guar gum can improve viscosity and Film-forming capacity (Garsuch and Breitkreutz, 2010); (Dinge and Nagarsenker, 2008); (Hariharan and Bogue, 2009). Colouring agents were not necessary for our product as the APIs yielded beautiful and acceptable colours. Preservatives were avoided as the APIs were self-preservatives in the solid dosage form and we preferred to keep the product as natural as possible. Saliva-stimulating agents and mucoadhesion were not desirable.

2.15 Ingredients of the Rice paper (IDF)

According to researcher Lindsay. A. Brown, “Resveratrol is a potent antioxidant mainly obtained from the skin of red grape fruits which has for a long time been known for its heart disease protecting properties” (Brown et al., 2008). At high doses, Resveratrol is remarkable for cancer protection and in small doses, it reverses Diabetes and obesity. It prevents brain damage and reduces age related diseases such as inflammation (Brown et al., 2008). After discovering the molecular mechanism of the sirtuins enzymes, it was established that these enzymes regulate the synthesis of cellular components in the nucleus and Resveratrol was found to remove the very reactive oxidants thus improving blood flow into the cells (Brown et al., 2008).

After establishing its cardio protective effects, recent data also gave enough evidence that resveratrol can act as a chemo preventive agent. Tumour initiation, promotion, and progression are reduced by resveratrol through a number of pathways. Resveratrol is anti-inflammatory exerts its action by antagonising NF-kappa B and AP-1 transcription. It also prevents bio activation of procarcinogens by interfering with drug metabolizing enzymes. In addition, resveratrol acts as an antioxidant, andhence contribute to the prevention of tumour initiation. Spreading or metastasizing of the carcinomas is inhibited by resveratrol by preventing angiogenesis and inhibiting VEGF and matrix metalloproteases. Apoptosis and cell cycle arrest, as important mechanisms for cancer therapy, are stimulated by resveratrol through different mode of actions, for example, activation of p53 and modulation of cell cycle proteins (Kraft et al., 2009).

It can be noted that though there has been enough evidence for resveratrol to act as a potent chemo preventive agent in vitro, its low oral bioavailability of resveratrol in humans is a drawback that could interfere with a successful in vivo treatment. Hence the study of intraoral drug delivery system to overcome this impediment. Nevertheless, resveratrol offers

two major advantages over conventional chemotherapy. The cytotoxic effects of resveratrol on healthy cells is negligible, and, as many pathways leading to chemotherapeutic effects are activated by resveratrol. This also overcomes chemo resistance-inducing mutations in cancer cells (Kraft et al., 2009).

Curcumin, has for a long time been expected to be a possible medicine or preventive agent for several major human diseases because of its ant oxidative, anti-inflammatory, and anticancer effects, (Lal et al., 2000)**.** Though pharmacological active concentrations of curcumin were obtained in colorectal tissue by taking curcumin orally, pharmacokinetic studies indicate its low oral bioavailability (Talwar et al., 2008).. This study attempts to enhance its bioavailability by formulating the rice paper IDF as a delivery system. Curcumin is a potent, non-toxic, natural virus killer (Talwar et al., 2008).

Curcumin as a natural anti-viral, and like all other anti-viral drugs, only helps the body to fight the viruses by preventing them from entering healthy cells. Several studies have examined the uses of turmeric in the treatment of HIV/AIDS but are outside the scope of this study. Talwar explained this phenomenon in The International Journal of Antimicrobial agents when he discovered that a herbal cream effectively prevented the HIV-1 virus from entering human cells. On examination, the main ingredient in the herbal cream was curcumin. Talwar concluded that the cream had significant anti-viral properties including HIV growth inhibition (Talwar et al., 2008).

Curcumin, a yellow substance that belongs to the polyphenols super family, and is the active component of turmeric, a common Indian spice, which is obtained from the dried rhizome of the plant *Curcuma longa.* Many studies have revealed that curcumin possesses anti-oxidant, anti-inflammatory, and anticancer properties (Vallianou et al., 2015). The molecular mechanism by which curcumin exerts its anti-tumour effects is explained in he following paragraphs;

Curcumin inhibits the STAT3 and NF-κB signalling pathways, which play key-roles in cancer development and progression. It inhibits Sp-1 and its housekeeping gene expression that are important genes to prevent cancer formation, migration, and invasion. Recent data shows that curcumin acts by suppressing the Sp-1 activation and its downstream genes, which include; ADEM10, calmodulin, EPHB2, HDAC4, and SEPP1 in a concentration-dependent manner in colorectal cancer cell lines. Other studies have reported that curcumin suppresses the Sp-1 activity in bladder cancer as well as a decrease in DNA binding activity of Sp-1 in non-small cell lung carcinoma cells. Recent additional data reveal that ER stress

and autophagy also play a role in the apoptosis process, which is induced by the curcumin analogue B19 in an epithelial ovarian tumour cell line. Autophagy inhibition could increase curcumin analogue-induced apoptosis by inducing severe ER stress. The ability of curcumin to induce apoptosis in tumour cells and its anti-angiogenic potential is the core of its molecular mechanism of action (Vallianou et al., 2015).

Rice starch could be one of the promising raw materials for the production of biodegradable natural polymers because is quite cheap, abundant, biodegradable and edible. Rice starch has two major types of molecules, about 25% linear amylose and 75% the branched amyl pectin. Waxy starches are made of mainly amyl pectin and 0–8% amylose; and high-amylose starches have between 40–70% amylose (Yost and Hoseney, 1986). Amylose contains D-glucose molecules, which are linked in the α-1, 4 conformation positions. Glucose monomers are responsible for the formation of the linear straight chain polymers. Amylose is a key component responsible for water absorption, swelling and gelation of starch the properties which the starch materials have (Yost and Hoseney, 1986).

The antioxidant Cocoa contains 54 % Fat (Cocoa Butter), whose composition is 34% Oleic acid, 33 % Stearic acid, 26 % Palmic acid, and 6 % other acids, 31 % carbohydrates, with approximately 1 % sugar, and 16 % Fibre, 11 % Proteins, including Arginine, Glutathione, and Leucine. 3 % Polyphenols, including Flavonals responsible for its flavour, Proanthocyanins, and 1% Minerals (Fe, Mg, P, K, and Cu). In the formulation the rice paper film, cocoa is used as a flavouring agent.

According to Phillip Neven et al, “A water soluble curcumin compound for treating proliferative and/or inflammatory disorders was characterised and in that the compound shown below, is a cyclodextrin complex of a curcumin compound of the general formula shown below: where M is lysine or arginine and R_1 and R_2 are each independently selected from hydrogen, hydroxy or alkoxy, and/or their stereo isomers.” Since cocoa has arginine, it may assist in making curcumin more water soluble. The stereo geometry or electrostatic bonding in the cyclodextrin complex does not affect the activity of the free core curcumin molecule (Neven et al., 2014). (See Figure 2.15) below;

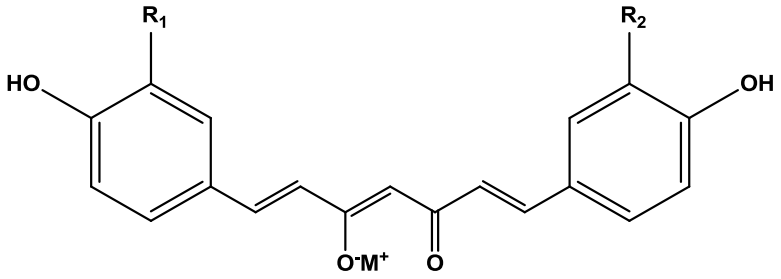


Figure 2.15: Substitutions on the Curcumin core molecule. (Neven et al., 2014).

The main ingredient in peppermint is menthol. Peppermint is a herb that is a cross between spearmint and water mint. In addition to the skin nourishing properties, peppermint is a good flavoring agent. The oil is used to treat a number of physical maladies. It is used in teas, in either in capsule or liquid forms, or mixed with a carrier. In the rice paper IDF, peppermint oil was used to give a good mouth feel. Stevia is a sweet herb in the sunflower family. It is known as Diabetic sugar. It is twice as sweet as sucrose and was used in the formulation of the IDF to accommodate Diabetic patients.

Glycerol or Glycerin is a carbon compound with three hydroxyl (OH) groups attached to its aromatic rings. At room temperature, it is a very viscous, colourless and odourless liquid, and has a sweet taste. It is synthesized as one of the by-products during the manufacture of soap. This involves reacting vegetable or animal fats with strong alkaline solutions. Glycerol is also produced as a byproduct of biodiesel manufacture. It can as well be manufactured from propene, the three carbon alkene. Glycerol was used as a plasticizer in the formulation of the rice paper IDF.

Mannitol powder, (D - Mannitol) is a polypyrol organic compound having 6 hydroxyl groups attached to it. It is a white crystal powder, ourdourless, with a sweet taste, and very soluble in water. It has the following molecular formula $C_6H_{14}O_6$ and a molecular weight of 182.17. Its sweetness degree is 55%-65% that of sucrose. It has Low calorie value of 1.6kc/g which is only 40% of sucrose's. Mannitol is Nonhygroscopic and is the only compound, which is nonhygroscopic among the hexahydric sugar alcohols. It is stable at room temperature and does not react with other pharmaceutical materials. It is used as filler in the formulation of the IDF to make it have a smooth finishing and confers to it sticking resistance property.

Honey is a sweet food produced by insect bees. It is consumed by humans and contains all the necessary substances to sustain life (Kelhoffer, 2005). The Bible Prophet John the Baptist survived on only bees honey and locusts in the wilderness (Kelhoffer, 2005). It is brown, thick and sticky. It is included in the rice paper film formulation as a plasticiser, sweetener and to improve the deserving mechanical properties of the rice starch to help stick its molecules together.

2.16 Biopharmaceutical aspects

Oral mucosa is described in detail elsewhere (Hao and Heng, 2003); (Pather et al., 2008); (Shojaei, 1998); (Smart, 2005). The mucosa of the oral gingival, i.e. the hard palate and the gums, are known to be the masticatory mucosa and are keratinized. The mouth mucosa floor and the buccal mucosa are known as lining mucosa and are non-keratinized (Pather et al., 2008). In general, permeation of oral mucosa occurs somewhere between the epidermis and the interstitial space. Permeability of the oral mucosa diminishes as the drug moves in the order from sublingual to buccal to palatal (Shojaei, 1998). Oral films possibly are applied on the tongue, the cheeks, the hard palate or sublingual, but the usual site of application for IDFs is the tongue, and for sustained release and mucoadhesive patches is the mucosa of the cheeks. Sublingual application is possible as well (Mashru et al., 2005).

Local delivery of triclosan has been described as well (Boateng et al., 2010). If the drug penetrates through the oral mucosa, it is absorbed into the reticulated and jugular veins and then drained into the blood circulation. First-pass metabolism in the liver is by passed (Smart, 2005). Therefore, this application site may improve bioavailability and reduce unwanted side effects. Absorption through the mucosa can be either transcellular or paracellular, lipophilic drugs mainly penetrate via the transcellular route, but more hydrophilic drugs penetrate via the paracellular route, usually both routes coexist (Hao and Heng, 2003); (Smart, 2005). An ideal API for absorption through oral mucosa should be soluble in the saliva an should be non-ionized in the pH of the saliva, which ranges between 6.5 to 7.5 and sometimes determined by the flow rate of the saliva and the meal taken at that time (Shojaei, 1998); (Reddy et al., 2009). Reddy stated that, “A moderate molecular mass facilitates absorption” (Reddy et al., 2009).

As ODFs have a very short residence time in the oral cavity, adsorption to the oral mucosa will not play an important role in most cases and is not desirable. Some APIs may be absorbed in part, whereas most of the drug is absorbed after swallowing and this transition

in the gastrointestinal tract leads to complex pharmacokinetic profiles (Breitkreutz and Boos, 2007). Selegiline film is an example whose bioavailability is improved by administering an orally disintegrating dosage form. It is absorbed pre-gastrically from a Zydus freeze dried formulation. First-pass metabolism is avoided. To yield similar plasma concentrations as conventional selegiline hydrochloride tablets, the dose had to be reduced to one-eighth in the oral lyophilisates (Clarke et al., 2003). A similar effect is seen when selegiline is delivered as IDF. Most IDFs on the market contain the API as particles, which are absorbed after disintegration of the film. Therefore, bioavailability is often approximately the same as immediate release.

The bioequivalence between Rapid film and an Oral lyophilisates, both containing ondansetron, was determined in a two way, randomized, single-dose, crossover study with 24 healthy volunteers (Reiner et al., 2010). Risperidon as before, is HEXAL SF. Schmelz film was shown to be bioequivalent to film coated tablets (Hexal, 2010). Comparable plasma level-time profiles for IDFs and tablets (dissolved in distilled water) with tianeptine sodium were obtained in a bioavailability study with rabbits (El-Setouhy and El-Malak, 2010). Shimoda et al. used a bioavailability study with rats to compare dexamethasone IDFs with a suspension (Shimoda et al., 2009). Nishimura et al. could not observe significant differences in pharmacokinetic parameters after administration of prochlorperazine IDFs or solution to rats but did not fulfil the FDA approval for bioequivalence, because the 90% confidence interval of the ratio of the means of Cmax and AUC between the test drug and the reference drug was not within the required range (Nishimura et al., 2009).

2.17 Marketed products and future potential

Since Listerine PocketPaks were introduced as breath fresheners, IDFs have become popular, especially in North America. Several OTC products with APIs have been available on the US market for years. These included Novartis, Basel, IDFs brands such as, Gas-X and Triaminic. Vitamins, nutraceuticals and many other lifestyle products have been commercialized. The first prescription products are already available or will soon be available in Europe. As the breath-fresheners are less popular in Europe, pharmacists have to educate patients in the handling of IDFs. Reckitt Benckiser Pharmaceuticals received FDA approval for Suboxone sublingual films containing Buprenorphine and naloxone, the drugs used in the treatment of opioids dependence.

However, only physicians certified under the Drug Addiction Treatment Act 2000 can prescribe these drugs (Şenel et al., 2012). The market for fast-disintegrating dosage forms

including IDFs, fast-disintegrating tablets and lyophilisates is fast growing. Between 2003 and 2007, over 50 different IDF brands were introduced on the US and Canada market. Falls states that a further increase is expected (Gupta et al., 2011). For companies, the IDF technology is suitable for life cycle management and extending patent protection of branded APIs (Barnhart and Vondrak, 2008).

We cannot say IDFs is the solution for all problems, because drug load is limited and pharmacokinetics is complicated. Nevertheless, IDFs out compete the orodispersible tablets, immediate-release dosage forms and oral lyophilisates. Gavaskar concluded that many pharmaceutical companies are moving their product franchise away from orally disintegrating tablets to IDFs (Gavaskar et al., 2010). Various APIs are on their way to the market as IDFs, for example, for CNS applications such as Parkinson's disease, depression, schizophrenia and Alzheimer's disease.

Future potential APIs include Midazolam, loperamide, oxycodon, fentanyl, triptans or even sildenafil (Siebenand, 2010). Allicin from garlic may also be considered a future potential drug. Nevertheless, future decisions of the authorities on equivalence of IDFs and orally disintegrating tablets as well as other dosage forms will influence the market (Barnhart and Vondrak, 2008). Johns Hopkins University in Baltimore developed a film for administering a vaccine (Saroja et al., 2011). Incorporation of pollen and antigens may improve therapy of allergies. Animal treatments may be another market in future as well (Barnhart and Vondrak, 2008). Meathrel and Moritz mentioned the benefit of IDFs for in vitro diagnostics Reagents incorporated into films improve manufacturing, handling and stability of *in vitro* diagnostics (Meathrel and Moritz, 2007).

2.18 Advances in orodispersible films for drug delivery

Orodispersible tablets and lyophilisates may be considered in future, especially if their manufacturing is cost-effective. Up to now, approvals of IDFs from the governments were realized by bioequivalence studies in comparison with lyophilisates or immediate release tablets. Many pharmaceutical companies seem to avoid the development of ODFs with APIs, which may have an improved bioavailability because it would require specific clinical trials. Improvement of bioavailability may be interesting for mucoadhesive, sustained-release films rather than for IDFs. Mucoadhesive patches may be a suitable dosage form for administering macromolecular therapeutics such as proteins. Although approved IDFs are bioequivalent to lyophilisates or immediate-release tablets, substitution is not allowed. Regulatory agencies seem to accept them as a different dosage form, considering the

compliance aspect. Substitution of an IDF with an immediate-release tablet would lead to enormous problems for patients with swallowing difficulties. Substitution of an immediate-release tablet with an IDF is less critical. In the near future, because of the advantages of IDFs over conventional dosage forms, IDF technology promise more applications and more IDF products are expected to increase on the market.

2.19 Concluding remarks

Over the Counter (OTCs), IDF formulations have been available in the US for years, but the first drug-loaded films have entered the prescription sector only recently. IDFs are a very suitable dosage form for children and the elderly, because they are not swallowed and involve no risk of choking. They usually consist of film-forming polymers, plasticizers and a few more excipients, for example, sweeteners to improve the taste. The main disadvantage of IDFs is the limited drug load. IDFs are commonly manufactured by solvent casting. Basic characterization methods are; Determination of mechanical properties such as disintegration behaviour. Validated methods and pharmacopoeial specifications are still lacking. Further research, in my own view, has to be done for more natural polymers, regarding their use and effectiveness, their composition, manufacturing and quality control in the pharmaceutical industry.

Drug loading of IDFs is limited and taste masking is challenging, IDFs will therefore not be the answer to all problems. Manufacturing of IDFs is very flexible. Different dose strengths can be obtained easily. Therefore, suitable doses, for example for children and the elderly can be manufactured. Small batch sizes of IDFs can be produced in a cost-effective manner using the solvent casting method. IDFs may also be a suitable dosage form for personalized medicine. It is the responsibility of the authorities to include a monograph on IDFs in the pharmacopoeias as soon as possible to define IDFs and their quality requirements. Characterization and quality control should be standardized. Specifications at least for mechanical properties like Tensile strength and disintegration time should be given.

Chapter 3. Formulation, design and optimization of the rice paper intraoral dispersible film

3.1. Introduction The experimental model was designed as outlined in (Mohamed et al., 2011) with some modifications and also the Box-Behnken experimental design has been applied. This chapter elaborates the methods that were used to design and optimize a pharmaceutical vehicle that is formulated to deliver the model natural drugs that have low oral bioavailability such as Resveratrol and Curcumin using the Box-Behnken experimental design. Parameters which were evaluated included; Thickness variation, Tensile strength, Disintegration time, and Folding Endurance among others.

3.1.1. Principles of experimental factorial design

Usually, research methods give the effect of a single variable at a time. This is so because it is statistically easier to calculate and to interpret. However, two factors have showed interdependence in many cases, and therefore, it becomes impractical or even false to try the traditional way to analyse them as a single entity. In this study, we employed factorial design to assess the effects of dependant and independent multi variables. It was not only impractical but also difficult to isolate and test each variable individually. The Factorial design allowed for a logical manipulation of a larger number of interdependent variables that played a significant role or that were key factors in the formulation of the rice paper IDF.

The method had limitations because only disintegration time, thickness variation, tensile strength and folding endurance were optimised. Other test like moisture content, Elongation percent, and others were not optimised and was therefore not conclusive. However, it was useful in streamlining the investigations and was a powerful statistical tool that highlighted all related variables. The Solver of the factorial design and the taste testing as well as robustness of the films were utilised in finding the optimal formulation.

3.1.1.1. The Basics of Factorial Design

A traditional design of the experimental study would involve selecting different variables contained within the population, for example none or 10%. We choose to use the factorial design to co-ordinate the additive trials of the different variables. Using the factorial experiment, we chose 3 factors on 3 levels that is 27 variations. The Box Behnken chose 16 experiments to be tested. The usual rules used in scientific writing and methodology were still implemented. Therefore, statistically, it required that every experiment be conducted in

triplicate for each of the model drug loaded into the film. Therefore a Box Behnken Design; 3 Factors, 4 Centerpoints, Quadratic Model with 10 items was adopted in this study (See Equation 3.1) quadratic equation.

3.1.1.2. The Pros and Cons of Factorial Design

These experimental designs are very useful in science because they act as a primary study tool. They allow the researcher to judge if there is a link between variables and reduces the possibility of errors in the experiments (Asplund Brattberg, 2015); (Parakh and Gothoskar, 2003). The factorial design simplifies the experimentation process and reduces experimental costs. It allows many levels of analysis and highlights the correlation between the different parameters (Siddharth et al., 2011). A single variable could still be isolated and analysed. However, as a disadvantage, it becomes difficult to experiment with more than two factors at many levels. The Box Behnken Design had to be planned very well, because a mistake at any one of the levels would greatly jeopardize a great amount of work.

3.2. Methods

3.2.1. The Box – Behnken experimental design

A factorial design was constructed to provide information on the direct effects of the study variables additives on the physical and chemical properties, and the pair-wise interaction effects of the plasticizers and sweeteners on the IDF. Sixteen (16) statistically derived formulations of various concentration combinations of polymers, and plasticizers, were performed for the plain film and for those loaded with curcumin and resveratrol respectively (See Table 3.1).

The 3 factors, 4 Centerpoints, quadratic model with 10 items gave the following response;

Equation 3.1: Response = b0 + b1 x Rice, %w/v + b2 x Plast, %w/v + b3 x Type + b4 x Rice, %w/v x Rice, %w/v + b5 Plast, %w/v x Plast, %w/v + b6 x Type x b7 x Rice, %w/v x Plast, %w/v + b8 x Rice, %w/v x Type + b9 x Plast, % w/v x Type

Equation 3.2

Where:

b0 is the intercept (constant)

b1 – b9 are confidents for different combinations of the factors

Table 3.1: The Rice paper film Box Behnken design of the generated formulations with 3 factors and 4 Centerpoints experimental factorial design (Two factors and three levels (2X3 or 2^3FACTORIAL).

Formula Codes			Exp. #	Rice grain	Plasticizer	Type of
Plain	Curcumin	Resveratrol		%	%	Plasticizer
P1	C1	R1	1	10	5	0
P2	C2	R2	- ◊ 2	15	10	0
P3	C3	R3	3	10	10	-1
P4	C4	R4	- ◊ 4	15	10	0
P5	C5	R5	- ◊ 5	15	10	0
P6	C6	R6	6	20	5	0
P7	C7	R7	7	15	15	-1
P8	C8	R8	8	20	10	1
P9	C9	R9	9	20	10	-1
P10	C10	R10	10	10	15	0
P11	C11	R11	- ◊11	15	10	0
P12	C12	R12	12	15	5	1
P13	C13	R13	13	20	15	0
P14	C14	R14	14	15	5	-1
P15	C15	R15	15	10	10	1
P16	C16	R16	16	15	15	1

Type of Plasticizer: 1 Glycerine
0 Honey
-1 Sorbitol
-◊ The 4 Centerpoints

P Plain formulation
C Loaded with Curcumin
R Loaded with Resveratrol

3.2.1.1. Formulation of rice paper IDF

As stated before, the experimental model used in this study is as outlined in (Mohamed et al., 2011) with some modifications. The solvent casting method was employed to prepare the IDFs of edible rice paper using the sol-gel principle. The sol-gel method is a process employed to make thin films. This method reduces the powders to fine particles by stirring the solution with a homogenizer (Brinker and Mukherjee, 1981). (See Figure 2.9) 2.9, to 2.12). The rice paper was made by means of steaming the rice starch for each formulation (Dalal, 2014). A detailed step by step method to prepare the product is included in (Appendix M). Briefly, rice starch was weighed, placed in a 600 ml beaker to which 150 ml of distilled water at 25 °C was added.

The rice starch-water mixture was subsequently subject to stirring for one hour at 500 rpm using a Silverson emulsifier (Vortex Mixers, England), in order to reduce the particle size which would help reduce adhesion forces between particles. Thereafter, this mixture was placed in a steam bath and heated at 95 - 100 °C for three hours to form a thick gel. In a separate beaker, a suspension of glycerin, cocoa, Stevia solution, D-mannitol were weighed according to the weighing record (see Appendix M) and mixed in a small volume of filtered hot water and added to the rice starch gel. Notice, hot water was added to disperse the cocoa. Filtered water was added to a final volume of 300ml. This was then mixed again together with the rice gel at room temperature (25 °C) for one hour at 500 rpm using the Silverson emulsifier (Vortex Mixers, England) in order to form a homogenous suspension with a viscosity of approximately 50 Ns/m^2 using the viscometer (Brookfield Engineering Laboratory. Inc. Stoughton Massachusetts, USA). The beaker was placed in an ice bath to keep the temperatures low as the homogenizer generated a lot of heat.
Natural plasticizers like glycerin, or honey, or Sorbitol, were used as plasticizer to adjust the consistency of the rice starch suspension. The thick dense suspension into which the drugs were loaded was formed first. This mixture was allowed to cool to approximately 40 °C in order to add flavoring agent in the form of two drops of peppermint oil (100 % from Aromatherapy oils of SA). Thereafter, 0.5 g of the active the drugs, either Curcumin or Resveratrol were accurately weighted on an analytical balance and loaded into the carrier. They were triturated on a white tile with a stainless spatula into 5ml of the thick gel.

This was then poured into a 10 cm (diameter) clean plastic Petri dish and placed on a perfect flat surface and left undisturbed in order to allow for the sol-gel process explained before to occur during the casting (See Figure 2.10) to eventually form the rice paper films. A thin wide flat sheet of rice paper film was carefully peeled from the Petri dish and securely wrapped in aluminum foil to prevent further moisture loss. The film was cut into 2 x1 cm, also wrapped in aluminum foil to prevent drying and stored at room temperature.

The lab preparation of the Rice paper IDF is graphically shown in (See Figure 3.1) below;



Mixing	Rice paper thick Suspension	Film Drug Loading	Solvent Casting on the Petri Dishes and wrapping in Aluminium foil

Figure 3.1: The lab preparation of the Rice paper IDF. Where P is the Plain rice paper film, R is the film loaded with Resveratrol, and C is the film loaded with Curcumin, which were cut into 2 cm x1 cm intraoral strips and wrapped in aluminum foil to protect them from light and moisture loss.

3.2.1.2. Optimization and statistical evaluation

In order to determine an optimal rice starch IDF, various formulations were prepared using a Box-Behnken factorial design. Essential Experimental Design® (Steppan et al., 1998) (Copyright version 2.2016) for Microsoft Excel® was used to determine the optimal formulation. The different formulations prepared are listed in Table 3.2. In order to determine the optimal formulation, the important output variables of disintegration time (seconds), tensile strength (Ns/m^2), folding endurance (number of folds) and film thickness variation (CV%) were used. The 3 input variables or factors used in the experimental design were % $^{w}/_{v}$ rice starch, type of plasticizer (glycerin, honey, or sorbitol) and % $^{w}/_{v}$ plasticizer.

Table 3.2: Rice Paper IDF Factorial Design Template

Experiment number	Rice %w/v	Plasticiser %w/v	Type
1	10	5	0
- ◊ 2	15	10	0
3	10	10	-1
- ◊ 4	15	10	0
- ◊ 5	15	10	0
6	20	5	0
7	15	15	-1
8	20	10	1
9	20	10	-1
10	10	15	0
- ◊ 11	15	10	0
12	15	5	1
13	20	15	0
14	15	5	-1
15	10	10	1
16	15	15	1

Type of Plasticizer: 1 Glycerine
0 Honey
-1 Sorbitol
- ◊ The 4 Centerpoints

Table 3.2 above is the template that was used to optimize the parameters of Thickness, Tensile strength, Folding Endurance, and Disintegration using the 3 variables factors of the experimental Design namely; Rice %w/v, Plasticiser %w/v, and Type of plasticizer. We optimised the rice paper IDF using only the above-mentioned parameters but other parameters like moisture content and could also be optimised using the same template of the experimental factorial design.

3.3 Results and discussion

The casting process occurs when the mixture undergoes air-drying for 14 - 23 hours. After 14 -23 hours, the films would lose robustness and became difficult to harvest from the Petri dish. (See Figure 3.2) below shows the individual time taken for each formulation to form during casting in dry air.

Table 3.3: Time taken for the films to from during the Casting process in the 16 different formulations. The films for the plain, and those loaded with the drugs form at the same time for each of the 16 formulations**.**

Film No.	Time at Start (hrs)	Time at Harvest (hrs)	Total time Taken (hrs)
1	04.00 pm	10.00 am	18
◊2	05.30 pm	12.00 noon	19
3	05.30 pm	01.25 pm	19.55
◊4	05.10 pm	12.00 noon	18.5
◊5	05.15 pm	12.00 noon	18.45
6	05.40 pm	07.44 am	14.04
7	05.50 pm	01.20 pm	19.3
8	06.00 pm	10.20 am	16.2
9	07.00 pm	01.30 pm	18.3
10	07.30 pm	10.10 am	14.4
◊11	05.20 pm	12.00 noon	18.4
12	08.00 pm	01.40 pm	17.4
13	09.30 pm	11.50 pm	14.2
14	10.00 pm	01.15 pm	15.15
15	11.00 pm	01.00 pm	14
16	03.40 pm	03.00 pm	23.2

The films ◊ 2, ◊ 4, ◊ 5, and ◊ 11 around the centerpoint form within similar range of time with a time difference of only 20 minutes. Films 6, 10, 13, and 15 have the shortest time to form of around 14 hrs and formulation 16 takes the longest time to form. This time lag may have its effect later during manufacture when the product is viewed in terms of cost effectiveness of industrial inputs.

Figure 3.2 below clearly shows the Time taken to cast the 16 different formulations and as a bar graph, it is easier to visualise and compare the setting times of the 16 formulations.

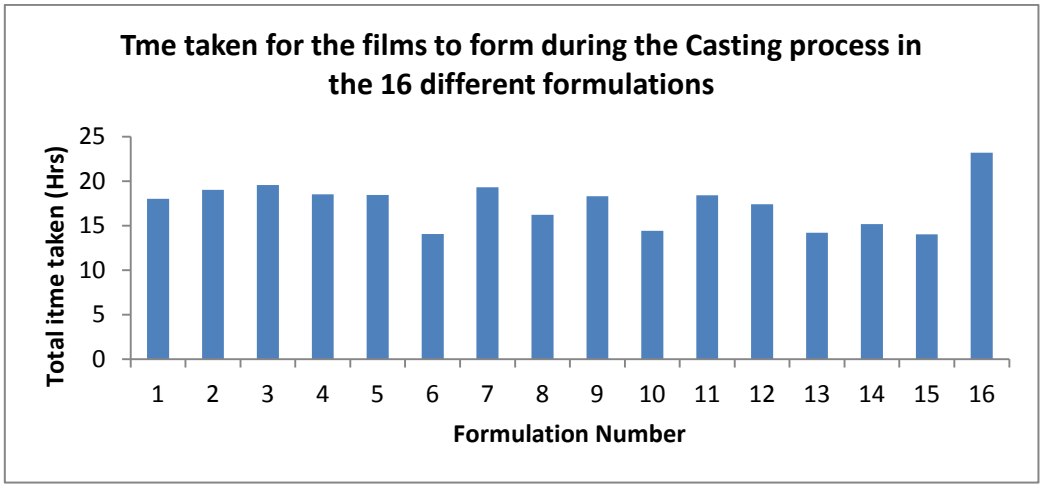


Figure 3.2: Shows the Time taken to cast the 16 different formulations

3.3.1 Surface Response Plots

3.3.2 Disintegration, Tensile Strength, Folding, and Thickness Response surface plots

Disintegration = b0 + b1*Rice, %w/v + b2*, Plast, %w/v + b3*Type - For Resveratrol Films

Where, b1 is the Rice %w/v, b2 is the Plasticizer %w/v and, b3 is the Type of Plasticizer. b0 is the start Response, and others are Response combination factors integrated in the Response plot Equation (See 4.2.2.1 Equation 1) but are not really significant in this study.

Table 3.4: Disintegration = b0 + b1*Rice, %w/v + b2*, Plast, %w/v + b3*Type - For Resveratrol Films

Summary	
|R|	0.737
R^2	0.543
R^2 adjusted	0.429
Standard Error	5.363
# Points	16
PRESS	624.20
R^2 for Prediction	0.174
Durbin-Watson d	1.780
First Order Autocorrelation	0.000
Collinearity	1.000
Coefficient of Variation	38.349
Precision Index	18.450

ANOVA						
Source	*SS*	*SS%*	*MS*	*F*	*F Signif*	*df*
Regression	410.89	54	136.96	4.761	0.02070	3
Residual	345.20	46	28.77			12
LOF Error	331.72	44 (96)	36.86	8.2019	0.05515	9
Pure Error	13.48	2 (4)	4.494			3
Total	756.10	100				15

Disintegration = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type							
		P value	Std Error	-95%	95%	t Stat	VIF
b0	-11.78	0.117	6.967	-26.96	3.403	-1.690	
b1	1.242	0.00665	0.379	0.415	2.068	3.274	1.000
b2	0.714	0.08426	0.379	-0.112	1.540	1.882	1.000
b3	0.278	0.886	1.896	-3.854	4.409	0.146	1.000

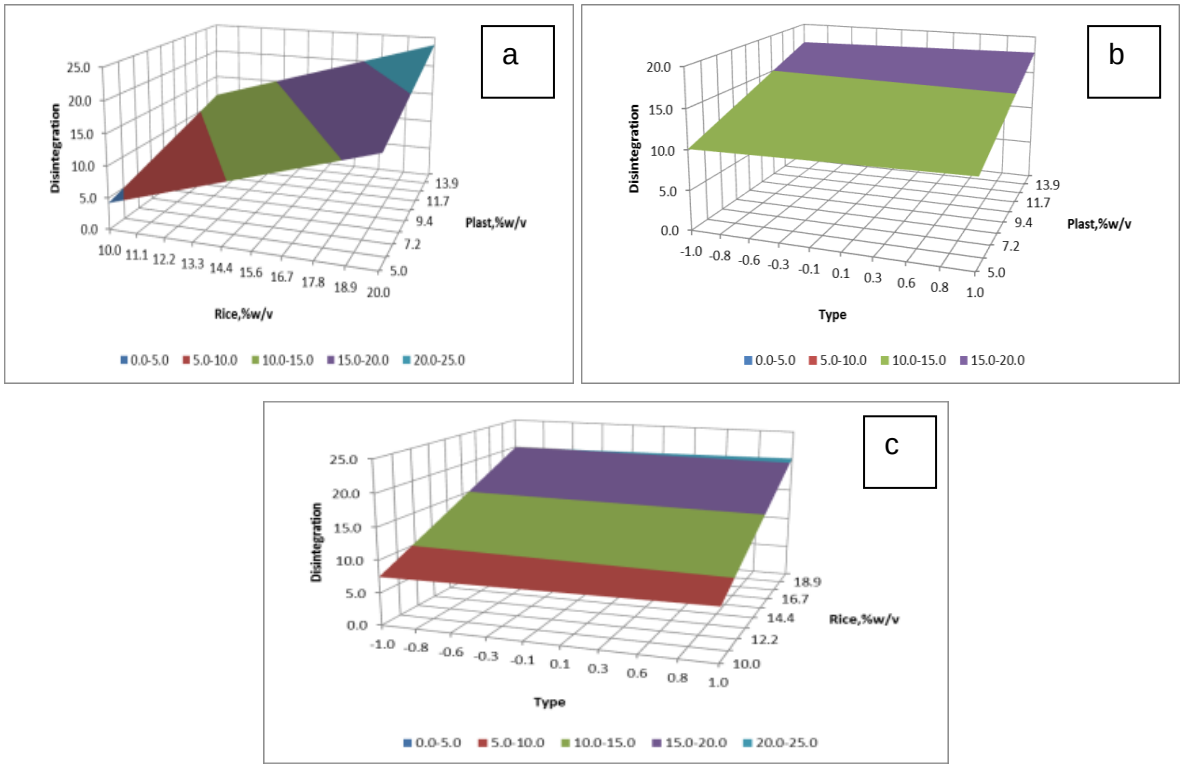


Figure 3.3: (a), (b), and (c) Shows the three dimensional graphs for Resveratrol Disintegration.

(a) The graph shows that for Resveratrol films, as the concentration of rice is increased, the Disintegration time increases.

(b) The graph shows that for Resveratrol films, as the concentration of the plasticizer is increased, the Disintegration time decreases.

(c) The graph shows that for Resveratrol films, the plasticizer type does not play ant significant role.

This means that the concentration of rice and plasticiser have a significant impact on the disintegration of the Resveratrol films while the type of the plasticiser does not.

Table 3.5: Folding = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type - For Resveratrol Films

Summary	
|R|	0.818
R^2	0.669
R^2 adjusted	0.586
Standard Error	0.423
# Points	16
PRESS	4.49
R^2 for Prediction	0.308
Durbin-Watson d	2.333
First Order Autocorrelation	-0.175
Collinearity	1.000
Coefficient of Variation	28.227
Precision Index	59.756

ANOVA						
Source	*SS*	*SS%*	*MS*	*F*	*F Signif*	*df*
Regression	4.343	67	1.448	8.075	0.00328	3
Residual	2.151	33	0.179			12
LOF Error	2.139	33 (99)	0.238	57.7500	0.00333	9
Pure Error	0.01235	0 (1)	0.00412			3
Total	6.494	100				15

Folding = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type							
		P value	Std Error	-95%	95%	t Stat	VIF
b0	1.181	0.05298	0.550	-0.01783	2.379	2.146	
b1	-0.06389	0.05417	0.02994	-0.129	0.00134	-2.134	1.000
b2	0.128	0.00109	0.02994	0.06255	0.193	4.268	1.000
b3	-0.181	0.251	0.150	-0.507	0.146	-1.206	1.000

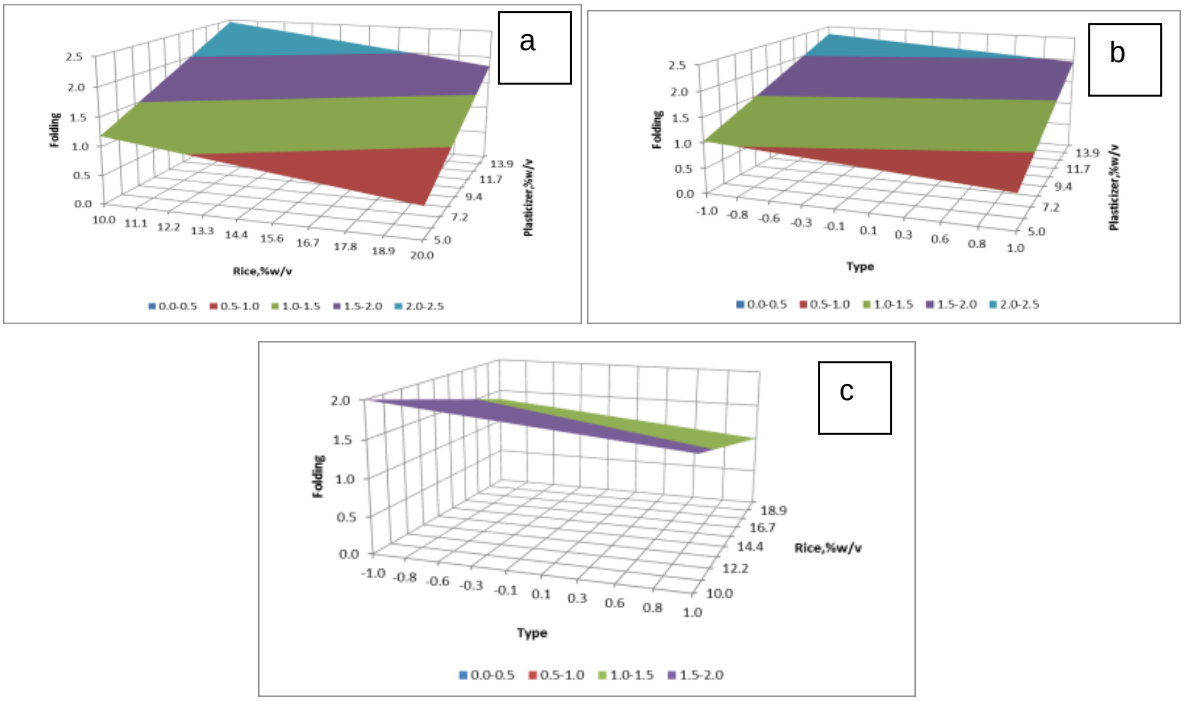


Figure 3.4: (a), (b), and (c) shows the three dimensional graphs for Resveratrol Folding Endurance.

(a) The graph shows that for Resveratrol films, as the concentration of rice is increased, the Folding Endurance increases.

(b) The graph shows that for Resveratrol films, as the concentration of the plasticizer is increased, the Folding Endurance decreases.

(c)The graph shows that for Resveratrol films, Sorbitol (-1) gives the least Folding Endurance.

This means that the concentration of rice and plasticiser have a significant impact on the Folding Endurance of the Resveratrol films while the type of the plasticiser does not.

Table 3.6: Tensile = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type – For Resveratrol Films

Summary	
|R|	0.608
R^2	0.369
R^2 adjusted	0.211
Standard Error	0.246
# Points	16
PRESS	1.45
R^2 for Prediction	-0.262
Durbin-Watson d	1.075
First Order Autocorrelation	0.362
Collinearity	1.000
Coefficient of Variation	54.424
Precision Index	6.851

ANOVA						
Source	*SS*	*SS%*	*MS*	*F*	*F Signif*	*df*
Regression	0.424	37	0.141	2.340	0.125	3
Residual	0.725	63	0.06042			12
LOF Error	0.641	56 (88)	0.07123	2.5435	0.239	9
Pure Error	0.08402	7 (12)	0.02801			3
Total	1.149	100				15

Tensile = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type							
		P value	Std Error	-95%	95%	t Stat	VIF
b0	-0.04437	0.892	0.319	-0.740	0.651	-0.139	
b1	0.04277	0.03000	0.01738	0.00490	0.08064	2.461	1.000
b2	-0.01455	0.419	0.01738	-0.05243	0.02332	-0.837	1.000
b3	0.04460	0.617	0.08691	-0.145	0.234	0.513	1.000

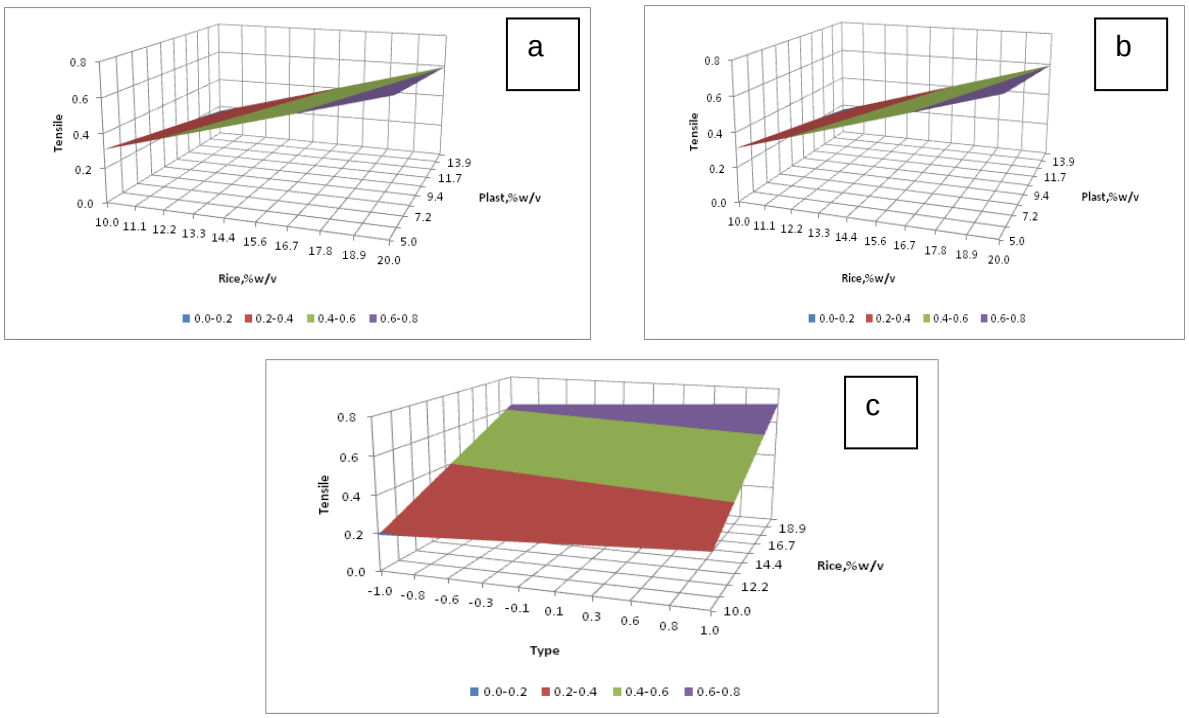


Figure 3.5: (a), (b), and (c) show the three dimensional graphs for Resveratrol Tensile strength

(a) The graph shows that for Resveratrol films, as the concentration of rice is increased, the Tensile Strength increases.

(b) The graph shows that for Resveratrol films, as the concentration of the plasticizer is increased, Tensile Strength decreases.

(b) The graph shows that for Resveratrol films, Glycerine (1) gives the highest tensile strength.

This means that the concentration of rice and plasticiser have a significant impact on the Tensile Strength of the Resveratrol films while the type of the plasticiser does not.

Table 3.7: Tensile = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type – For Resveratrol Films

Summary	
|R|	0.608
R^2	0.369
R^2 adjusted	0.211
Standard Error	0.246
# Points	16
PRESS	1.45
R^2 for Prediction	-0.262
Durbin-Watson d	1.075
First Order Autocorrelation	0.362
Collinearity	1.000
Coefficient of Variation	54.424
Precision Index	6.851

ANOVA						
Source	*SS*	*SS%*	*MS*	*F*	*F Signif*	*df*
Regression	0.424	37	0.141	2.340	0.125	3
Residual	0.725	63	0.06042			12
LOF Error	0.641	56 (88)	0.07123	2.5435	0.239	9
Pure Error	0.08402	7 (12)	0.02801			3
Total	1.149	100				15

Tensile = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type							
		P value	Std Error	-95%	95%	t Stat	VIF
b0	-0.04437	0.892	0.319	-0.740	0.651	-0.139	
b1	0.04277	0.03000	0.01738	0.00490	0.08064	2.461	1.000
b2	-0.01455	0.419	0.01738	-0.05243	0.02332	-0.837	1.000
b3	0.04460	0.617	0.08691	-0.145	0.234	0.513	1.000

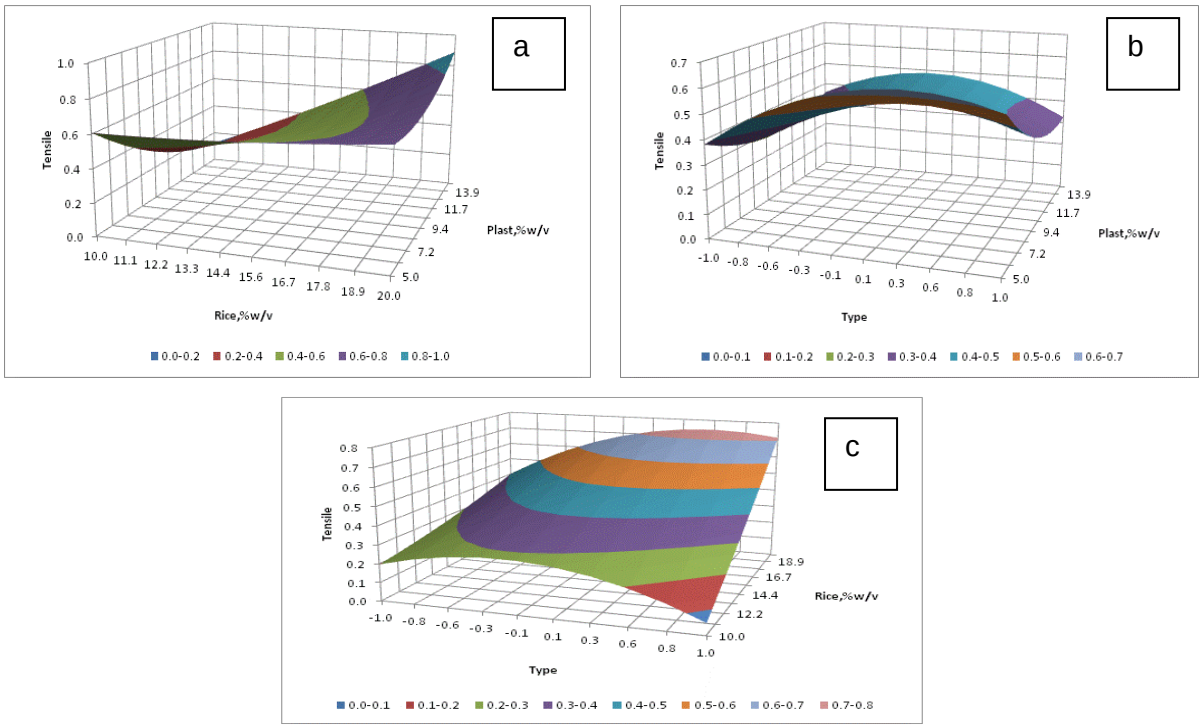


Figure 3.6: (a), (b), and (c) show the Quadratic three-dimensional graphs for Resveratrol Tensile strength.

(a) The graph shows that for Resveratrol films, as the concentration of rice is . increased, Tensile strength the time does not increase.

(b) The graph shows that for Resveratrol films, as the concentration of the plasticizer is increased, the Tensile strength. time does not increase.

(c) The graph shows that for Resveratrol films sorbitol (-1) is the best as it gives the highest Tensile Strength..

This means that the concentration of rice and plasticiser have no significant impact on the Tensile Strength of the Resveratrol films while the type of the plasticiser only slightly does. Generally the quadratic equation yields no meaningful results probably because many factor are integrated at the same time.

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Table 3.8: Thickness_ = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type for Resveratrol films

Summary	
|R|	0.494
R^2	0.244
R^2 adjusted	0.05540
Standard Error	3.948
# Points	16
PRESS	364.65
R^2 for Prediction	-0.473
Durbin-Watson d	2.709
First Order Autocorrelation	-0.368
Collinearity	1.000
Coefficient of Variation	53.042
Precision Index	4.983

ANOVA						
Source	*SS*	*SS%*	*MS*	*F*	*F Signif*	*df*
Regression	60.47	24	20.16	1.293	0.322	3
Residual	187.03	76	15.59			12
LOF Error	159.27	64 (85)	17.70	1.9128	0.323	9
Pure Error	27.76	11 (15)	9.252			3
Total	247.50	100				15

Thickness_ = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type							
		P value	Std Error	-95%	95%	t Stat	VIF
b0	9.388	0.09209	5.128	-1.786	20.56	1.831	
b1	0.123	0.667	0.279	-0.485	0.731	0.441	1.000
b2	-0.379	0.199	0.279	-0.987	0.229	-1.358	1.000
b3	-1.894	0.200	1.396	-4.935	1.147	-1.357	1.000

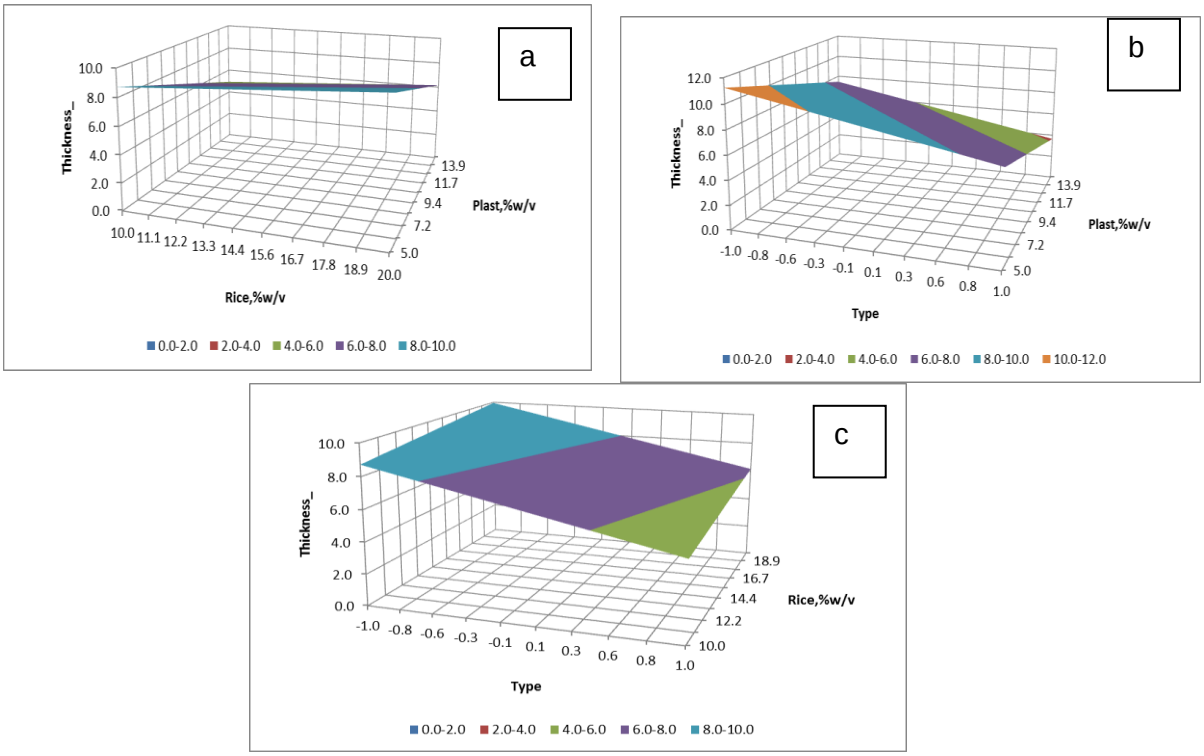


Figure 3.7: (a), (b), and (c) show the three dimensional graphs for Resveratrol Thickness.

(a) The graph shows that for Resveratrol films, as the concentration of rice is increased, the Thickness does not increase.

(b) The graph shows that for Resveratrol films, as the concentration of the plasticizer is increased, the thickness does not change.

(c) The graph shows that for Resveratrol films Glycerine (1) is the best the plasticizer

This means that the concentration of rice and plasticiser have no significant impact on the Thickness of the Resveratrol films.

Table 3.8: Disintegration = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type For Curcumin films

Summary	
|R|	0.737
R^2	0.543
R^2 adjusted	0.429
Standard Error	5.363
# Points	16
PRESS	624.20
R^2 for Prediction	0.174
Durbin-Watson d	1.780
First Order Autocorrelation	0.000
Collinearity	1.000
Coefficient of Variation	38.349
Precision Index	18.450

ANOVA						
Source	*SS*	*SS%*	*MS*	*F*	*F Signif*	*df*
Regression	410.89	54	136.96	4.761	0.02070	3
Residual	345.20	46	28.77			12
LOF Error	331.72	44 (96)	36.86	8.2019	0.05515	9
Pure Error	13.48	2 (4)	4.494			3
Total	756.10	100				15

Disintegration = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type							
		P value	Std Error	-95%	95%	t Stat	VIF
b0	-11.78	0.117	6.967	-26.96	3.403	-1.690	
b1	1.242	0.00665	0.379	0.415	2.068	3.274	1.000
b2	0.714	0.08426	0.379	-0.112	1.540	1.882	1.000
b3	0.278	0.886	1.896	-3.854	4.409	0.146	1.000

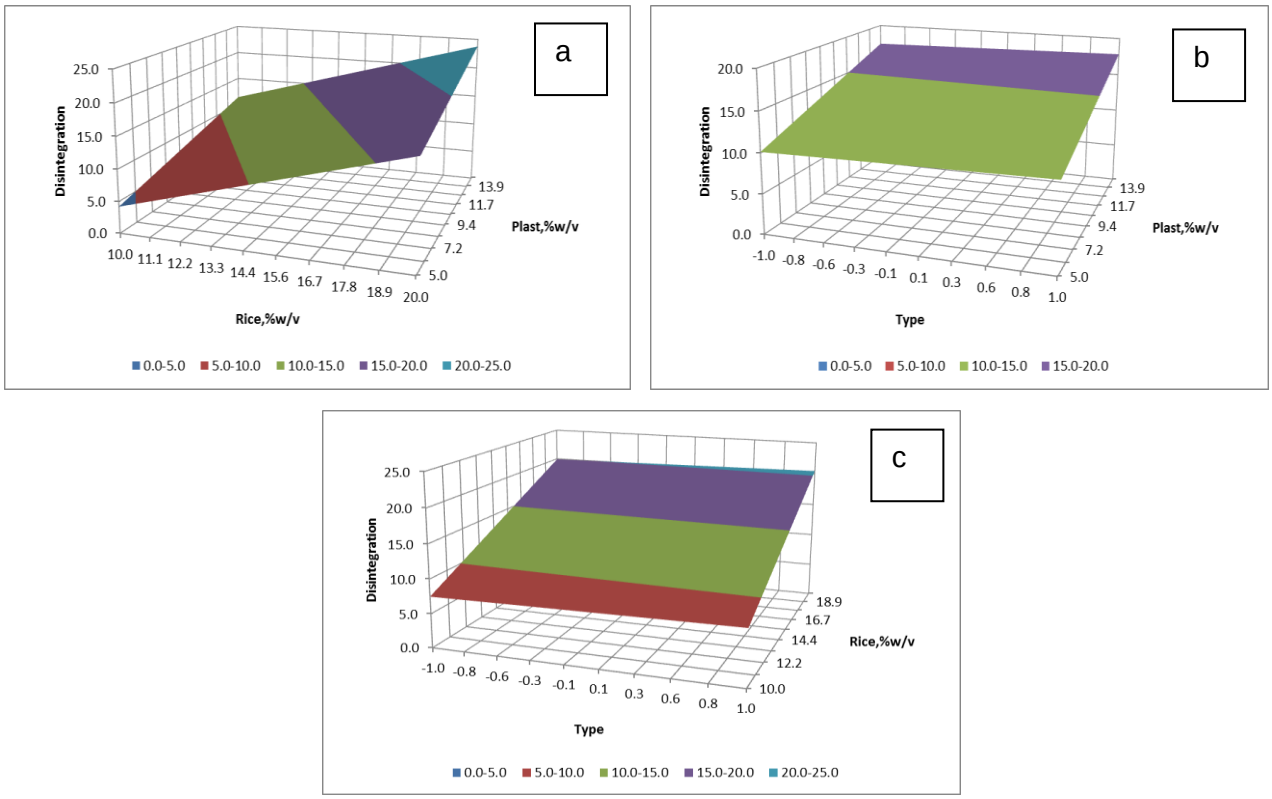


Figure 3.9: (a), (b), and (c) show the three dimensional graphs for Curcumin Disintegration

(a) The graph shows that for Curcumin films, as the concentration of rice is increased, the Disintegration time increases.

(b) The graph shows that for Curcumin films, as the concentration of the plasticizer is increased, the Disintegration time decreases.

(c) The graph shows that for Curcumin films, the plasticizer type does not play any significant role as it is almost horizontal from (-1) through (0) to (+1).

This means that the concentration of rice and plasticiser have a significant impact on the disintegration of the Resveratrol films while the type of the plasticiser does not.

Table 3.9: Folding = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type + b4*Rice, %w/v*Rice, %w/v + b5*Plast, %w/v*Plast, %w/v + b6*Type*Type + b7*Rice, %w/v*Plast, %w/v + b8*Rice, %w/v*Type + b9*Plast, %w/v* Type For Curcumin films

Table 3.10: Folding

Summary	
|R|	0.900
R^2	0.811
R^2 adjusted	0.527
Standard Error	2.583
# Points	16
PRESS	640.50
R^2 for Prediction	-2.026
Durbin-Watson d	1.608
First Order Autocorrelation	0.080
Collinearity	0.000
Coefficient of Variation	209.974

ANOVA						
Source	*SS*	*SS%*	*MS*	*F*	*F Signif*	*df*
Regression	171.64	81	19.07	2.858	0.107	9
Residual	40.03	19	6.672			6
Total	211.67	100				15

Folding = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type + b4*Rice, %w/v*Rice, %w/v + b5*Plast, %w/v*Plast, %w/v + b6*Type*Type + b7*Rice, %w/v*Plast, %w /v + b8*Rice, %w/v*Type + b9*Plast, %w/v*Type						
		P value	Std Error	-95%	95%	t Stat	VIF
b0	15.75	0.322	14.58	-19.94	51.43	1.080	
b1	-2.920	0.126	1.644	-6.942	1.103	-1.776	81.00
b2	1.837	0.209	1.304	-1.355	5.029	1.408	51.00
b3	12.63	0.03745	4.745	1.018	24.24	2.662	27.00
b4	0.09841	0.105	0.05166	0.02799	0.225	1.905	73.00
b5	-0.04593	0.408	0.05166	-0.172	0.08048	-0.889	33.00
b6	1.148	0.408	1.291	-2.012	4.308	0.889	1.000
b7	-0.05249	0.349	0.05166	-0.179	0.07392	-1.016	27.00
b8	-0.722	0.03141	0.258	-1.354	0.08965	-2.794	19.00
b9	-1.49013E-16	1.000	0.258	-0.632	0.632	-5.76903E-16	9.000

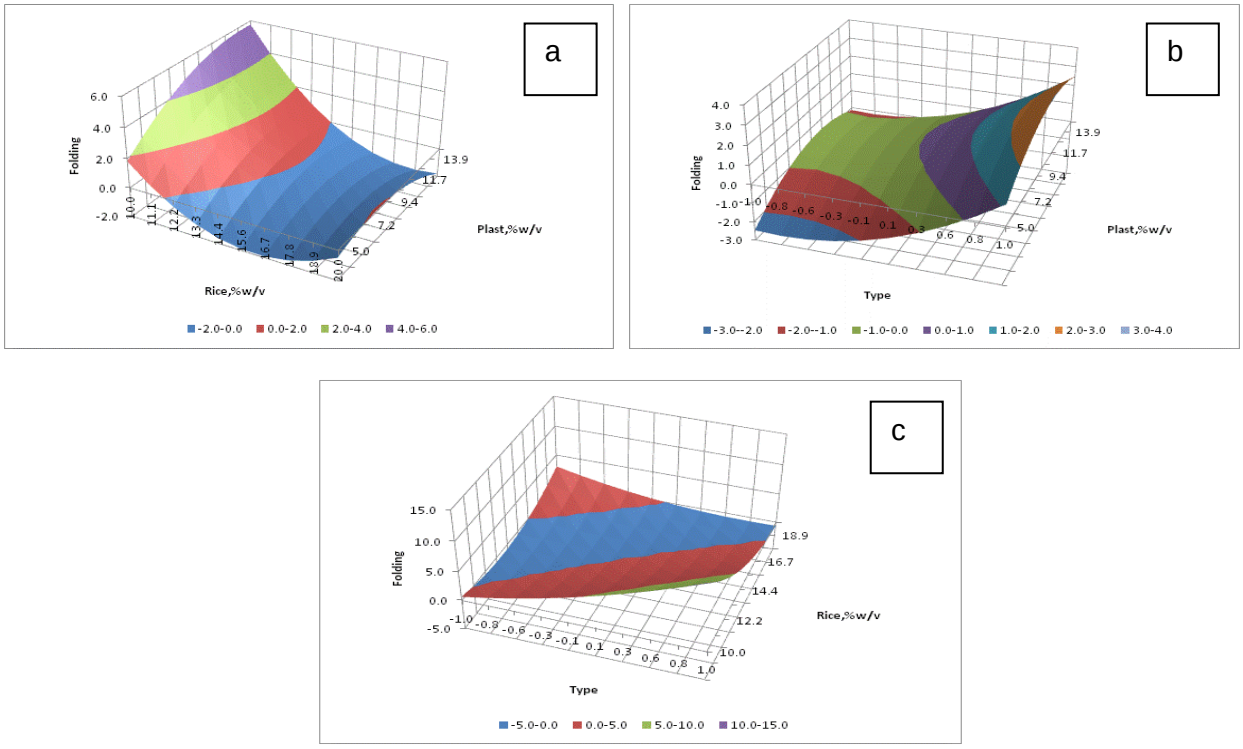


Figure 3.8: (a), (b), and (c) show the three dimensional Quadratic graphs for Curcumin Folding Endurance.

(a) The graph shows that for Curcumin films, as the concentration of rice is increased, the Folding Endurance increases.

(b) The graph shows that for Curcumin films, as the concentration of the plasticizer is increased, the Folding Endurance decreases.

(c)The graph shows that for Curcumin films, Sorbitol (-1) gives the least Folding Endurance.

This means that the concentration of rice and plasticiser have a significant impact on the Folding Endurance of the Curcumin films while the type of the plasticiser does not.

Table 3.10: Tensile = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type + b4*Rice, %w/v*Rice, %w/v + b5*Plast, %w/v*Plast, %w/v + b6*Type*Type + b7*Rice, %w/v*Plast, %w/v + b8*Rice, %w/v*Type + b9*Plast, %w/v* Type For Tensile Strength

Summary	
|R|	0.793
R^2	0.628
R^2 adjusted	0.07033
Standard Error	0.267
# Points	16
PRESS	5.64
R^2 for Prediction	-3.910
Durbin-Watson d	1.002
First Order Autocorrelation	0.394
Collinearity	0.000
Coefficient of Variation	59.090
Precision Index	6.245

ANOVA						
Source	*SS*	*SS%*	*MS*	*F*	*F Signif*	*df*
Regression	0.722	63	0.08021	1.126	0.459	9
Residual	0.427	37	0.07123			6
LOF Error	0.343	30 (80)	0.114	4.0868	0.139	3
Pure Error	0.08402	7 (20)	0.02801			3
Total	1.149	100				15

		P value	Std Error	-95%	95%	t Stat	VIF
b0	1.524	0.351	1.507	-2.163	5.211	1.012	
b1	-0.04833	0.786	0.170	-0.464	0.367	-0.285	81.00
b2	-0.188	0.213	0.135	-0.518	0.142	-1.395	51.00
b3	-0.167	0.745	0.490	-1.367	1.033	-0.341	27.00
b4	0.000774	0.889	0.00534	-0.01229	0.01383	0.145	73.00
b5	0.00358	0.528	0.00534	-0.00948	0.01664	0.670	33.00
b6	-0.145	0.317	0.133	-0.472	0.181	-1.090	1.000
b7	0.00679	0.251	0.00534	-0.00627	0.01985	1.272	27.00
b8	0.02225	0.436	0.02669	-0.04306	0.08755	0.834	19.00
b9	-0.01219	0.664	0.02669	-0.07750	0.05311	-0.457	9.000

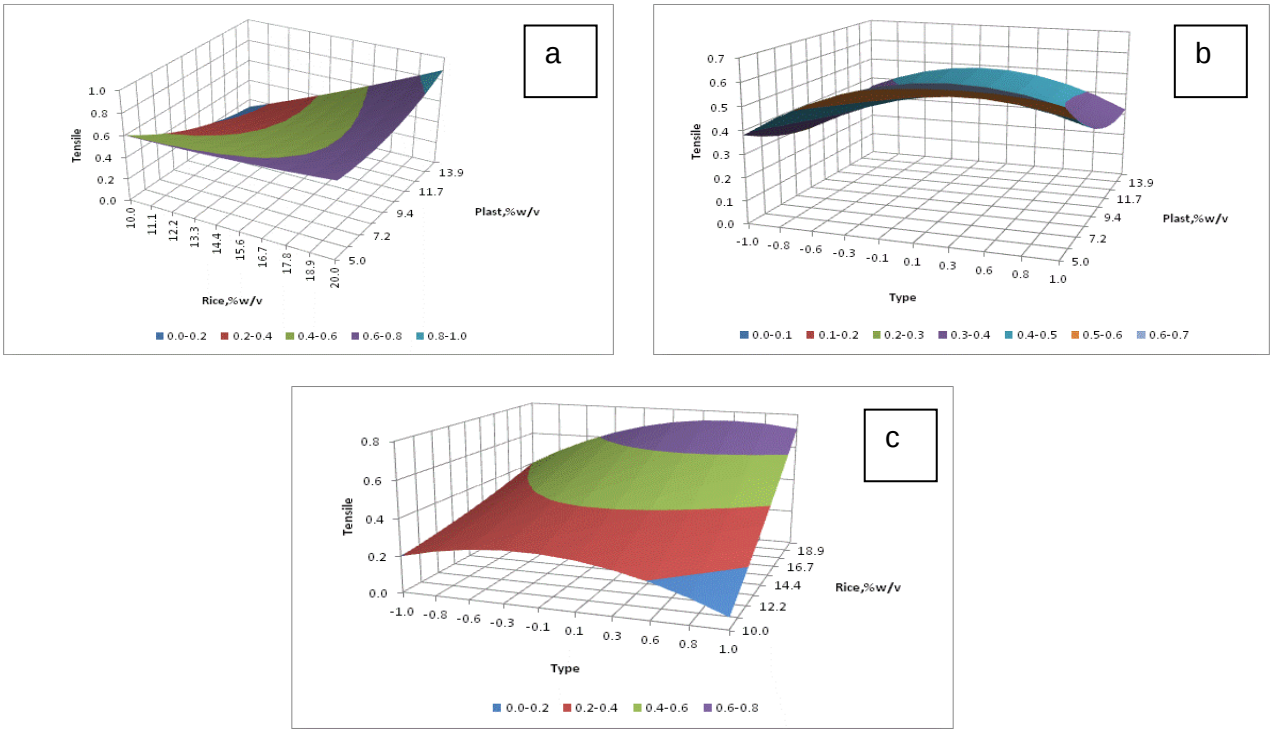


Figure 3.9:(a), (b), and (c) show the three dimensional graphs for Curcumin Tensile Strength.

(a) The graph shows that for Curcumin films, as the concentration of rice is . increased, Tensile strength the time does not increase.

(b) The graph shows that for Curcumin films, as the concentration of the plasticizer is increased, the Tensile strength. time does not increase.

(c) The graph shows that for Curcumin films Honey (0) is the best as it gives the highest Tensile Strength.

This means that the concentration of rice and plasticiser have no significant impact on the Tensile Strength of the Curcumin films while the type of the plasticiser only slightly does. Generally, the quadratic equation yields no meaningful results as no factor is of any significance, probably because many factor are integrated at the same time.

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Table 3.11: Thickness_ = b0 + b1*Rice, %w/v + b2*Plast, and a p-value of %w/v + b3*Type - For Curcumin films

Summary	
|R|	0.190
R^2	0.03600
R^2 adjusted	-0.205
Standard Error	5.092
# Points	16
PRESS	628.80
R^2 for Prediction	-0.948
Durbin-Watson d	1.603
First Order Autocorrelation	-0.002
Collinearity	1.000
Coefficient of Variation	69.198
Precision Index	2.379

ANOVA						
Source	*SS*	*SS%*	*MS*	*F*	*F Signif*	*df*
Regression	11.62	4	3.874	0.149	0.928	3
Residual	311.19	96	25.93			12
LOF Error	287.48	89 (92)	31.94	4.0418	0.139	9
Pure Error	23.71	7 (8)	7.903			3
Total	322.81	100				15

Thickness_ = b0 + b1*Rice, %w/v + b2*Plast, %w/v + b3*Type							
		P value	Std Error	-95%	95%	t Stat	VIF
b0	7.349	0.288	6.615	-7.065	21.76	1.111	
b1	0.134	0.716	0.360	-0.650	0.919	0.373	1.000
b2	-0.200	0.588	0.360	-0.985	0.584	-0.556	1.000
b3	0.00169	0.999	1.800	-3.921	3.925	0.000937	1.000

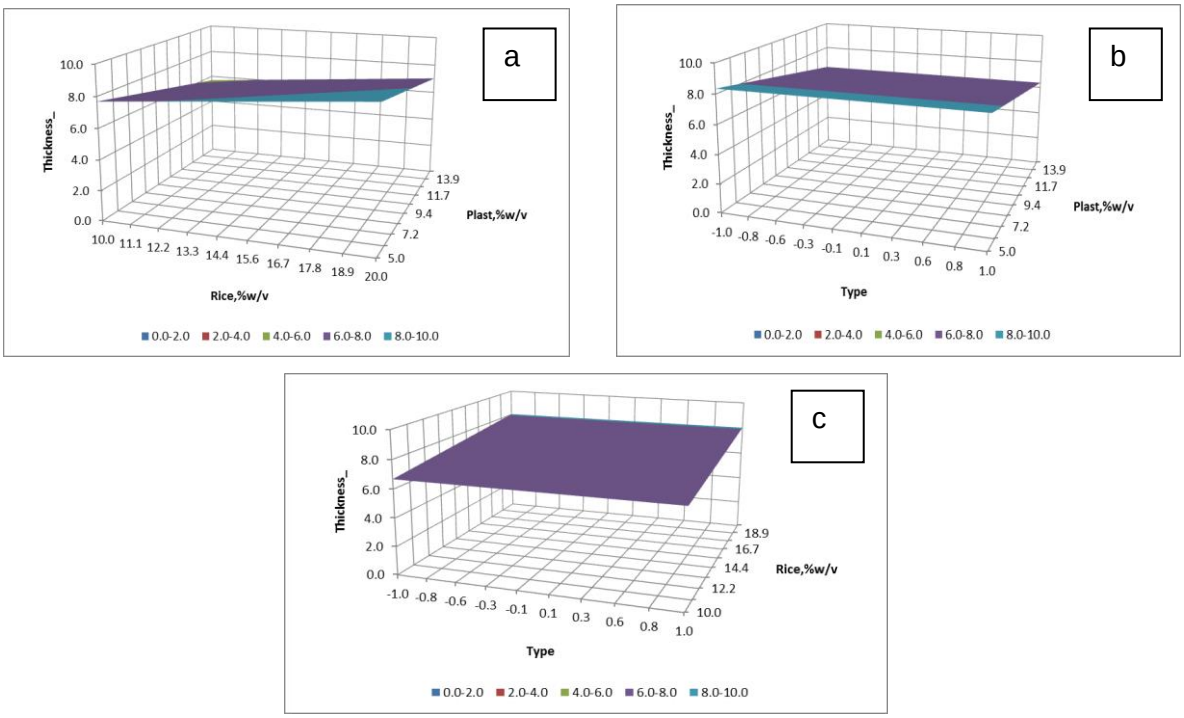


Figure 3.13: (a), (b), and (c) shows the Quadratic three dimensional graphs for Curcumin Thickness.

(b) The graph shows that for Curcumin films, as the concentration of rice is increased, the Thickness does not increase.

(b) The graph shows that for Curcumin films, as the concentration of the plasticizer is increased, the thickness does not change.

(c) The graph shows that for Curcumin films Glycerine (1) is the best the plasticizer

This means that the concentration of rice and plasticiser have no significant impact on the Thickness of the Curcumin films.

The Biostatistics Regration analysis in the tables (SeeTable 3.4) 3.4 – 3.12, describes the significance of the formulation constraints. The Dependant and independent variables, R^2 Adjusted R^2, Standard Error, R^2 for Prediction, Durbin-Watson (d), First Order Autocorrelation, Collinearity, Coefficient of Variation, Precision Index, **ANOVA** to show the significance of the variables (*F Signif*) and the degree of freedom *(df*), The Beta and the (t Stat)indicators explain the significance of the formulation variables and a highly statistical justification of the study. (See Table 3.12), page 77, has been extracted to only summarise and explain the p – value of less than 0.05 which emphasises the significance of the formulation factors.

The surface response plots (SeeTable 3.4) Tables 3.4. – .3.12 above showed that the concentration of rice played a significant role on the films integrity with a p-vale of 0.03000 for the tensile strength, and a p-value of 0.00665 for disintegration. It was also noted that the concentration of the plasticizer significantly affected the folding endurance with a p-value of 0.00109. The thickness of the films played no significant role in the film's integrity as its p-values were greater than 0.05.The summary of the integrated results is given in
(See Table 3.12) below.

The three dimensional graphs represented by (SeeFigure 3.3) Figures 3.3. – 3..13 above helped the visual influence of the different polymeric concentrations on the film integrity as well as release characteristics of the model drugs namely, Resveratrol and Curcumin loaded in the rice paper films.

Table 3.12: Factors that have a significant impact on the physical and mechanical properties

Variable factors			Rice %		Plasticizer %		Plasticizer Type			ANOVA Regression
Outcome Responses	B_0	p-value	B_1	p-value	B_2	p-value	B_3	p-value	Adjusted R^2	F Sign
Disintegration	-11.78	0.117	1.242	0.007**	0.714	0.084	0.278	0.886	0.429	0.021*
Tensile Strength	-0.044	0.892	0.043	0.030*	-0.015	0.419	0.045	0.617	0.211	0.125
Folding Endurance	1.181	0.053	-0.064	0.054	0.128	0.0011**	-0.181	0.251	0.586	0.003**
Thickness	7.349	0.288	0.134	0.716	-0.200	0.588	0.002	0.999	-0.205	0.928

*Represents noteworthy p-values, noteworthy p-values are < 0.05.

Significant p values lie below 0.05

Table 3.13: Show the 4 Responses namely, Disintegration, Tensile Folding Endurance, and Thickness could not be depended on To optimize the formulation utilizing the solver Essential Experimental design.

Factor	Curcumin				Resveratrol			
	Disintegration Not significant (N/S)	Tensile (N/S)	Thickness (N/S)	Folding Endurance (N/S)	Disintegration (N/S)	Tensile (N/S)	Thickness (N/S)	Folding Endurance (N/S)
Rice	Low Rice	High Rice	None	None	Low Rice	Low Rice	None	Low Rice
Plasticizer	Low Plasticizer	None	Low Plasticizer	High Plasticizer	Low Plasticizer	High Plasticizer	None	High Plasticizer

(SeeTable 3.13) below summarizes the report. The four parameters chosen namely, Tensile strength, Disintegration, folding endurance, and Thickness were problematic as these four Responses chosen did not yield an optimum formula. Other variables including, Moisture content, Swelling Index, Appealingness, Taste, Softness and robustness had also to be considered to optimise the formulation.

Table 3.14: Altering the percentages of Rice, percentage of Plasticizer, and Type of plasticizer in the Solver for the Experimental Design yielded brittle films.

Factor	Curcumin				Resveratrol			
	Disintegration	Tensile	Thickness	Folding Endurance	Disintegration	Tensile	Tensile	Folding Endurance
Rice	10 %	15 %		10 %	11 %	None		
Plasticizer	10 %	15 %		15 %	6 %	None		
Type	Honey	Honey			Glycerin	None		

(SeeTable 3.14) summarizes the impact of varying concentrations of rice polymer, plasticizer and type of plasticizer on mechanical properties of the produced films. Optimisation was performed using only the four (4) mechanical properties of disintegration, tensile strength, folding endurance and thickness. Changing the concentration of rice had a significant impact on disintegration ($p<001$) and tensile strength ($p<0.05$). Plasticiser concentration had a significant positive association with folding endurance ($p<0.01$). None of the ingredients had any significant associations with thickness.

3.3.3 Solver for factorial design results

Table 3.15: Curcumin disintegration

Term	**Plast, %w/v**	**Rice, %w/v**	**Type**
Data Min	5	10	-1
Data Avg	10	15	0
Data Max	15	20	1
Cur Value	**5**	**15**	**0**
Disintegration 7.76			

Table 3.16: Curcumin tensile strength

Term	**Plast, %w/v**	**Rice, %w/v**	**Type**
Data Min	5	10	-1
Data Avg	10	15	0
Data Max	15	20	1
Cur Value	**5**	**15**	**0**
Tensile 1.26			

Table 3.17: Curcumin thickness

Term	***Plast, %w/v***	***Rice, %w/v***	***Type***
Data Min	5	10	-1
Data Avg	10	15	0
Data Max	15	20	1
Cur Value	**5**	**15**	**0**
Thickness_ 7.10			

Table 3.18: Curcumin folding

Term	Pla /v	Rice, %w/v	Typ
Data Min	5	10	-1
Data Avg	10	15	0
Data Max	15	20	1
Cur Value	**5**	**15**	**0**
Folding 1.19			

Table 3.19: Resveratrol disintegration

Term	Plast, %w/v	Rice, %w/v	Type
Data Min	5	10	-1
Data Avg	10	15	0
Data Max	15	20	1
Cur Value	**6**	**11**	**1**
Disintegration 6.84			

Table 3.20: Resveratrol tensile strength

Term	Plast, %w/v	Rice, %w/v	Type
Data Min	5	10	-1
Data Avg	10	15	0
Data Max	15	20	1
Cur Value	**6**	**11**	**1**
Tensile 4.80			

Table 3.21: Resveratrol thickness

Term	Plast, %w/v	Rice, %w/v	Type
Data Min	5	10	-1
Data Avg	10	15	0
Data Max	15	20	1
Cur Value	**6**	**11**	**1**
Thickness 8.59			

Table 3.22: Resveratrol folding

Term	**Plast, %w/v**	**Rice, %w/v**	**Type**
Data Min	5	10	-1
Data Avg	10	15	0
Data Max	15	20	1
Cur Value	**6**	**11**	**1**
Folding			
0.74			

Table 3.23: Resveratrol and Curcumin disintegration

Term	***Plast, %w/v***	***Rice, %w/v***
Data Min	5	10
Data Avg	10	15
Data Max	15	20
Cur Value	**15**	**15**
Ln Disintegration		
2.813089		
Disintegration		
16.66		

Table 3.24: Resveratrol and Curcumin disintegration

Term	***Rice, %w/v***	***Type***
Data Min	10	-1
Data Avg	30	0
Data Max	50	1
Cur Value	**10**	**1**
1/ Disintegration		
0.118484		
Disintegration		
8.44		

The four parameters of Tensile Strength, Disintegration, Folding endurance, and thickness considered during the optimisation for the formulation did not give conclusive results on the optimum formulation. The four mechanical test results were further analysed in the Solver (See Table 3.15) Tables 3.16 to 3.23 for factorial design. Two optimal formulations for Curcumin and Resveratrol were suggested as indicated below. The two formulas for curcumin and Resveratrol were manufactured again in the laboratory (For Curcumin, Rice 15%, Plast 5% and Type Honey. For Resveratrol Rice 11%, Plast 6% and Type Glycerine). The Curcumin film was brittle though it yielded the best disintegration time in simulated saliva of the predicted 7.76 seconds, and a folding endurance of one (1) fold at break. The film for Resveratrol could not even be harvested from the Petri dish.

The solver again was used to optimise the Ln Disintegration at 15% Rice starch and 15% glycerine (See Table 3.14) and (See Table 3.23)Tables 3.24 – 3 25 and the reciprocal of Disintegration yielded an acceptable robust film for both Resveratrol and Curcumin with 10% Rice starch with predicted Disintegration time of 8.439962 seconds. This could have been the preferred formulation because it could allow the incorporation of 15% honey which would act as a natural preservative for the solid formulation however, its plain film folded over itself which is an undesirable characteristic (See Table 3.23) tables 3.24 and 3.25.Stability of the product will be a subject of future research through suitable preservation using natural preservatives, aseptic technique, and gamma radiation of the finished product, suitable packaging, and stability studies to obtain a stable formulation for the predicted shelf life.

In the circumstance, other desirable characteristics of the IDF that included moisture content, flexibility, and Taste testing using Human volunteers as panellist for Appealingness were considered for the optimum formulation 16. The solver coincidently also optimised the Ln Disintegration at 15% Rice starch and 15% glycerine and the reciprocal of Disintegration predicted a Disintegration time of 16.66 seconds for both Resveratrol and curcumin (See Table 3.23). This yielded an acceptable robust film for both Resveratrol and Curcumin. None the less, the factorial design helped to generate the 16 formulations for the variables and it proved that the three factors Rice percentage, Plasticizer percentage, and Type of plasticizer had significant effect. Thickness though indirectly, Tensile Strength, Folding Endurance, and Disintegration eventually influenced the Release characteristics of the rice paper drug Delivery system.

3.4 Concluding remarks

A statistical experimental factorial design was employed in order to develop and optimize the novel approach for the formulation of an Intraoral Rice paper Film intended for the delivery of essential natural drugs with low oral bioavailability. A range of formulations varying in release characteristics was obtained. Response surface design was employed in order to identify the relationships between the responses (i.e. Rice starch and the experimental factors, Plasticisers, sweeteners). Optimization of experimental factors resulted in the generation of an optimised rice paper formulation possessing maximal release characteristics of the model drugs Resveratrol and Curcumin capable of displaying a zero-order rate of drug release.

The cross-linked polymeric matrix (Rice starch + Plasticisers + Model drugs) seen in the ESM film plate, gave a geometrical optimization using very minimum energy between bonds that are easy to break and resulting in excellent intraoral drug release. Thus, stress transduction forces were mainly interpreted to be the collective interactions of electrostatic energies, van der Waals forces, and H-bonding contributing to the binding energy that put the thin rice paper film together. The results obtained from the characterization give much promise that the developed drug delivery system may find a good application in the delivery of not only the model natural drugs but also some essential drugs exhibiting low oral bioavailability as well.

Chapter 4. Characterization of rice paper intraoral drug delivery system (IDF) loaded with the model drugs

4.1. Introduction

IDFs are not yet listed in any pharmacopoeia. Besides typical parameters such as content, content uniformity and impurity profile, disintegration or dissolution properties and mechanical properties are investigated to ensure a robust manufacturing process and a good patient compliance. Many of the ideal tests used for solid oral dosage forms for example, orodispersible tablets are not transferable to IDFs. Having to comply, for example, with content specifications for conventional oral dosage forms, in house specifications need to be developed. Lists 4.1 and 2.2 give an overview on possible pharmacopoeial and alternative characterization methods for IDFs.

4.1.1 Alternative characterization methods.

List 4.1: List of Pharmacopoeial and alternative characterisation methods
Property, Pharmacopoeial and Alternative methods are found in the USP 34 NF 29 Ph.Eur. 7.0, but are not specifically tied to IDFs but applied to other dosage forms. These tests, as they may apply to IDF characterisation, are listed below with their references;
‘‘[Appearance and API distribution. Optical microscopy. Scanning electron microscopy. Visual inspection. Near-infrared chemical imaging (Boateng et al., 2009a); (Boateng et al., 2010); (Kulkarni et al., 2010).. Content uniformity. Uniformity of dosage units. Uniformity of mass of single-dosage preparations. Uniformity of content of single-dose preparations. Uniformity of dosage units (Cilurzo et al., 2008); (Cilurzo et al., 2011); (Dinge and Nagarsenker, 2008); (Nishimura et al., 2009); (Shimoda et al., 2009).

Crystallinity and Glass transition temperature. Crystallinity. Crystallinity determination by solution Calorimetry. Thermal analysis. X-ray diffraction. Characterization of crystalline and partially crystalline solids by XRPD (Boateng et al., 2009b, Boateng et al., 2010); (Cilurzo et al., 2008); (Dinge and Nagarsenker, 2008); (Gaisford et al., 2009); (Garsuch and Breitkreutz, 2009); (Mashru et al., 2005); (Repka et al., 2003)..

Disintegration. Contact angle measurement. Thermal mechanical analysis of the swelling behaviour Slide frame method Petri dish method. Stainless steel wires mesh Swirling Swelling behaviour. Modified dissolution apparatus. Modified disintegration tester Diffusion apparatus

(Garsuch and Breitkreutz, 2009); (Mishra and Amin, 2009) (Bi et al., 1996); (Garsuch and Breitkreutz, 2010); (Peh and Wong, 1999); (Sakuda et al., 2010).
Dissolution. Drug release*. In vitro* and *in vivo* evaluation of dosage forms
In vivo bioequivalence guidance. The dissolution procedure: Development and Validation. Dissolution test for solid dosage forms. FIP/AAPS guidelines EMA BE guideline.

Sinkers Stainless steel wire mesh Modified USP type 1 apparatus Continuous flow-through cell Fibre-optic sensor system Diffusion apparatus (Mishra and Amin, 2009); (Barnhart and Sloboda, 2007, Barnhart and Vondrak, 2008);; (Boateng et al., 2010, Dinge and Nagarsenker, 2008); (Dinge and Nagarsenker, 2008); (Garsuch, 2009); (Hughes and Gehris, 2003);; (Mashru et al., 2005); (Nishimura et al., 2009); (Patel et al., 2010); (Sharma et al., 2007); (Shimoda et al., 2009); (Siewert et al., 2003). Water content, Hygroscopicity and Moisture uptake.

Loss on drying. Water determination. Loss on drying. Water-semi-micro determination. Water-micro determination. Wettability of porous solids including powders Dynamic vapour sorption. Determination of water uptake by weight; (Boateng et al., 2010); (Dinge and Nagarsenker, 2008); (Garsuch and Breitkreutz, 2010); (Peh and Wong, 1999, Wong et al., 1999).
We did Moisture content and Swelling Index to evaluate the rice paper films.

[AAPS: American Association of Pharmaceutical Scientists; API: Active pharmaceutical ingredient; ASTM: American Society for Testing and Materials; BE: Bioequivalence; DIN EN ISO: Deutsches Institut fu¨ r Normung, Europa¨ ische Norm, International Organization for Standardization; EMA: European Medicines Agency; FIP: International Pharmaceutical Federation; ICH: International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH); Ph. Eur. European Pharmacopoeia; USP: United States Pharmacopoeia; All of these professional bodies recommend XRPD: X-ray powder diffraction]''.

4.1.2 Other alternative characterization methods

List 4.2: List of other Pharmacopoeial and alternative characterisation methods
Property and alternative methods to characterise IDFs found in the USP 34 NF 29 and the Ph.Eur. 7.0 but are not specifically tied to IDFs but applied to other dosage forms. These include; Local tolerance, Sensitization testing in Animals and humans (Barnhart and Vondrak, 2008); (Dixit and Puthli, 2009); (Hao and Heng, 2003). Mechanical properties tested include

Tensile strength and dryness or tack test. Puncture and shear test and Vertical strength. Folding endurance as well as Stickiness are tests also done on IDFs (Mishra and Amin, 2009, Cilurzo et al., 2011); (Dixit and Puthli, 2009); (El-Setouhy and El-Malak, 2010); (Garsuch, 2009); (Gavaskar et al., 2010); (Malke et al., 2010); (Mashru et al., 2005); (Peh and Wong, 1999);; (Radebaugh et al., 1988); (Sakuda et al., 2010).

Microbiology; Microbiological examination can be done on IDFs as for all non-sterile products: Microbial enumeration tests for microbiological examination of non-sterile products must be taken as mandatory acceptance criteria for pharmaceutical preparations and substances for pharmaceutical use especially for the Rice paper IDFs. Honey is a weak natural preservative (Aliyazicioglu and Boukraa, 2015) and later in the production stages of the IDF, it would be safer to prepare the it under sterile conditions and probably sterilize the final packed product by Gamma radiation. Microscopic examination using Scanning Electron Microscope (SEM) revealed presence of fungal microscopic growth on the finished product as illustrated in the images below. This emphasises the point that the Rice paper film is a purely natural organic product and could be food for micro- organisms. Therefore, the films must be freshly prepared, casted, and packaged in a sterile environment, in sterile containers. Gamma radiation of the finished sealed product is highly recommended. The containing jars of the suspensions were pre sterilized at 100^0C in the water bath to avoid microbial growth in the suspension and the final product during manufacture. The films were sealed off immediately in foil to avoid contamination and further loss of moisture as well. (See Figure 4.1) Figures 4.1, and 4.2, below show evidence of microbial growth. The SEM images revealed when the precautions were not taken. It is emphasized therefore, that the films should be manufactured under strict aseptic conditions and aseptic technique should be applied at all stages of production. Our rice paper IDF was desired to be natural and did not contain synthetic preservatives but later, the addition of a smaller validated percentage of honey to the optimal formulation may be considered as a natural preservative to enhance the product stability.

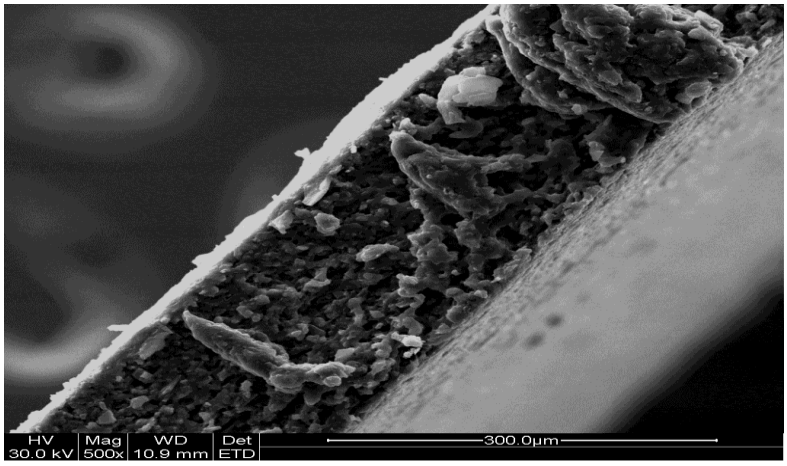


Figure 4.1: Fungal growth as revealed by SEM imaging of the finished product.

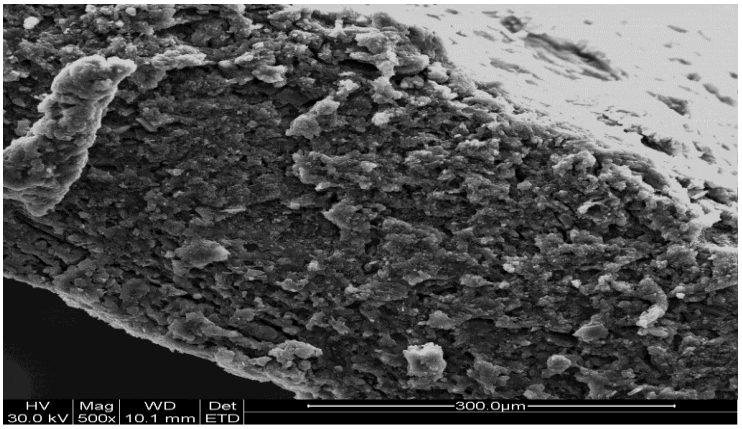


Figure 4.2: Fungal growth as revealed by SEM imaging of the finished product.

Microbiological quality methods of non-sterile pharmaceutical preparations and substances are based on a texture analysis and can be done on mucosal tissues from animals. A friable texture could mean a degeneration due to microbial digestion (Dinge and Nagarsenker, 2008); (Asane et al., 2008); (Hao and Heng, 2003); (Pather et al., 2008); (Shojaei, 1998).. Permeation using a Diffusion cell (Franz cell) can be done on mucosal tissues from animals (Barnhart and Vondrak, 2008),; (Hao and Heng, 2003); (Pather et al., 2008); (Shojaei, 1998).

Qualification and quantification of the API, Spectrophotometric identification tests, Spectrophotometry and light scattering, Raman spectrophotometry, and Absorption spectrophotometry, are among the test done on IDFs. Infrared, Near-infrared spectrophotometry is also a test done on IDFs (Mishra and Amin, 2009); (Cilurzo et al., 2011); (Haag et al., 2009); (Tumuluri et al., 2004); (Tumuluri et al., 2008).

Residual solvents, is a test performed to identify and control of residual solvents.
Stability Test. Pharmaceutical stability studies is required by the ICH Guidelines Q1 A -- F (Borsadia et al., 2003); (Hao and Heng, 2003); (Nishimura et al., 2009); (Sakuda et al., 2010); (Shimoda et al., 2009)..
Taste testing using Human healthy volunteer taste panel. Animal preference test are tests usually done on IDFs as Taste-sensing systems (Cilurzo et al., 2008); (Cilurzo et al., 2011); Cilurzo et al., 2011; (Dinge and Nagarsenker, 2008); (Klancke, 2003); (Woertz et al., 2011).

Taste testing was done for our films as well to determine acceptability by the patients.

4.1.3 Mechanical properties

Determination of thickness and weight are routine tests. A typical film has thickness that ranges from 12 to 100 µm (Barnhart and Vondrak, 2008). Furthermore, acceptable mechanical parameters ensure flexibility and robustness of the films during manufacturing and handling. As the USP describes only a tensile strength test for surgical sutures and patches, technical regulations from other industries such as the plastic industry can be used as templates. Tensile strength has been described as a test for IDFs according to the Standards for American Society for Testing and Materials (ASTM). Deutsches Institut fu¨r Normung, Europa ische - Norm, International Organization for Standardization (DIN EN ISO 527-1 and 527-3 regulations), state that Tensile strength can be used as a Test Method for Thin Plastic Sheeting (Cilurzo et al., 2008); (DIN, 1996); (Garsuch and Breitkreutz, 2009).

Tensile strength of the films was measured. The films held between two clamps were pulled and the force and elongation at break point were measured (Mishra and Amin, 2009); (Cilurzo et al., 2008); (El-Setouhy and El-Malak, 2010); (Garsuch and Breitkreutz, 2009); (Peh and Wong, 1999). Further parameters such as tear resistance have also been mentioned (Cilurzo et al., 2011);(Dixit and Puthli, 2009); (Gavaskar et al., 2010). An ideal IDF should have moderately high tensile strength, high elongation at break and strain, but a low elastic modulus (Garsuch and Breitkreutz, 2009); (Mashru et al., 2005); (Peh and Wong, 1999).

To measure the folding endurance, the films were repeatedly folded at the same position until they broke (Patel et al., 2009). Cilurzo et al. determined film flexibility by adapting the ASTM bend mandrel test D 4338 – 97 (Cilurzo et al., 2008). Further tests are the dryness / tack test according to the ASTM paint testing manual, puncture and shear tests, vertical strength and stickiness determination (Cilurzo et al., 2008); (Dixit and Puthli, 2009); (Gavaskar et al., 2010); (Radebaugh et al., 1988); (Sakuda et al., 2010).

4.1.4 Viscosity rotating viscometer method

The American Association of Pharmaceutical Scientists (AAPS), American Society for Testing and Materials (ASTM), Bioequivalence (BE), Deutsches Institut fu¨ r Normung, Europa¨ ische Norm, International Organization for Standardization (DIN EN ISO), European Medicines Agency (EMA), International Pharmaceutical Federation (FIP), and the International Conference on Harmonization of Technical (ICH), all state that, viscosity test of the thick suspension should be done as an in process test before the casting process as a requirements for Registration of Pharmaceuticals for Human Use (Ali and Quadir, 2007). The European Pharmacopoeia (Ph.Eur) and the United States Pharmacopoeia (USP) need X-ray powder diffraction (XRPD) to be done (Ali and Quadir, 2007).

4.1.5 Disintegration and dissolution

IDFs are developed to disintegrate or dissolve rapidly in the mouth. Therefore, disintegration and dissolution tests have to be performed. A disintegration time of less than 3 minutes is specified for orodispersible tablets in the European Pharmacopoeia using the pharmacopoeial disintegration tester. A disintegration time of not more than 1 min is required for ODFs in the Guidance for Industry. 2008, FDA, CDER, and, the European Directorate for the Quality of Medicines & HealthCare., 2010.

IDFs should disintegrate rapidly *in vivo* as well. However, as a disintegration test does not exactly mimic the physiological conditions in the oral cavity, it is inappropriate for orally disintegrating dosage forms in general and particularly for IDFs. Nevertheless, it is still often used. Typical disintegration times range from 5 to 30s (Barnhart and Vondrak, 2008). Efforts have been made to simulate the *in vivo* disintegration, such as contact angle measurements and thermal mechanical analysis of the swelling behaviour of the films (Garsuch, 2009). Further on, simple tests, such as the slide the frame method, and the Petri dish method have been described in literature. These tests work out disintegration time in a small volume of buffer medium simulating the small volume of saliva in the mouth.

In another test, a slide frame holding the IDF is laid on a Petri dish and distilled water is added drop by drop until the drop forms a hole. Time for the drop forms a hole in the film is measured. For the Petri dish method, the IDFs are placed on the surface of 2 ml of distilled water in a small glass beaker and stirred then the time for complete dissolution is recorded (Garsuch and Breitkreutz, 2010). Amin and Mishra used 10 ml of distilled water. They placed the IDFs on a stainless steel wire mesh dipped in a small glass beaker. Disintegration is the time taken for the film to break (Mishra and Amin, 2009). In another test, disintegration was

established using 25 ml distilled water. The dish was swirled every 10 seconds and the time when the film started to break was recorded (Arya et al., 2010).

Peh and Wong described the measurement of swelling behaviour (Peh and Wong, 1999). Bi developed a disintegration test for orodispersible tablets using a modified dissolution apparatus, which might work for IDFs as well, although an un physiologically high volume of dissolution medium was applied (Bi et al., 1996). Sakuda used the disintegration tester in a modified set up. The film is clipped onto the arm of the tester without using its basket. Then the film is dipped in and out of the medium (Sakuda et al., 2010). Boateng measured the hydration of films in a diffusion apparatus. The films were placed on a stainless steel mesh and dipped in distilled water. Hydration was observed by a digital camera until the formulation completely disappears (Boateng et al., 2010).
Most of the test methods used are sufficient to discriminate between different film formulations and may be useful for quality control. However, none of them imitates the physiological conditions of the oral cavity sufficiently. In particular, the mechanical force of the tongue acting on the films is not simulated. In most cases, distilled water is used as the disintegration medium.

There is no simulated saliva described in the European Pharmacopoeia. However, a phosphate buffer at a pH of 6.0 is recommended for the dissolution test for medicated chewing gum and was used by Garsuch and Breitkreutz (Garsuch and Breitkreutz, 2009). Other groups used phosphate buffers at pH 6.8, for example, the European Medicines Agency (EMA) and the Guideline on the investigation of bioequivalence, London 2010 (Mashru et al., 2005); (Nishimura et al., 2009); (Patel et al., 2009); (Shimoda et al., 2009).

In this study, we worked with saliva at a pH 7.4 since simulated saliva at his pH dissolved both Curcumin and Resveratrol and was used to simulate plasma as well. Therefore, simulated saliva at a pH of 7.4 has been adopted to determine disintegration time of the rice paper films. If a drug is dispersed in a film, the limiting factor for that drug dissolution is the time it takes to disintegrate. Therefore, a disintegration test may be done as a test for fast-dissolving films. This was recommended for orodispersible tablets in the FIP/AAPS guidelines for dissolution and may be transferred to IDFs (Siewert et al., 2003). The drug should be stable both in saliva and in blood plasma, which is at pH 7.4 because this same drug will be absorbed in the blood stream, as we shall see in the permeation studies later on.

When drug particles are dispersed in the film to be absorbed after film disintegration, the solubility and the dissolution rate of the drug particles are the limiting factors. In this case a

paddle or basket apparatus can be used for the dissolution test. For quality control, a single point measurement may be sufficient to assure complete drug release for immediate-release systems (Siewert et al., 2003). As the films tend to float, sinkers may be necessary (Barnhart and Vondrak, 2008). Stainless steel wire meshes or the paddle-over-disk apparatus were used to prevent the films from floating. (Mishra and Amin, 2009); (Mashru et al., 2005); (Sharma et al., 2007). Dinge and Nagarsenker used 20 ml of phosphate buffer at a pH of 6.4 as medium in a 50 ml glass beaker. They utilized only the shaft of the USP type 1 apparatus without the basket attached to it for agitation (Dinge and Nagarsenker, 2008). Hughes and Gehris characterized the buccal dissolution of drugs using the method developed by Rohm and Haas. The apparatus that they used was made of a single, stirred, continuous flow-through a cell, dipped in 10 ml of a phosphate buffer. Small disintegrated particles were withdrawn from the vessel during testing using a dip probe to simulate swallowing (Hughes and Gehris, 2003).

Garsuch and Breitkreutz compared manual sample withdrawing with automatic sample withdrawing using a peristaltic pump in fibre optic sensor equipment. The sensor makes online measurements at high sample rates per minute possible and may be very helpful for dissolution measurements of fast-releasing dosage forms. They observed square root kinetic release profile for caffeine films using the fibre optic system (Garsuch, 2009). Patel et al. assumed a zero-order kinetic of a film formulation of ondansetron (Patel et al., 2009). Boateng used his diffusion apparatus to determine dissolution. He fitted his dissolution profile of paracetamol films to fit that of Korsmeyer-Peppas, and Highuchi, zero and first-order equations. They generally yielded sustained release profiles dependent on polymer content (Boateng et al., 2010).

For the evaluation of sustained-release films or buccal patches, permeation studies may be useful. Different *in vivo* or *ex vivo* and *in vitro* models have been investigated to quantify permeation, including diffusion cells such as the so-called Franz cells. Usually mucosal tissue from animals is used as surrogates for human mucosa, for example, porcine mucosa (Hao and Heng, 2003); (Pather et al., 2008); (Shojaei, 1998). Studies to quantify permeation were done for the rice paper films. The donor compartment contained simulated saliva at pH 7.4 and as explained before, the receiving compartment contained simulated plasma at pH 7.4 as the drug was going to be absorbed into the blood veins.

4.1.6 Organoleptic tests.

4.1.6.1 Taste

Taste is one of the critical properties of IDFs influencing patient acceptability. There are different approaches for taste assessment that have been made (Shimoda et al., 2009). Dissolution taste testing with pharmacopoeial dissolution testers is possible, if saliva-resistant coated particles are incorporated in the IDFs. Methods and suitable dissolution media have to be validated carefully in this kind of test (Klancke, 2003). Dissolution testing is not suitable if taste masking is based on flavours and sweeteners or complex forming ingredients. In this case volunteer taste panels have to be employed (Cilurzo et al., 2011); (Dinge and Nagarsenker, 2008). Animal preference tests have been mentioned but may be potentially invalid. (Anand et al., 2007)

There are some disadvantages of using human taste panels. In fact, this has been adopted in this study by setting up a taste-testing panel of healthy Human volunteers. The main problem is the ethical concern, especially for toxic drugs or for drugs intended for paediatric use. It is difficult to get children as volunteers. Further, there is often a lack of objectivity. Taste preferences depend on ethnicity and age. Younger people favour sweeter and exciting flavours, whereas the elderly may prefer traditional flavours, such as mint (Brown, 2003). Children may totally reject a formulation preferred by adults.

An European taste by the use of a Taste-sensing system, the so-called electronic tongues, has been developed as an in vitro alternative. Recently, Woertz gave an overview on these systems in this pharmaceutical area (Woertz et al., 2011). Cilurzo evaluated the suppression of bitterness in sodium Diclofenac IDF using taste-sensing equipment. He thought that the equipment could assist in the complexicity of human ethics to replace human sensory evaluation (Cilurzo et al., 2011). However, as grittiness and mouth-feel also play a major role in patient's acceptability, human taste panels cannot completely be replaced.

4.1.6.2 Others

Other methods for characterization and quality control of IDFs include viscosity measurement of the casting mass, content uniformity and determination of residual solvents (Nishimura et al., 2009); (Cilurzo et al., 2008); (Barnhart and Sloboda, 2007); (Shimoda et al., 2009). Further, visual inspection, optical microscopy and Scanning Electron Microscopy (SEM) evaluate the appearance of the films (Boateng et al., 2010).

Garsuch and Breitkreutz found out that caffeine recrystallized out of the IDFs using electron scanning microscopy, X-ray diffraction, and near-infrared chemical imaging. Variation occurred between the upper and lower surfaces (Garsuch, 2009). Near-infrared spectroscopy and Raman spectroscopy are suitable technologies to qualify and quantify APIs within the films (Haag et al., 2009); (Tumuluri et al., 2004). Further, Fourier transformation infrared spectroscopy has been used to eliminate any reaction between the vehicle and the API (Mishra and Amin, 2011); (Cilurzo et al., 2011).

Tumuluri investigated online measurements with Raman spectroscopy and suggested its suitability as a process analytical tool (Tumuluri et al., 2004). Differential scanning Calorimetry, thermal mechanical analysis and X-ray diffraction are used to investigate crystallinity and glass transition temperature (Boateng et al., 2010); (Cilurzo et al., 2010); (Dinge and Nagarsenker, 2008); (Mashru et al., 2005); (Repka et al., 2003). Gaisford et al. monitored crystallization of drugs from IDFs with isothermal Calorimetry (Gaisford et al., 2009).

Hygroscopicy and residual water content are investigated by dynamic vapour absorption or by weight ((El-Setouhy and El-Malak, 2010); (Garsuch and Breitkreutz, 2010).

Further, microbiological studies and stability tests should be investigated regarding common guidelines (Boateng et al., 2010); (Garsuch and Breitkreutz, 2010); (Nishimura et al., 2009); (Patel et al., 2010); (Shimoda et al., 2009). For the development of sustained-release films or oral patches, some tests on bio adhesion can be helpful (Asane et al., 2008); (Hao and Heng, 2003); (Pather et al., 2008); (Wong et al., 1999); (Shojaei, 1998). Barnhart suggested that local oral mucosal irritation studies in animals and humans should be investigated as well (Barnhart and Vondrak, 2008); (Borsadia et al., 2003); (Dixit and Puthli, 2009); (Hao and Heng, 2003), hence we established the surface pH of our rice paper IDFs.

4.2. Materials

4.2.1 Materials

Curcumin 77% $^{w}/_{w}$ HPLC grade, (Sigma Aldrich, South Africa), Resveratrol (98%w/w HPLC grade, from DB Fine Chemicals, SA) Sorbitol 98% $^{w}/_{w}$ from Sigma Aldrich, SA) and glycerine (99% $^{v}/_{v}$, Unilab, SA) were purchased from Sigma Aldrich. Rice starch (Health Connections, SA), Honey (Rynfield Agricultural Holdings, SA) and Stevia (Merisant company SA) were used as sweeteners. In addition, honey was also used as a plasticizer. D-mannitol 98 % (Tedro Products SA) was used as a filler. Cocoa powder (Mondelez International) was bought from

Shoprite SA, Both cocoa powder and peppermint oil (Aromatherapy oils SA) were used as flavourants in this study. Methanol (99% $^{v}/_{v}$, Romil, SA) and Acetonitrile (99% v/v, Romil, SA) used in this study were analytical grade and HPLC grade respectively.

4.2.2 Equipment

Table 4.1 shows the list of equipment used in the formulation of the Rice paper IDF.

Table 4.1: List of equipment used in the formulation of the Rice paper IDF.

Equipment	Source
UV Spectrophotometer	Cary 50 USA
Thermostatically controlled water bath	Grant instrument Cambridge Ltd
Modified disintegration apparatus	Grant instrument Cambridge Ltd
Silverson emulsifier / Magnetic stirrers	Vortex Mixers, England
Plastic Petri dishes/ Analytical electronic balance/ pH meter	Mettler Toledo (Switzerland)
Digital Micrometer Screw Gauge	Besto (England)
Texture Analyser / Bifocal microscope / Scanning Electron Microscope (SEM)	Nikon, Japan
Differential Scanning Calorimeter (DSC)	Mettler Toledo (Switzerland)
Infrared spectroscopy (FT-IR)	Perkin Elmer, Beaconsfield, Bucks, UK
High-performance liquid chromatography (HPLC) with UV-VIS.detector	Perkin Elma South Africa (Pty) Ltd. SA.
Franz diffusion cell.	United Scientific (Pty) Ltd. SA.
Rotating Digital viscometer	Brookfield Engineering Laboratory. Inc. Stoughton Massachusetts, USA

4.3. Methods

4.3.1. Physico-mechanical tests

4.3.1.1. Method for measuring the Tensile Strength

The tensile strength (Ns/cm^2) is the property of the film that requires a load to cause load deformation failure of film. This mechanical property was evaluated by using the Texture analyzer instrument. Film strips in dimension of $2cm^2$ and free from air bubbles or physical imperfections were held between two clamps positioned at a distance of 2 cm. apart. During measurement, the strips were pulled by the top clamp at a rate of 100 mm/min; the force and elongation were measured when the film broke. Results from film samples, which broke at and not between the clamps, were not included in the calculations. Measurements were run in triplicate for each film. Tensile strength is also defined as the maximum stress applied to a point at which the film specimen breaks and can be computed from the applied load at rupture as a mean of three measurements and cross-sectional area of the fractured film from the following equation;

Tensile strength (Ns/cm^2) = breaking force (N) / cross sectional area of sample (cm^2). The force and elongation at break point were measured (Garsuch and Breitkreutz, 2009); (Mishra and Amin, 2011); (El-Setouhy and El-Malak, 2010).. The tensile strength was calculated using the following equations;

Tensile strength $\sigma TS = FA \max$ **Equation 4.1**

E is elastic modulus $E\ F\ A = \times 1\varepsilon$ **Equation 4.2**

Percentage Elongation at break $\varepsilon = \times \Delta L L_0\ 100\%$ **Equation 4.3**

where σTS is tensile strength, Fmax is maximum load, A is the initial cross-sectional area of the sample, E is elastic modulus, which is Young's modulus, F is force at the corresponding strain, $\%\varepsilon$ is the percentage Elongation at break, ΔL is increase in length and L_0 is original length in mm. Further parameters such as tear resistance have also been mentioned (Dixit and Puthli, 2009); (Gavaskar et al., 2010). An ideal IDF should have moderately high tensile strength, high elongation at break and strain, but a low elastic modulus (Peh and Wong,

1999). Typical Tensile Strength for oral film is 1.80 ± 0.20 MPa (Tumuluri et al., 2008). 1Mpa = 1Ns/cm^2. (1Mpa = 106 neutons/cm^2 = 1.45 x 105 lb/in^2).

(See Figure 4.3) below shows the Texture analyser that was used to measure the Tensile strength.



Figure 4.3: The Texture Analyzer was used for measuring Tensile Strength.

4.3.1.2. Method for measuring the percentage (%) age elongation

The percent elongation was measured when the film snapped as sufficient force was applied that exceeded the elastic limit. Percentage elongation was calculated by using the following equation;

$$(\%)Elongation = \frac{Increase\ in\ length\ at\ breaking\ (mm)}{original\ length\ (mm)} \times 100$$

(% Elongation) **Equation 4.4**

The percentage Elongation data was observed and collected from the Texture Analyzer Equipment. Formulation 16 had the highest % age Elongation. This is one of the attributes to a good film.

4.3.1.3 Method for measuring Folding Endurance

For measuring the folding endurance, the films were repeatedly folded at the same position (through a 180° angle) until they broke apart (Patel et al., 2009); (Cilurzo et al., 2008). Film flexibility was determined by adapting the Stands for American Society for Testing and Materials (ASTM) bend mandrel test D 4338 - 97. For our films only the folding endurance

was done. The test was done in triplicate on three films of each drug from the 16 formulations and the mean numbers of folds with their standard deviation at break were recorded.

4.3.1.4 Method for measuring Surface pH

The surface pH of the IDFs was determined because any change in pH to either highly acidic or to highly alkaline might cause irritation to the buccal mucosa. As a procedure, the 16 films were each placed on a Petri dish and moistened with 0.5 ml of distilled water for 1 hr. The pH was recorded by inserting the electrode of the pH meter on the surface of the film (Bottenberg et al., 1991).

4.3.1.5 Method for measuring thickness Variation

Oral film thickness determination and weight variation are routine tests. A typical film thickness ranges from 12 to 100 µm (Barnhart and Vondrak, 2008). The thickness of ten films that were selected at random from the centre and the four sides of a single Petri dish of every formulation was measured using a Digital Micrometer screw gauge (Besto, England). The thickness of the left, centre and right sections of the rectangular film were measured and the average was calculated. The idea was to produce a film of even thickness and as thin as possible (Nafee et al., 2003)..

Standard deviation and the coefficient of Variance were also calculated using an excel program. Samples with air bubbles, nicks or tears and having mean thickness variation of greater than 5% were excluded from analysis. (Nafee et al., 2003). Laying the Petri dishes on a perfectly levelled table during casting of the films was critical for this step

4.3.1.6 Method for measuring the Swelling Index

The studies for swelling index of the film were conducted in stimulated salivary fluid. The film sample was weighed and submerged in a glass dish containing 50 ml of stimulated salivary medium at a pH 7.4. The films were immediately removed and blotted with tissue to remove the excess fluid. The increase in the weight of the film was noted and the swelling index of film was calculated (Thimmasetty et al., 2008); (Semalty et al., 2005).

The degree of swelling was calculated using the formula:

Swelling Index $SI = wt. - w_0 / w_0$ **Equation 4.5**

Where SI is the swelling index, wt. is the weight of the film at time "t", w_0 is the weight of film at t = 0.

The percentage swelling was determined by using the following formula;

Percentage Swelling (%) = $(X_t - X o / X o) \times 100$ **Equation 4.6**

Where X_t is the weight of the swollen film after time t, X o is the initial film weight at zero time.

4.3.1.7 Method for measuring Weight Variation

The weight of ten IDFs cut out to a standard size of 2 cm^2 from a single Petri dish of 10 cm diameter and from 3 batches of the optimum formulation using a small surgical blade was determined. The 2 cm^2 strips of the film were weighed using the Metler Toledo analytical balance to test reproducibility (Nafee et al., 2003). Laying the Petri dishes on a perfectly levelled table during casting of the films was critical for this step as it significantly could affect film uniformity. This could be used as an in process test for the films during future manufacturing stages of the rice paper films.

4.3.1.8 Viscosity - Rotating viscometer Method for measuring Viscosity

The International Pharmaceutical Federation (IPF) and the International Conference on Harmonization of Technical procedures (ICH) state that, viscosity test of the thick suspension should be done as an in process test before the casting process as a requirement for Registration of Pharmaceuticals for Human Use (Ali and Quadir, 2007).

The average viscosity in Newton second/m^2 (Ns/m^2) was determined. This was done by measuring 100 ml of the formulated gel suspensions in a 250 ml beaker. The instrument was set at (6. 0.3) rotation. Then the viscosity all the 16 formulation suspensions were measured to establish that of the optimal formulation using the Rotating Digital viscometer (Brookfield Engineering Laboratory, Inc. Stoughton Massachusetts, USA). Viscosity measurements were recorded in triplicate. This could be used as an in process quality control check during the manufacture of the rice paper IDFs.

4.3.1.9 Method for Disintegration

Disintegration as a parameter was measured because it relates to the release characteristics of the of the rice paper drug delivery system. The films first disintegrate before the drugs dissolve and eventually get absorbed. The modified tablet disintegration apparatus was used for this test (See figure 4.4).

The basket where the films were suspended was immersed in a beaker of simulated saliva pH 7.4. This was heated to 37^0 C with water circulating in a warm water bath. The basket moving in an upward and downward motion was set at 400 revolutions per hour. The passing time when the film first showed signs of deterioration or break was displayed on a digital meter and the experiments were done in triplicate.

Disintegrating is defined as the time (in seconds) at which a film breaks when brought into the contact with water or saliva and disintegration time is the time when a film starts to break or disintegrate (Dahiya et al., 2009) (Vishwkarma et al., 2011). Thickness and mass or viscosities of the suspension play a key role in determining the dissolvable films physical properties. Disintegration test was done by using a modified Disintegration apparatus. A beaker containing the simulated saliva where the basket moved was inserted inside the water jacket and the films were placed vertically in the tubes with prestrike to suspend them as shown in (See Figure 4.4) below.



Figure 4.4: The Modified Disintegration Apparatus that was used for Testing Disintegration Time.

The results of calibration for Resveratrol were used to determine the Lowest Limit of Quantification. The calibration was calculated using only the least recorded absorbance to get the lowest limit of quantification (LOQ) and the concentration ranged from 10 µg/ml – 100 µg/ml. The Regression Coefficient was good at 0.9964.The lower limit of quantification (LOQ) in simulated plasma for Resveratrol in this study was determined to be (n) = 7.95 µg/ml.

4.3.1.10 Calibration curve

The results of calibration for Curcumin were also used to determine the Lowest Limit of Quantification. The calibration was calculated using only the least recorded absorbance to get the lowest limit of quantification (LOQ) and the concentration ranged from 10 µg/ml – 100 µg/ml. The Regression Coefficient was still good at 0.9916. The lower limit of quantification (LOQ) in simulated plasma for Resveratrol in this study was determined to be (n) = 7.377 µg/ml.

In vitro dissolution studies of the rice paper films loaded with the model drugs was done using simulated saliva at pH 7.4 also for Resveratrol films , Solubility: Soluble in water (less than 0.1mg/ml) for uniformity purposes, and in simulated saliva at pH 7.4 for Curcumin due to its low solubility: Soluble in water (3 mg/100mL) (Langmuir, 1916); (Modasiya and Patel, 2012) The experiments were done in triplicate in the same saliva buffer at pH of 7.4 for both Resveratrol and Curcumin. The normal pH of saliva is a range of 6.5- 7.5 up to 1 minute. See figure 4.5. The Human digestive tract pH range chart is shown below; (Gohel et al., 2009); (Jantratid and Dressman, 2009); (Marques et al., 2011).

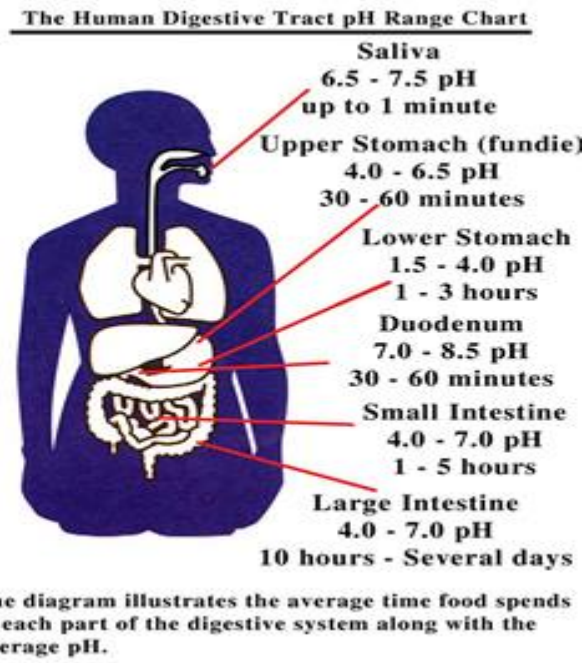


Figure 4.5: The normal pH range of the gastrointestinal tract.
(Gohel et al., 2009) (Jantratid and Dressman, 2009) (Marques et al., 2011).

“The pH in the human digestive tract varies greatly. The pH of saliva is usually between 6.5 - 7.5. After we chew and swallow food it then enters the fundic or upper portion of the stomach, which has a pH between 4.0 - 6.5. This is where "predigestion" occurs while the lower portion of the stomach is secreting hydrochloric acid (HCL) and pepsin until it reaches a pH between 1.5 - 4.0. After the food mixes with these juices it then enters the duodenum (small intestine) where the pH changes to 7.0 - 8.5 (Gohel et al., 2009) (Jantratid and Dressman, 2009) (Marques et al., 2011).

This is where 90% of nutrients absorption takes place in the GIT, while the waste products are passed out through the colon (pH 4.0 - 7.0).” Most probably, the films will have to be taken 1 hour before or 1 hour after food for the best absorption at the fasting pH of 7.4, slightly above neutral. (Gohel et al., 2009) (Jantratid and Dressman, 2009) (Marques et al., 2011).

This could be verified later in the clinical trials in the advanced stages of product development. Drug absorbance was measured spectrophotometrically at 315 nm and 420 nm for resveratrol and Curcumin respectively.

The increased starch concentration in formulation 16 would have enhanced hydration and this could have led to the higher rate and extent of swelling of the larger proportion of the hydrophilic polymer, hence enhanced release of Resveratrol and Curcumin from the films. In addition formulation 16 had the highest moisture content which might have lead to easier disintegration and subsequently high drug release. Though formulation 16 did not have the lowest disintegration time, the factor of robustness when put into consideration, made it optimal. Its disintegration time was 19 seconds for Resveratrol and 15 seconds for Curcumin in simulated saliva pH 7.4 at 37^0C (See Table 4.6). This is still within the published disintegration range of 5 - 30 seconds (Setia et al., 2011).

In vitro Dissolution studies were conducted on the films loaded with the model drugs using the USP 32 apparatus dissolution test (Erweka DT 700, Heusenstamm, Germany). This was made by immersing the samples under a round wire mesh to prevent them from floating and suspended under the 900 ml of simulated saliva p H 7.4 in a beaker for both Resveratrol and Curcumin and maintained at 37°C by slow heating circulating water in the equipment and set at a rotation speed of 100 rpm.

Samples of 5ml were removed at predetermined time intervals of time, i.e., 0 hrs, 2hrs, 4hrs, 6hrs, 8hrs, 10hrs, and 12 hrs. The samples were filtered through a 0.22µm Millipore Millex filter paper. Equal volumes of fresh simulated saliva pH 7.4 were replaced in order to maintain sink conditions.
Samples were then analyzed with the Avian UV-Vis spectrophotometer at wavelength of 315nm and 420nm for Resveratrol and Curcumin respectively. All experiments were conducted in triplicate. The release data was subjected to a model-independent analysis known as the time-point approach. The application of the MDT12 approach proved to be a more precise analysis for the release performance of both Resveratrol and Curcumin over a period of 12 hours and was employed in this regard (Hopfenberg and Hsu, 1978).

4.3.1.11 Method for *in vitro* Dissolution

The calibration curve for the Avian Spectrophotometer was drawn according to the following calculations;
The solubility of Curcumin in water is 3mg/100ml i.e. 0.03mg/ml.
The solubility of Resveratrol in water is 0.01mg/ml.
100mg each of Curcumin and Resveratrol from were accurately weighed on a Metler Toledo analytical balance and dispersed in 5ml of methanol in a 100 ml volumetric flask. Simulated saliva at pH 7.4 was added to the make to make 100 ml of the solution (A) of concentration

1mg/ml. 1 ml was drawn for Resveratrol Standards and 10ml of curcumin and each diluted to 100 ml to make solution (B) of 0.01mg/ml. 0,5 ml, 1ml, 1,5 ml, 2ml, 2,5 ml, and 3ml of Stock solution (B).

Each was further diluted with a 95; 5 Simulated saliva pH 7.4: Methanol to 10 ml to yield 0.005mg/ml. 0.01mg/ml, 0.015mg/ml, 0.020mg/ml, 0.025mg/ml, and 0.03mg/ml standard Solution respectively. This was done in triplicate from 3 batches and the Average absorbance was measured on the Avian Spectrophotometer with Cary 50 software. Mean and Standard Deviation were calculated and standard error bars were included on the calibration curve of Concentration versus Absorbance that was drawn to show reproducibility of results.
The absorbance of the sample drawn at 0hrs, 2hrs, 4hrs, 6hrs, 8hrs, 10hrs, and 12hrs respectively were measured which was plotted against time to get the y value that was substituted in the linear equation of the calibration curve to calculate the respective concentrations. The log of the concentrations was plotted against time to calculate the flux (Gradient) which is the dissolution rate. The Cummulative curve was also drawn to ascertain the dissolution rate.

4.3.1.12 Drug -vehicle excipie*n*ts interaction studies

Examining the possible incompatibilities that could occur between the Active Pharmaceutical Ingredient (API) and the formulation excipients has an important role in the formulation particularly during the development stage of solid dosage forms. Therefore, Fourier Transformer Infra-Red Spectrum (FTIR), Differential scanning calorimeter (DSC), and chromatography have been used to assess possible drug excipient interaction in this study. DSC allows the fast evaluation of possible incompatibilities or interaction. Changes in appearance shift of melting endotherms and exotherms, and variation in the corresponding enthalpies of the reactions would reveal vehicle – drug interactions. Spectroscopy on FTIR was performed on the rice paper films in their native form as well when they were loaded with the model drugs and showed no interactions.

4.3.1.13. Method for Differential Scanning Calorimetry studies (DSC)

Samples were analyzed using a Mettler Toledo DSC spectrometer with an MIRTGS detector.
The spectrum of 16 sample scans against 16 background (blank) scans with a sample weight of each sample below 10 mg. was done. Each sample was weighed individually and the weight entered in the programme. The background was scanned to eliminate noise on the spectrum. The samples were analyzed at a temperature range from 25^0C – 300^0C and the

heating rate was 10^0C / min. The exotherms were recorded and examined for similarity to rule out any interactions.

4.3.1.14. Method for Fourier Transforms Infrared (FTIR).

Samples were analyzed using a Spectrum 2000 FTIR spectrometer with an MIRTGS detector (Perkin Elmer Spectrum 100, Beaconsfield, UK). The spectrums of 16 samples were scanned with a resolution of 4 cm-1. The samples were analyzed at wave numbers ranging from 600 - 4000 cm-1.

4.3.1.15 Method for Drug content uniformity

Content uniformity was determined by dissolving one Rice paper film of 2cm x1 cm area containing 20 mg of Resveratrol or Curcumin in 10 ml of phosphate buffer solution (pH 7.4). The contents were stirred with the help of magnetic stirrer to dissolve the film. The contents of solution were filtered through a 0.22µm filter and transferred to the (10 ml) polytopes pending analysis. The absorbance of the solutions was measured at 315 nm and 420 nm for resveratrol and Curcumin respectively using Perkin Elmer HPLC with a UV/Vis detector. The experiments were carried out in triplicate for each formulation and average value was calculated (Balamurugan et al., 2001). The area under the curve in all formulation was uniform and it was found to be in the range of 3.125 ± 0.008.

4.4 Results and discussion

4.4.1. Physical and Mechanical evaluation

(See Table 4.4) and (See Figure 4.6) give the viscosity of the 16 suspension formulations. As an in process parameter, the most optimal formulation had a suspension with a viscosity of **49.3 N/m^2.** Therefore a suspension of about **50N/m^2** would yield a good film.

Table 4.2: Viscosity measurements of Resveratrol films (Average viscosity in Newton second /m^2 (Ns/m^2).

Form No.	Batch 1	Batch 2	Batch 3	Average viscosity	Standard deviation	CV %
1	3.0	2.0	1.5	2.2	0.76	35.25
2	4.0	3.0	2	3.0	1.00	33.33
3	11.5	13.0	7	10.5	3.12	29.74
4	4.0	3.0	2	3.0	1.00	33.33
5	4.0	3.0	2	3.0	1.00	33.33
6	11.0	7.0	23.5	13.8	8.61	62.22
7	27.5	68.0	38	44.5	21.02	47.23
8	100.0	100.0	54	84.7	26.56	31.37
9	100.0	99.0	25	74.7	43.02	57.61
10	3.0	2.5	3	2.8	0.29	10.19
11	4.0	3.0	2	3.0	1.00	33.33
12	76.0	100.0	15	63.7	43.82	68.83
13	9.0	5.5	21.5	12.0	8.41	70.09
14	55.0	82.0	80	72.3	15.04	20.80
15	8.0	3.5	7	6.2	2.36	38.32
16	24.0	85.0	39	**49.3**	31.79	64.43

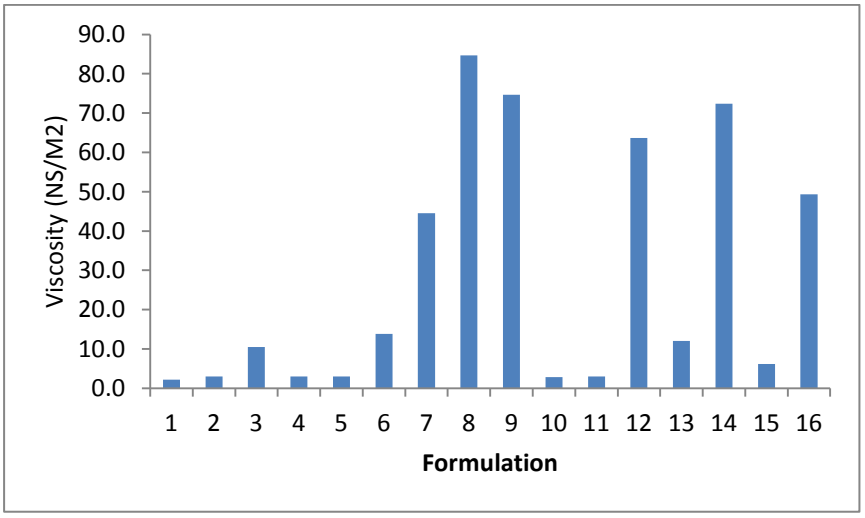


Figure 4.6: Viscosity measurement of Resveratrol films.

Table 4.6 below gives the summary of the physical mechanical test results from the rice paper films loaded with Curcumin and Resveratrol model drugs. The figures represent the median and the range between which the measured parameter lied. There are currently no Pharmacopoeial standards for this type of dosage form. However, from literature, few test specifications were published. Vondrac and colleagues published that a typical oral film thickness ranges from 12 to 100 µm (Barnhart and Vondrak, 2008). Venkata. S. Tumuluri gave the range for the oral film thickness to be 130 ± 3 µm (Tumuluri et al., 2004). All films were in the normal range of acceptable thickness of 0.20mm to 0.30mm ± 0.01mm. They all fall in the range of 0.23 mm ± 0.03 mm to 0.26mm ± 0.01mm. The Type of plasticizer was not significant as all p-values were above 0.05. Our rice paper films fell out of range (0.16 – 0.36 mm). This solely depended on the polymer used to prepare the films.

Venkata and Vondrac used other polymers, In the International Journal of Biomedical sciences, 2014 October; 5 (4): P 54-67, Venkata. M. Anupama and colleagues gave the following specifications for fast dissolving oral films.

The disintegration time together with the mean plus or minus standard deviation was recorded from *in vitro* studies of rice paper films that were loaded with the model drugs, Curcumin and Resveratrol. Figure shows the results of disintegration of the films as observed from the *in vitro* experiment. Disintegration Time 5 - 30 sec (Setia et al., 2011). Disintegration test was first done in simulated saliva pH 6.8, which was the average pH of the oral cavity, but later the optimal formulation 16 was tested in simulated saliva pH 7.4 as Curcumin could just dissolve in simulated saliva pH 7.4. This suggested that the films for optimal absorption should be taken at fasting oral pH of 7.4 one hour before or after meals. The disintegration time for formulation 16 was 19 seconds for Resveratrol and 15 seconds for Curcumin in simulated saliva pH 7.4 at 37^0C (See Tables 4.13**).**

Tensile Strength (Ns/cm2) (1.8 ± 0.20 MPa), 1 MPa equals 1 Ns/cm^2, Elongation (%) (32.24 ± 63.3%) (Barnhart and Vondrak, 2008); (Tumuluri et al., 2004). For the other tests the specifications were in- house as found during the experiments. Comparing the mechanical test results from literature and our results, the Tensile Strength of the optimal formula 16 was 0.180833 for the plain film, 0,133300 for Resveratrol and 0.138422 for Curcumin. Though this laid below the published limits of 1.8 ± 0.20 Mpa (Tumuluri et al., 2008) the films were robust enough to be handled confortartblly during manufacture.

Table 4.3: Disintegration for the Plain films in simulated saliva pH 6.8.

	B/N0.1(1)	2	3	B/N0. 2(1)	2	3	B/N0.3(1)	2	3	AVG	STDEV	CV %
	5	5	5	4	2	3	6	10	5	5	2.24	44.72
2	3	4	3	4	5	3	15	15	15	7.44	5.70	76.61
3	5	5	5	5	6	4	5	5	5	5	0.5	10
4	5	5	4	5	5	5	16	20	11	8.44	5.88	69.58
5	5	5	6	5	5	4	6	5	4	5	0.71	14.14
6	5	5	5	10	9	7	4	5	5	6.11	2.09	34.17
7	10	9	11	20	21	18	7	8	9	12.56	5.50	43.83
8	18	16	18	18	18	17	15	17	19	17.33	1.22	7.07
9	28	30	29	27	30	28	24	25	20	26.78	3.27	12.21
10	9	10	12	17	16	13	15	10	11	12.56	2.88	22.92
11	12	11	14	10	11	8	13	8	10	10.78	2.05	19.00
12	8	9	9	8	6	9	8	8	5	7.78	1.39	17.93
13	28	21	27	26	29	26	20	23	26	25.11	3.10	12.35
14	22	20	19	22	26	20	21	26	21	21.89	2.52	11.52
15	5	6	6	8	8	7	6	7	8	6.78	1.09	16.12
16	25	27	24	27	28	28	22	26	23	25.56	2.19	8.55
										Mode	5	
										Median	9.61	
										Mean	12.76	
										Min	5	
										Max	26.78	
										Range	5 - 26.77	
										SD	7.97	

Table 4.4: Disintegration for the Resveratrol films in simulated saliva pH 6.8

B/N0.1(1)		!2	3	B/N0 2(1)		3	B/N0.3(1)	2	3	AVG	STDEV	CV (%)
1	4	2	3	9	10	12	25	30	27	13.56	10.92	80.57
2	4	5	3	7	7	8	4	4	4	5.11	1.76	34.51
3	5	6	4	30	25	26	20	20	17	17	9.79	57.56
4	5	5	5	21	20	25	24	25	19	16.56	8.92	53.87
5	5	5	4	5	5	4	10	15	9	6.89	3.72	54.04
6	10	9	7	12	14	11	29	30	30	16.89	9.78	57.90
7	20	21	18	15	15	13	15	14	11	15.78	3.27	20.73
8	18	18	17	13	12	12	20	17	21	16.44	3.36	20.42
9	27	30	28	29	27	30	14	15	12	23.56	7.54	31.99
10	17	16	13	11	12	10	17	20	21	15.22	3.93	25.81
11	10	11	8	11	8	9	9	9	8	9.22	1.20	13.03
12	8	6	9	9	10	10	12	13	9	9.56	2.07	21.64
13	26	29	26	13	15	16	12	14	15	18.44	6.58	35.67
14	22	26	20	14	16	15	22	20	16	19.00	4.00	21.05
15	8	8	7	5	5	5	9	5	5	6.33	1.66	26.18
16	27	28	28	27	26	25	27	25	27	26.67	1.12	4.19

Mode	#N/A
Medi	16.11
Mean	14.76
Min	5.11
Max	26.67
Rang	5.11 - 66
SD	6.07

Table 4.5: Disintegration for the Curcumin films in simulated saliva pH 6.8.

B/N0.1 (1)	2	3	B/N0.2 (1)	2	3	B/N0.3(1)	2	3	**AVG**	STDEV	CV (%)
1	5	5	5	2	2	2	5	5	5	4	1.50	37.50
2	5	5	5	14	15	15	4	4	4	7.89	5.10	64.77
3	3	3	3	23	20	25	5	5	5	10.22	9.46	92.52
4	5	4	9	11	8	10	19	15	20	11.22	5.70	50.76
5	7	9	8	3	5	5	8	5	11	6.78	2.49	36.72
6	7	6	5	21	28	27	25	30	29	19.78	10.66	53.91
7	24	29	30	16	15	14	15	19	17	19.89	6.21	31.24
8	25	26	22	26	29	28	27	30	26	26.56	2.35	8.85
9	26	30	30	29	30	26	11	10	9	22.33	9.39	42.06
10	12	16	18	14	14	15	8	9	9	12.78	3.49	27.33
11	16	11	10	12	10	9	9	10	8	10.56	2.35	22.27
12	7	6	8	8	10	7	11	9	8	8.22	1.56	19.02
13	18	19	17	15	11	13	16	15	15	15.44	2.46	15.90
14	10	14	12	12	13	15	16	18	17	14.11	2.62	18.56
15	7	8	5	5	13	10	7	7	5	7.44	2.65	35.61
16	27	26	30	25	30	25	25	26	25	26.56	2.07	7.79
									Mode	26.56		
									Median	12.00		
									Mean	13.99		
									Min	4.00		
									Max	26.56		
									Range	4 - 26	55.00	
									SD	7.10		

Table 4.6: Disintegration for the optimal formulation 16 of Resveratrol and Curcumin films in simulated saliva pH 7.4

	1	2	3	Average Disintegration	STDEV
Resveratrol Disintegration Time in sec	19	17	20	18.67	1.53
Curcumin Disintegration Time in sec	15	15	14	14.67	0.58

The Disintegration test was repeated for the optimal formulation 16 and the results show that Resveratrol films disintegrated in only 19 seconds and curcumin films in only 15 seconds (See Table 4.6).

It is very important to note that, since the maximum drug there can be in the film is the drug load, which is 20mg, the graph can be drawn in terms mg of the drug that is released per time in seconds using the rate. In this way, it can be easily seen that all the 20 mg were released in about 47.6 seconds for Resveratrol and in about 71.4 seconds for Curcumin. However, in a practical sense, the film must first be hydrated in a small amount of saliva entrapped between the tongue and the upper palate and the rice starch will swell, disintegrate/dissolve because of the crashing effect of the tongue, then release the drug. The swelling index study revealed the average swelling index was to be 91.7 % for the plain films, 79.4 % for Resveratrol films and 73% for the Curcumin loaded films. This can explain the difference in the time lag that may occur in the practical sense.

The experiment was done in a large volume of saliva (900 ml) and for a long time of 12 hrs due to practicality reasons. However, the rate of drug release is independent of the volume at Steady state saturation point or maximum drug release and can be used the calculate the flux in mg/ second (Ritschel, 1989).

Swelling index was higher in film 16, which contained higher amount of Rice starch. This was because of higher swelling capacity of the rice starch. The average swelling index was found to be 91.7 % for the plain films, 79.4 % for Resveratrol films and 73% for the Curcumin loaded films.

For weight variation, ten films loaded with the drug from each batch of the three batches of formulation 16 were cut out with a surgical blade into a size of 2 cm x 1 cm and weighed individually on a Metler Toledo electronic balance to determine uniformity of weight. Their average weight was calculated. Their weight was found to be in the range of 64.4 mg. ±1.51 Standard deviation and CV % 2.34 for Resveratrol and 72.2 mg. ± 1.56 Standard deviation and CV % 2.16 for Curcumin.

Table 4.7: Resveratrol weight variation

B/No.16	1	2	3	AVG Wt. (mg)	STDEV	CV (%)
Film No. 1	64.1	62.5	67.3	64.63	2.44	3.78
2	62.2	62.7	66.3	63.73	2.24	3.51
3	63.5	64.7	65.1	64.43	0.83	1.29
4	64.2	66.1	65.9	65.40	1.044	1.60
5	64.4	65	64.3	64.57	0.38	0.59
6	65	63.7	67.1	65.27	1.72	2.63
7	62.5	63	65.8	63.77	1.78	2.79
8	63.4	62.6	67	64.33	2.34	3.64
9	66.2	64.1	66	65.43	1.159	1.77
10	62.3	62.4	64.3	63	1.13	1.79

Mode	#N/A	Avg CV%	2.34
Median	64.5		
Mean	64.46		
Min	63		
Max	65.43		
Range	63.00-65.43		
SD	0.79		

Table 4.8: Curcumin weight variation

B/No 16	1	2	3	AVG Wt. (mg)	STDEV	CV (%)
Film No.1	70.5	72	72.7	71.73	1.12	1.57
2	70.7	72.2	74.6	72.5	1.97	2.71
3	70.1	73	75.1	72.73	2.51	3.45
4	70.8	74.1	73.8	72.9	1.82	2.50
5	70.7	72.3	72.4	71.8	0.95	1.33
6	70.9	72.1	72.5	71.83	0.83	1.16
7	70.1	73.3	72.8	72.07	1.72	2.39
8	70.2	73.5	72.7	72.13	1.72	2.39
9	70.3	72.5	72.4	71.73	1.24	1.73
10	70.8	74.2	72	72.33	1.72	2.38

Mode	71.73	Avg CV%	2.16
Median	72.1		
Mean	72.18		
Min	71.73		
Max	72.9		
Range	71.73-72.90		
SD	0.43		

Any change in pH of administered dosage of the film could irritate the buccal mucosa. However, the surface pH of all films was found to be close to neutral in all the formulations. These formulations have less potential to irritate the buccal mucosa. The surface pH was found to be in the range of 5.00 ± 0.1 to 6.90 ± 0.02.

(See Figure 4.7) below is the background to the interpretation of the IR spectra. The functional groups are allocated specific wave numbers for their spectral identity on the spectra.

4.0 Infrared spectrophotometry

4.1 Introduction

When a molecule absorbs Infrared radiation, energy is covered into molecular vibrations. When the radiant energy matches the energy of a specific molecular vibration, absorption occurs. These bonds can vibrate with stretch motions or bend motions.

1. Stretching vibrations involve bond length change, i.e., the distance between atoms increase or decreases but the bond angle remains unchanged.
2. Bending vibrations involve a change in bond angle, i.e., atoms change positions while maintaining a constant distance between atoms. Bending vibrations are also called '*deformations*'.

Stretching vibrations		Bending vibrations
Symmetric Stretch	Asymmetric Stretch	
A ⟵ B ⟶ C	A ⟵ B ⟵ C	B, A ⟶, C ⟵

Most organic functional group absorptions occur in the mid IR range, between 4000 and 400 cm^{-1}. We will focus on this region in the course. A typical IR spectrum is given in Figure 1. The percent transmittance is given on the y-axis. The x-axis is linear in wavenumber ($\bar{\upsilon}$), and has units of reciprocal centimetres (cm^{-1}). The bands in the spectrum can be assigned to specific movement of bond (s) that makes up functional groups. The position of each band depends on:

a) Type of vibration (stretching occurs higher wavenumber relative to bending)

b) Strength of bond (stronger the bond, higher the wavenumber)

c) reduced mass of atoms in bond (heavier the atoms lower the wavenumber)

Regions for reading functional groups are from 1-3 on the spectrum and section 4 is the fingerprint region.

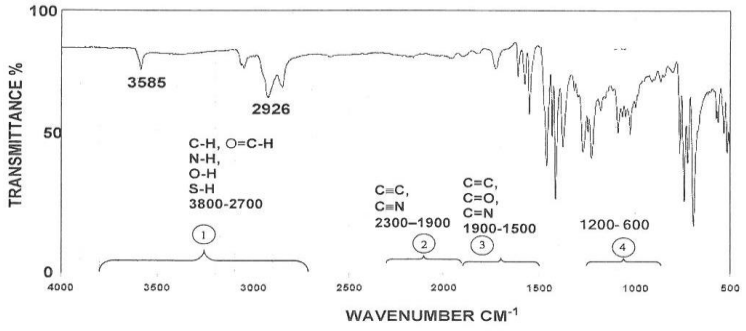
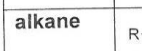
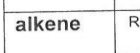
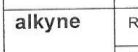
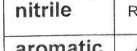
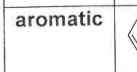
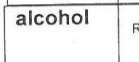
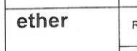
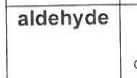
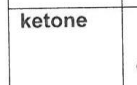


Figure 1 A typical IR spectrum

Table 1 gives the IR absorption frequencies that are characteristic of various functional groups.

Group		Bond & Molecular Motion	Wavenumber (cm^{-1})
alkane		C-H stretch	2850-2960
	R-HC(CH_3)$_2$	CH_3 bend, isopropyl-	doublet, 1370 and 1385
	R-C(CH_3)$_3$	CH_3 bend, tert-butyl	unsymmetrical doublet: 1370 (*s*) and 1395 (m)
alkene		=CH stretch	3000-3100
		C=C stretch (isolated)	1640-1680
		C=C stretch (conjugated)	1610-1640
alkyne		≡C-H stretch (acetylenic)	~3300 (sharp)
		C≡C triple bond stretch	~2150 (*v*,)
nitrile		C≡N	~2250
aromatic		C-H stretch	3000-3100
		C=C stretch	~1600 & ~1475 (*v*)
alcohol	 	O-H stretch	~3650 (monomeric, *v*) or 3600-3200 (H bonded alcohol, broad)
	H_3C—O—H	C-O stretch	1080-1300
		phenol	~1230
ether		C-O stretch (dialkyl)	1060-1150 (s, broad)
		C-O stretch (aryl)	~1250 & ~1120
aldehyde		C-H aldehyde stretch	~2850 & ~2750
		C=O stretch (alkyl)	~1725
		C=O stretch (aryl)	~1700
ketone		C=O stretch (alkyl)	~1710
		C-C stretch	1300-1100
		C=O stretch (aryl)	~1690
		C=O stretch (conjugated)	~1675

[a]**All bands are strong unless marked *m*, moderate; *v*, variable, s strong**
υ = stretch, δ = bend

Figure 4.7: Background to the FTIR interpretation.

Source: Wits B. Pharm. (III) Pharmaceutical Analytical Chemistry Course notes on IR. Spectrophotometry 2016 work book.

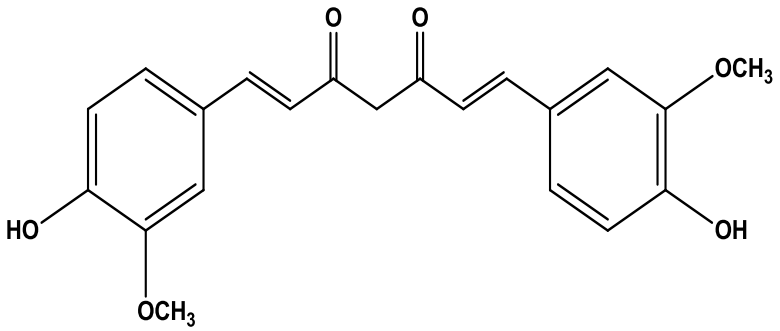
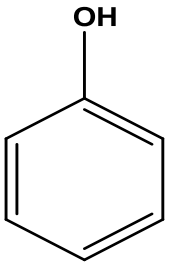


Figure 4.8: Structure of Curcumin showing identity functional groups with a molecular ($C_{21}H_{20}O_6$).
Source:www.google.co.za/?gfe_rd=cr&ei=h_WV_ucAo2p8weM7oyoBQ&gws_rd=ssl#q=structural+images+of+curcumin (Accessed on 23/ 07/16).

The structure of curcumin contains R- CHO=CHO-R stretch (conjugated double bonds) di-ketone which should appear between 1680 – 1750 (cm^{-1}) Wave number. Then 2 (stretch Aryl) bonds that



phenol

Figure 4.9: Phenol

Vibrate at around 1675 - 1690 (cm^{-1}) Wave number. Then 2 Phenol groups which should vibrate at about 3200 – 3550 (cm^{-1}) Wave number. Then the Aromatic bend of Benzene

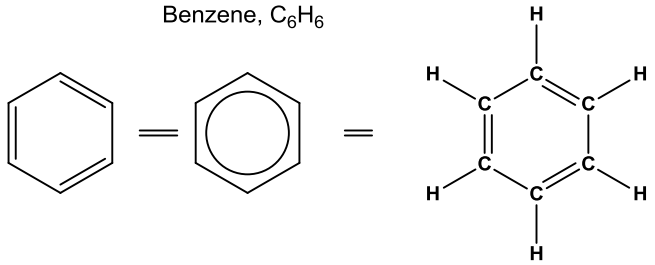


Figure 4.10: Benzene

which vibrates at around 1500 - 1600 (cm-^{1}) Wave number. All these bonds are identified on our Curcumin film FTIR spectra with almost similar wave numbers in the control (plain film) and only a slight shift in the melting point due to the homogeneous admixture in the film. The finger print region is also clearly seen between 660 - 1550 (cm-^{1}) Wave numbers in the sample and the pure drug.

This indicates no interaction between Curcumin and the plain film ingredients.

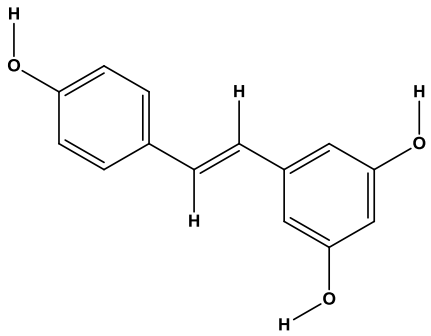


Figure 4.11: Structure of Resveratrol showing identity functional groups with a molecular ($C_{14}H_2O_3$).

www.pubchem.ncbi.nlm.nih.gov/compound/resveratrol#section=2D-Structure

(Accessed on 12 /08/16)

The structure of Resveratrol contains 2phenol group stretch bonds that vibrate between 3200 – 3550 (cm-^{1}) Wave numbers, it also contains aromatic bend double bonds at about 1500 – 1700 (cm-^{1}) Wave numbers, and alkenyl C=C stretch double bonds at about 1620 – 1680 (cm-^{1}) Wave numbers.

The fingerprint region is specifically identical of the individual molecule the IR spectra (See Figure 4.12) figures 4.12 – 4.16. This clearly appeared in the same region of the pure molecule as well as that in the drug formulation, i.e. Resveratrol and Curcumin films. This is clearly indicative of no interaction between the drugs and the vehicle.

4.4.2. The FTIR Spectra of the rice paper

(See Figure 4.12) Figures 4.12 – 4.16 below show exotherms of the Fourier Transforms Infra-Red Spectrum (FTIR) studies of the rice paper films.

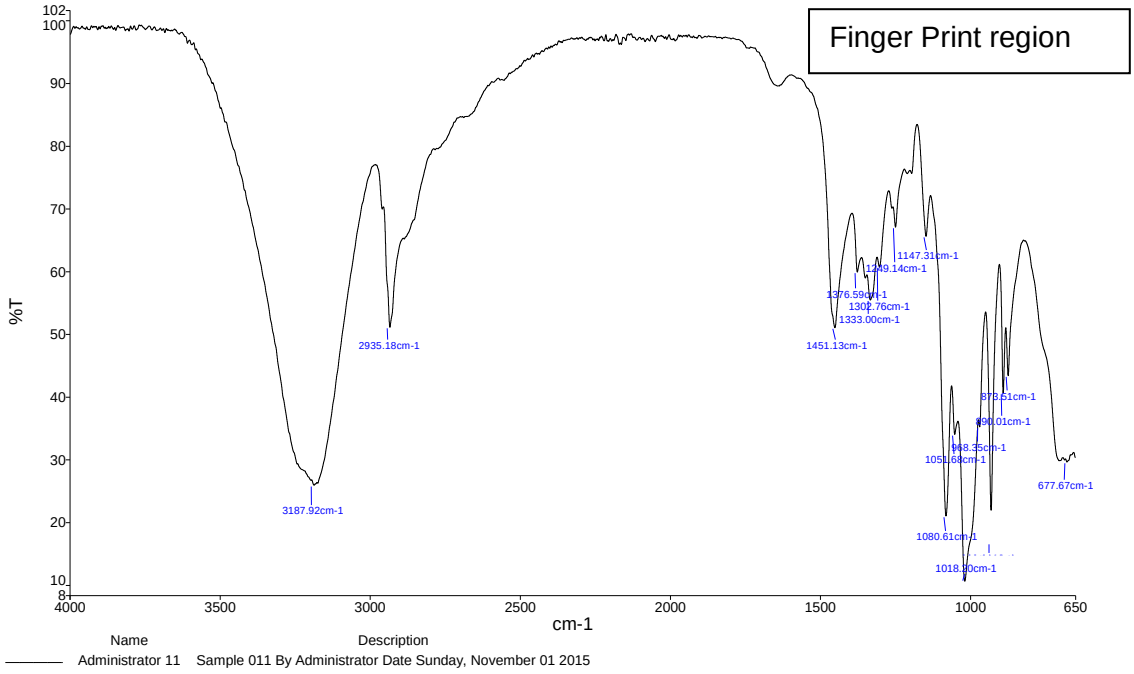


Figure 4.12: The IR Spectra for the Plain rice paper film

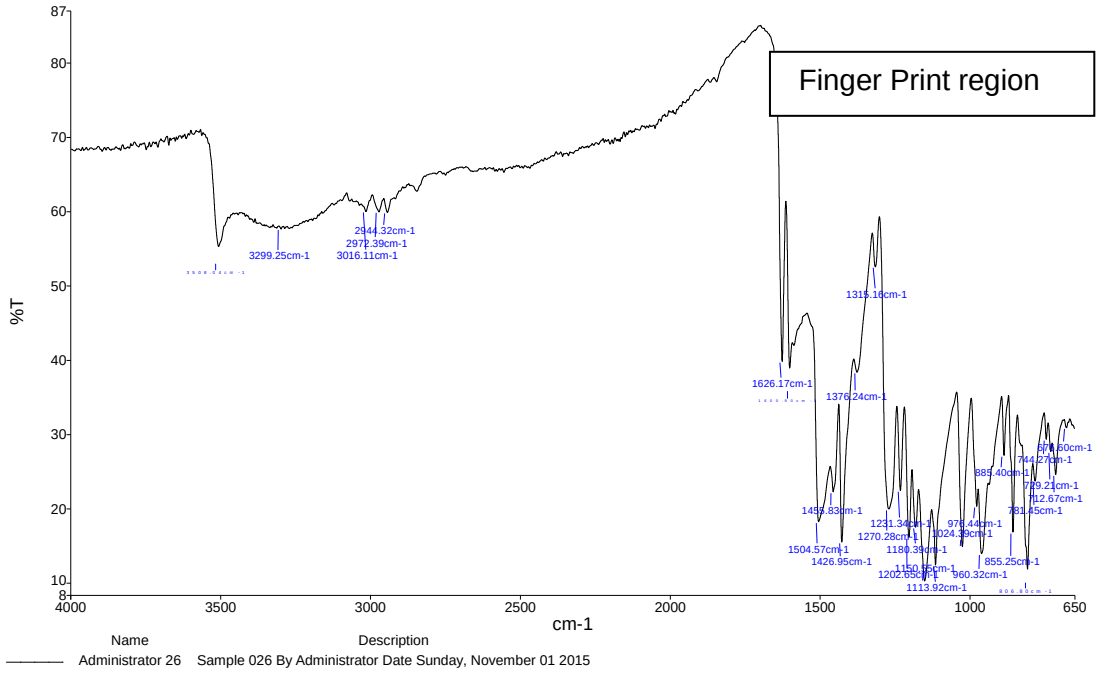


Figure 4.13: The IR Spectra for Pure Curcumin

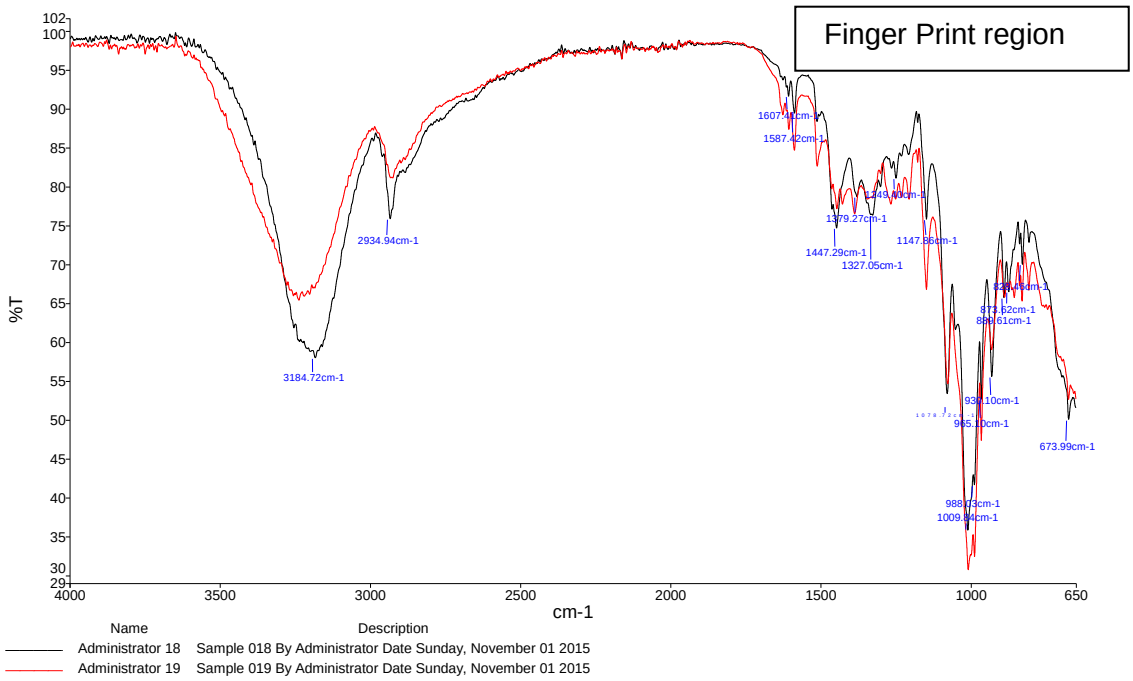


Figure 4.14: IR Spectra for Curcumin rice paper film super imposed with the plain film

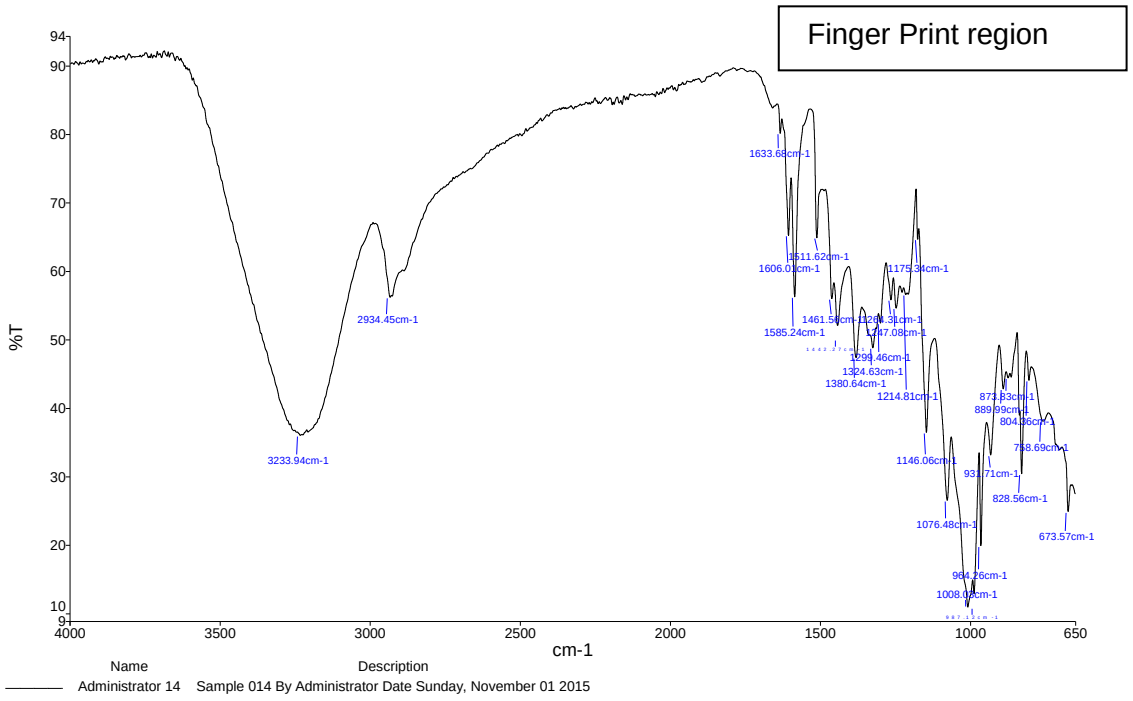


Figure 4.15: The IR Spectra for Pure Resveratrol

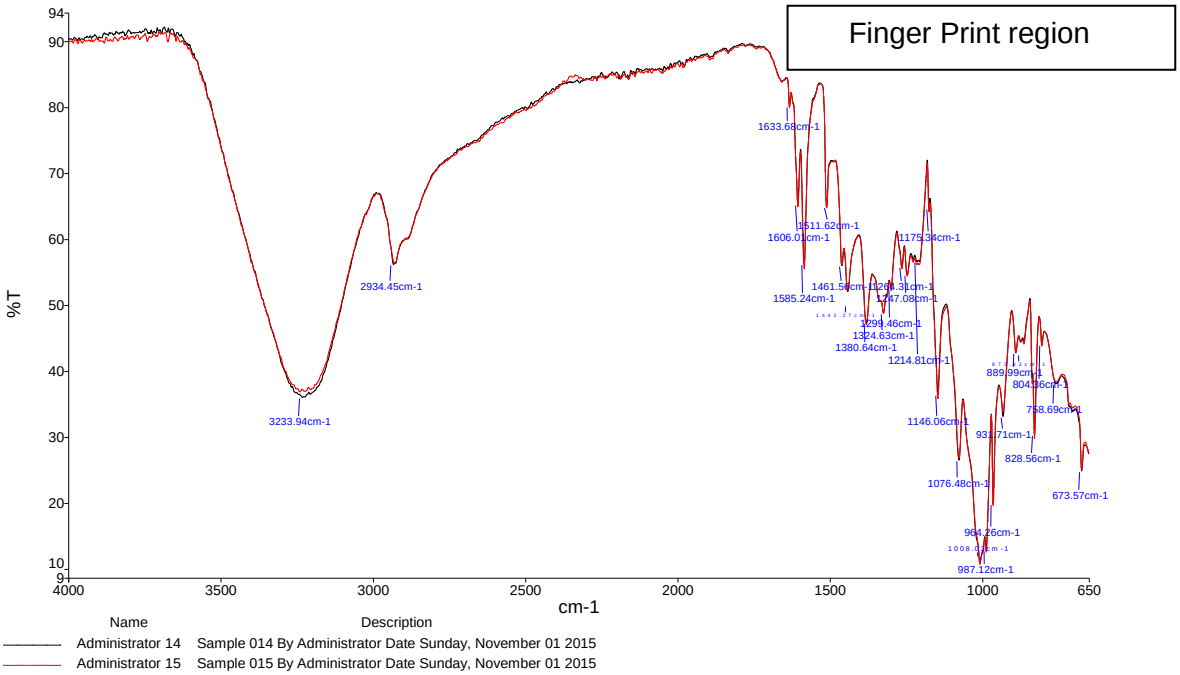


Figure 4.16: The IR Spectra for Resveratrol rice paper film super imposed with the plain film

All these bonds are identified on our Curcumin film FTIR spectra with almost similar wave numbers in the control (plain film) and only a slight shift in the melting point due to the homogeneous admixture in the film. The finger print region is again clearly seen between 660 - 1550 (cm^{-1}) Wave numbers in the sample and the pure drug.

This indicates no interaction between Resveratrol and the plain film ingredients

4.4.3 The DSC Thermograms for Rice paper

(See Figure 4.17) below show the DSC Thermograms for the rice paper films

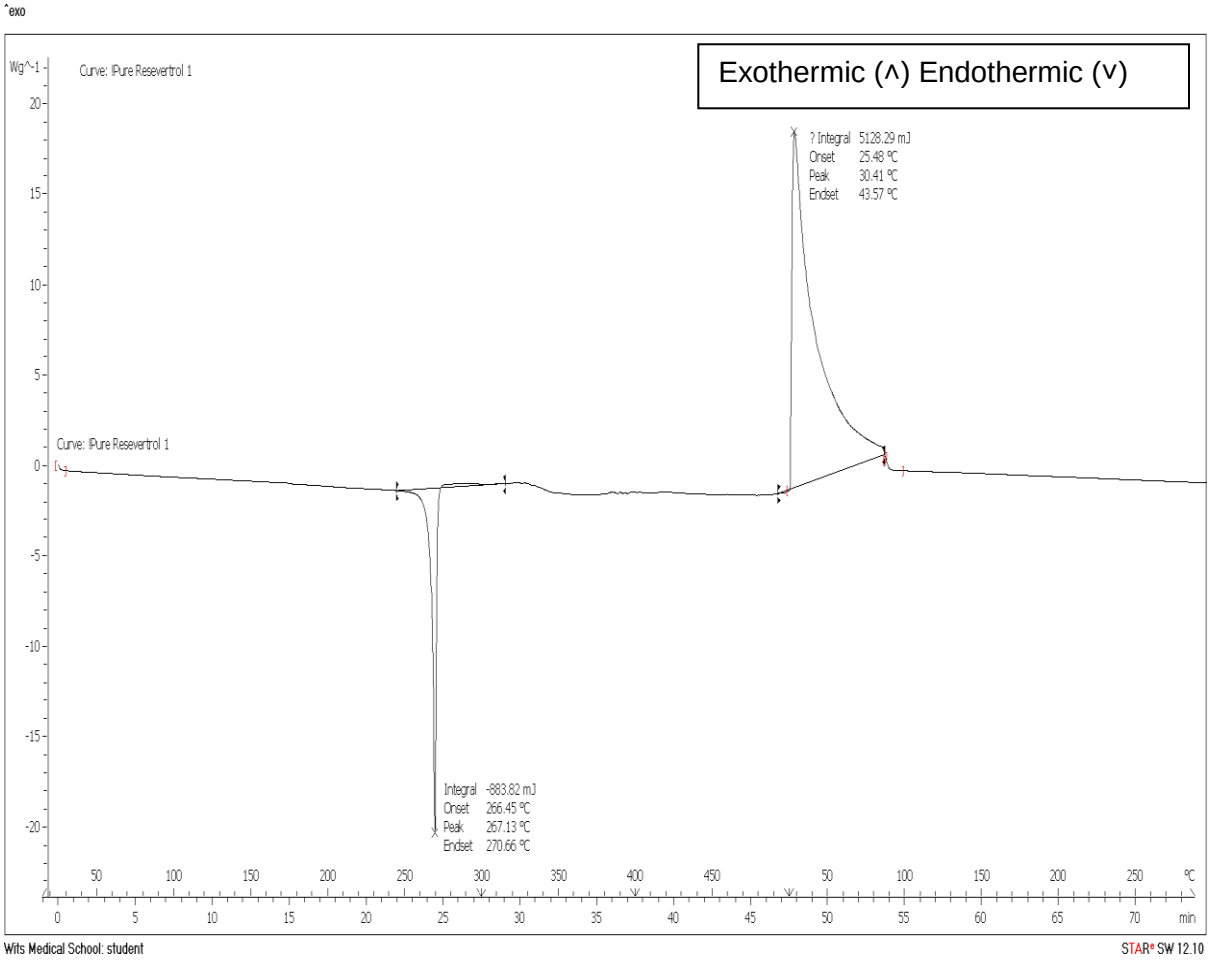


Figure 4.17: The DSC Thermogram for Resveratrol pure drug.

- A downward Thermogram represents an endothermic reaction and an upward Thermogram represents an exothermic reaction in Thermo analysis.
- Glass Transition of Resveratrol occurred between 0^0C – 100^0C.
- The melting point of Resveratrol was at 257.13^0C.
- The reaction was endothermic (Melting Endotherm).

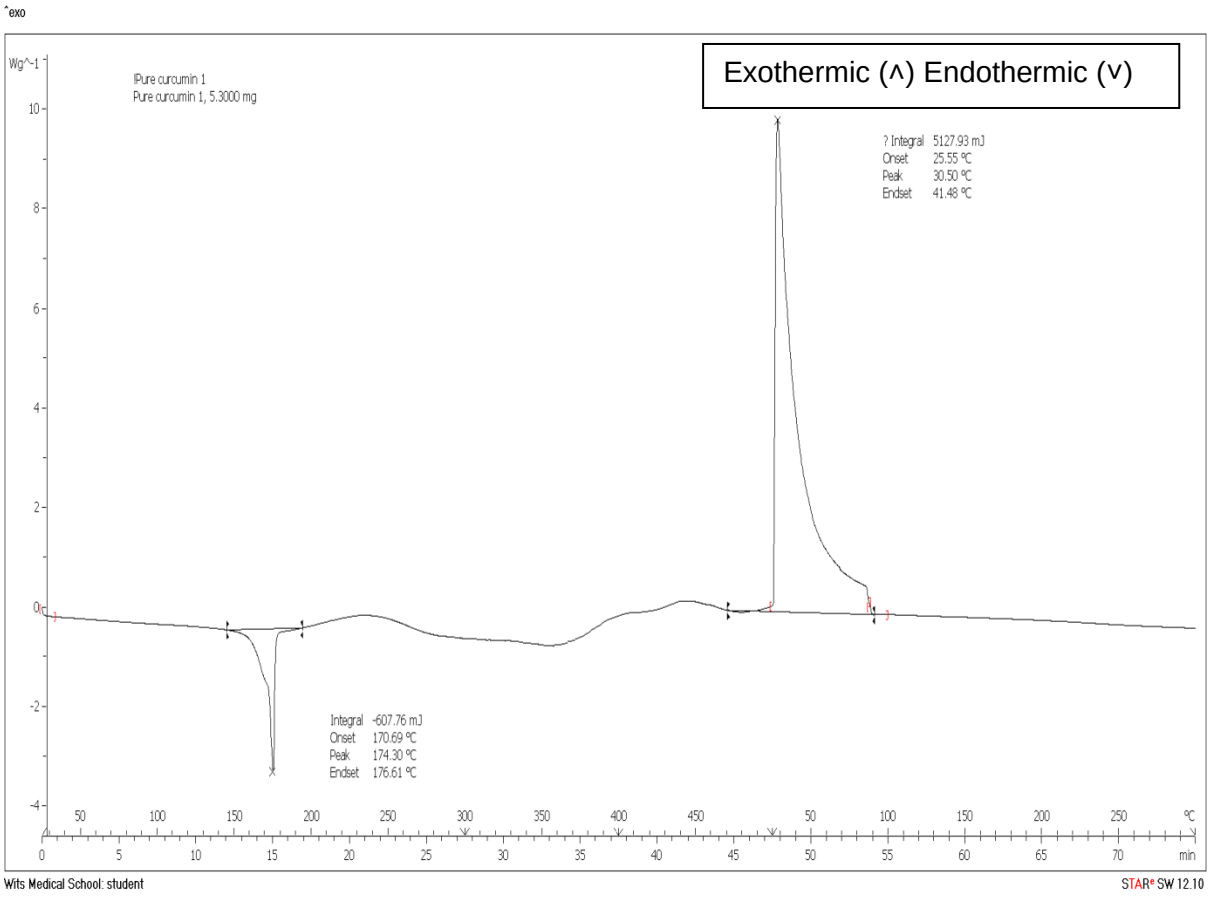


Figure 4.18: The DSC Thermogram for Curcumin pure drug

- A downward Thermogram represents an endothermic reaction and an upward Thermogram represents an exothermic reaction in Thermo analysis.
- Glass Transition of Curcumin occurred between 0^0C – 100^0C.
- The melting point of Resveratrol was at 174.30^0C.
- The reaction was endothermic (Melting Endotherm).

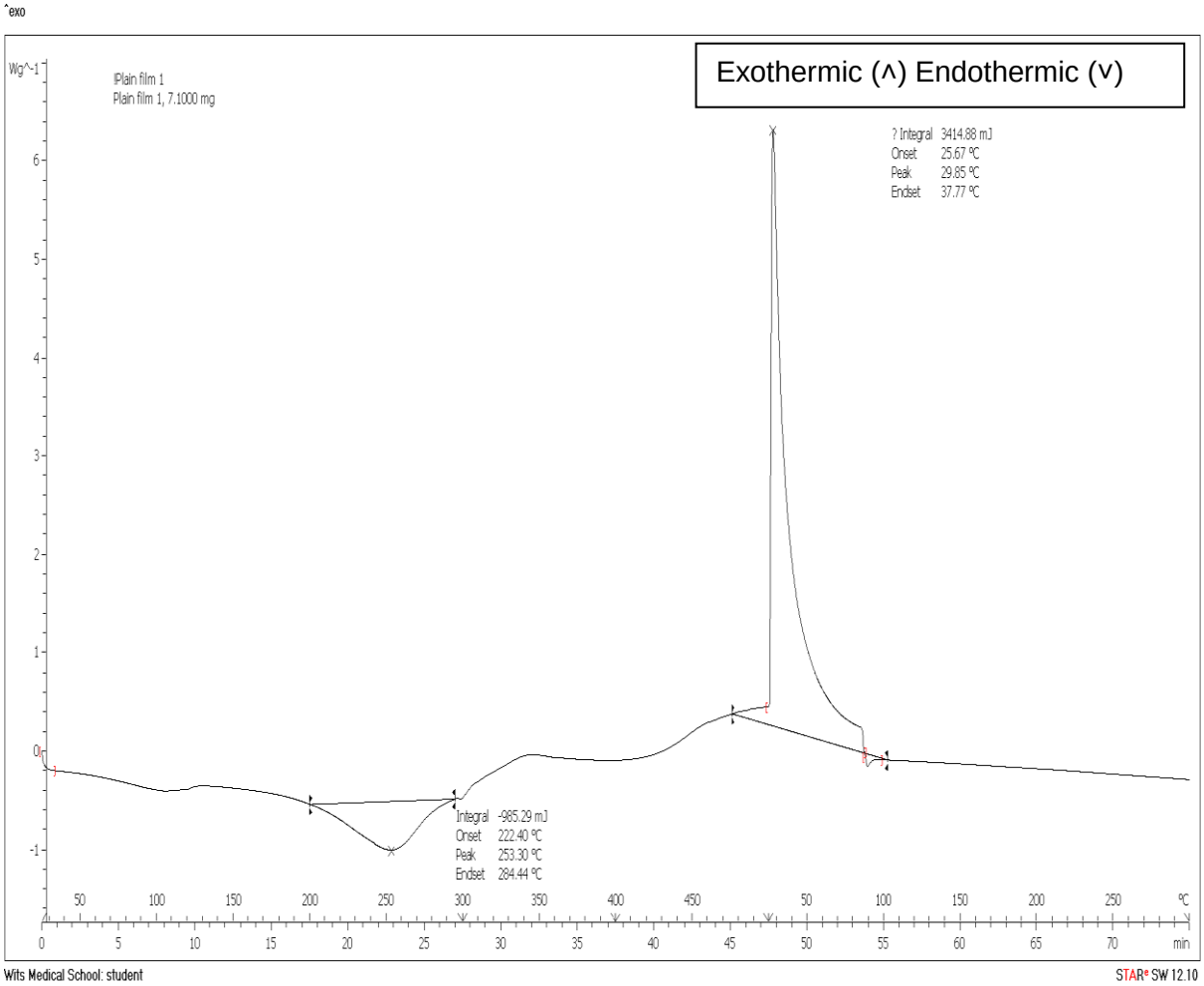


Figure 4.19: The DSC Thermogram for the rice paper plain film.

- A downward Thermogram represents an endothermic reaction and an upward Thermogram represents an exothermic reaction in Thermo analysis.
- Glass Transition of Plain film occurred between 0^0C – 100^0C.
- The melting point of Resveratrol was at 253.30^0C.
- The reaction was endothermic (Melting Endotherm).

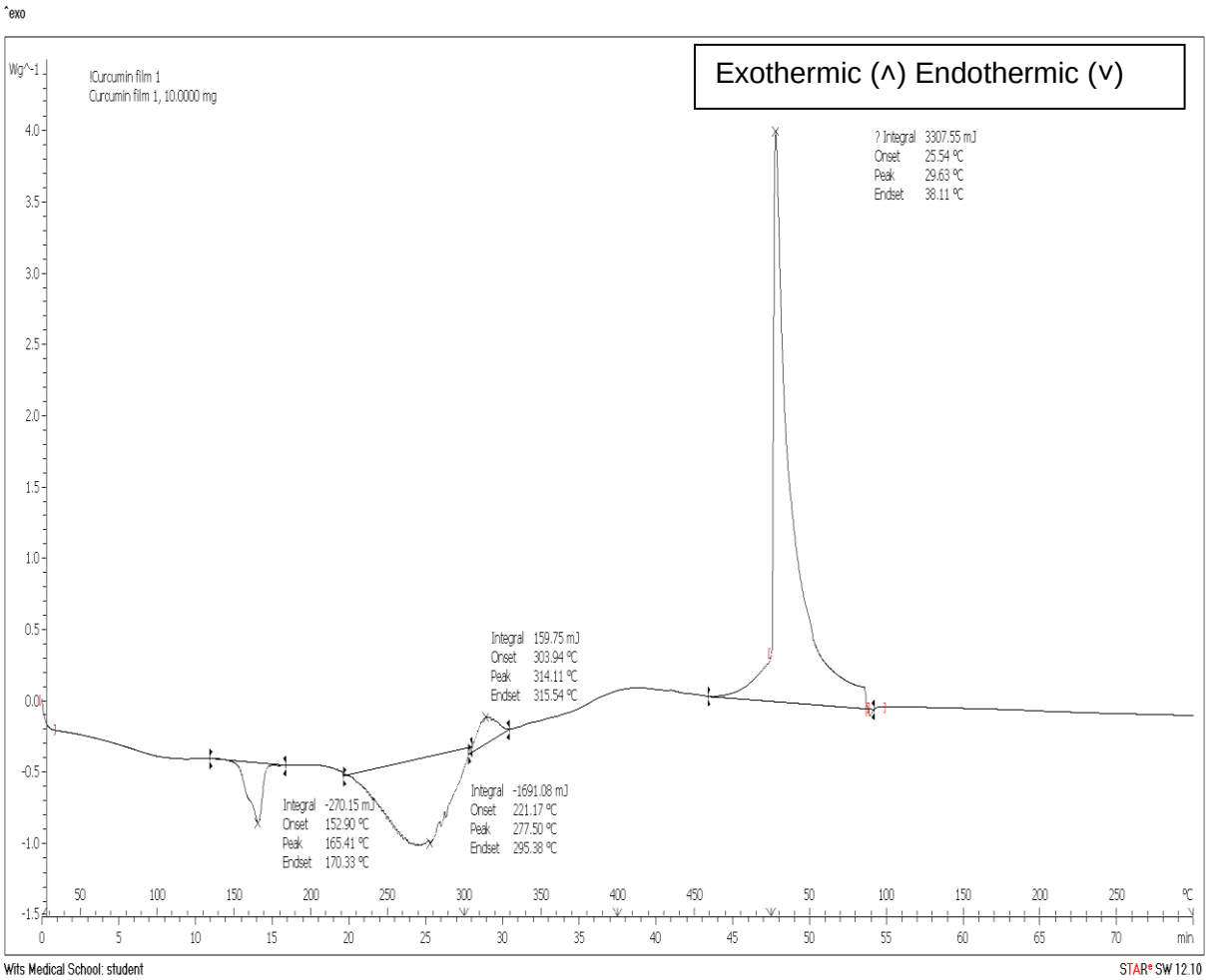


Figure 4.20: The DSC Thermogram for the Curcumin film

- A downward Thermogram represents an endothermic reaction and an upward Thermogram represents an exothermic reaction in Thermo analysis.
- Glass Transition of Resveratrol occurred between 0^0C – 100^0C.
- The peak is at 29.53^0C. (Exothermic).
- The melting point of Resveratrol was at 155.41^0C.
- The reaction was endothermic (Melting Endotherm).
- Decomposition occurred between 220^0C – 300^0C with a peak at 277.50^0C (Endothermic)
- Change of phase occurred at Onset temperature of 303.94^0C – End set of 315.54^0C.with a peak at 314.11^0C.

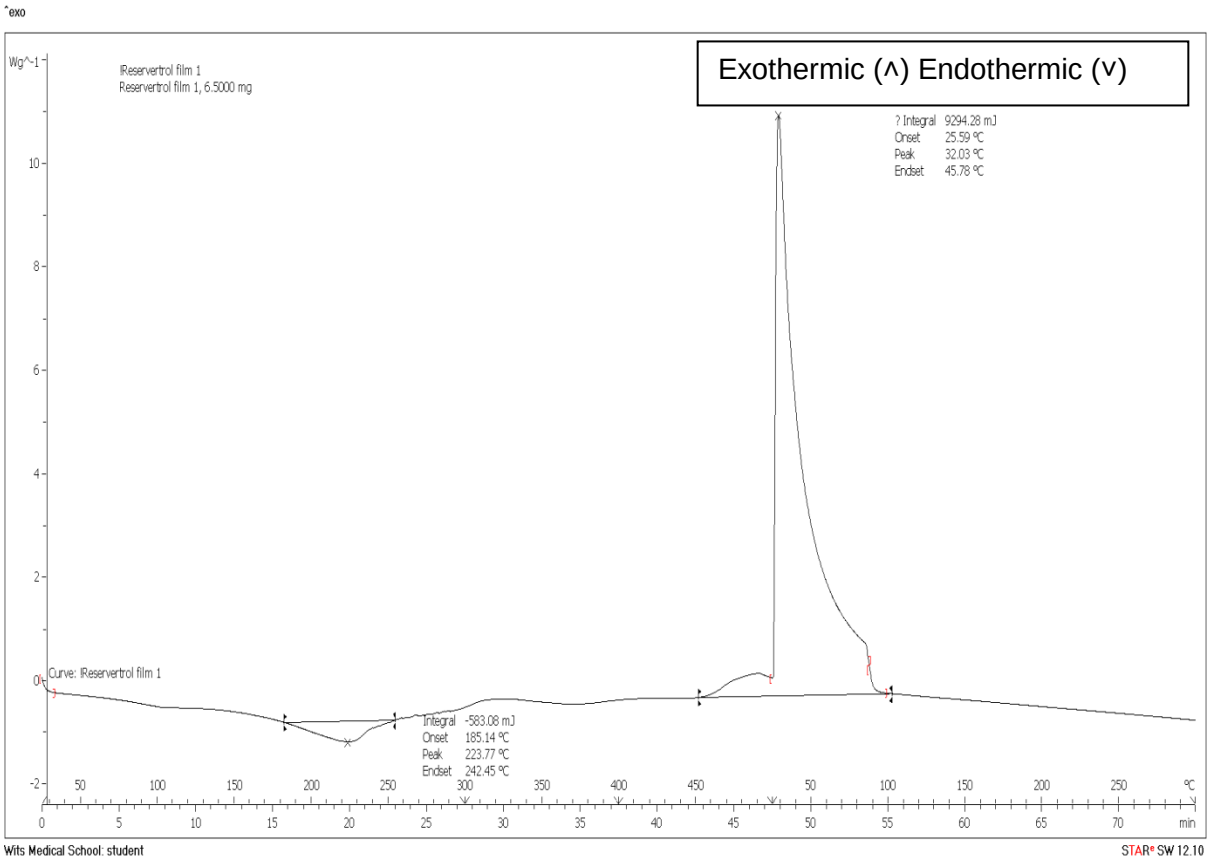


Figure 4.21: The DSC Thermogram for Resveratrol film.

- A downward Thermogram represents an endothermic reaction and an upward Thermogram represents an exothermic reaction in Thermo analysis.
- Glass Transition of Resveratrol occurred between 0^0C – 100^0C.
- The melting point of Resveratrol was at 223.77^0C. A slight lowering in the melting point because of the even distribution in the film.
- The reaction was endothermic (Melting Endotherm).
- The Endotherm is not as sharp as the pure drug because it is evenly distributed in the film.

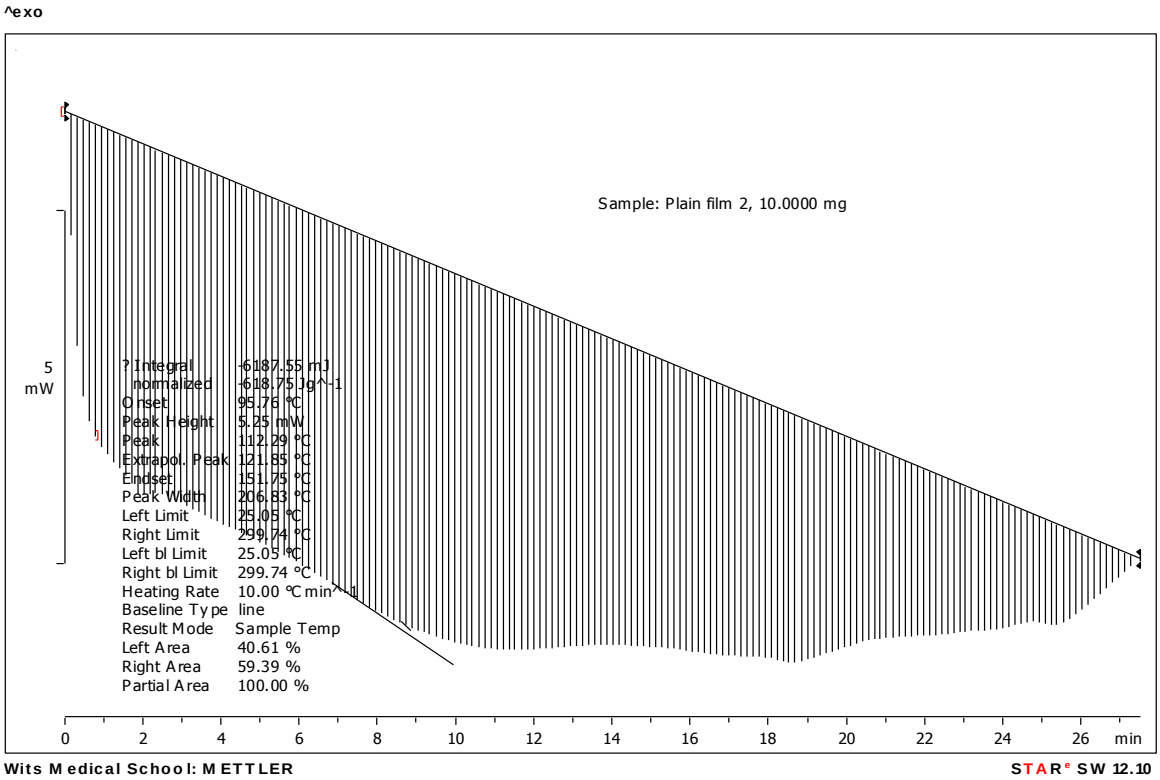


Figure 4.22: The DSC Thermogram for the plain film

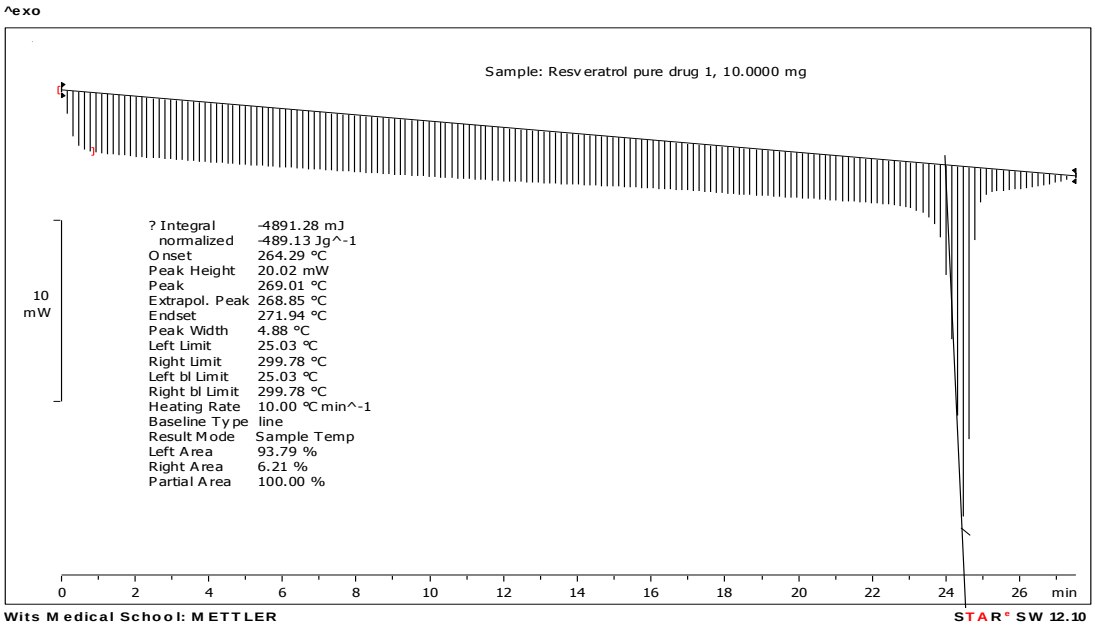
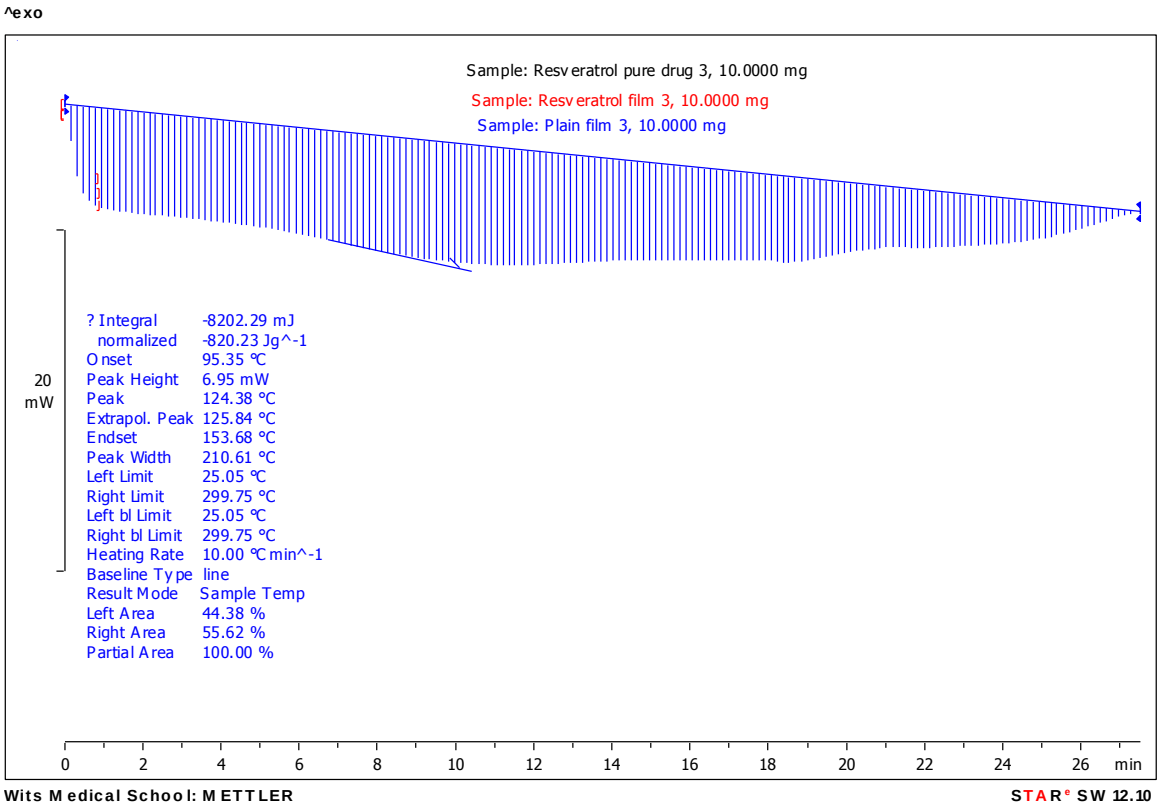


Figure 4.23: The DSC Thermogram for pure Resveratrol



:

Figure 4.24: The super imposed DSC Thermogram for Resveratrol film with the plain film and the pure drug

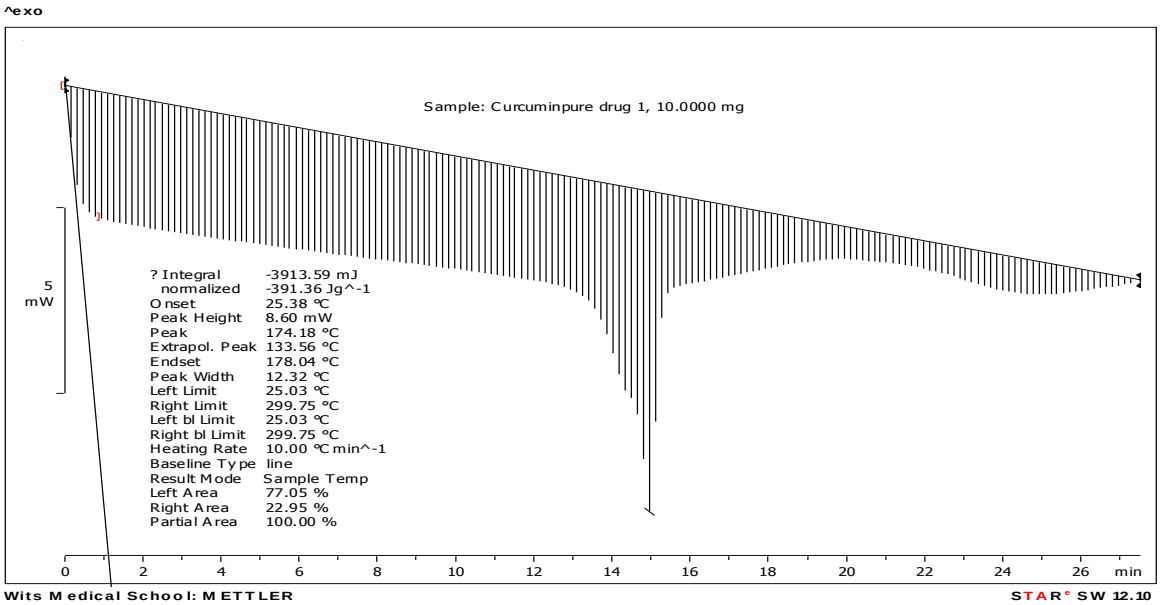


Figure 4.25: The DSC Thermogram for Curcumin pure drug

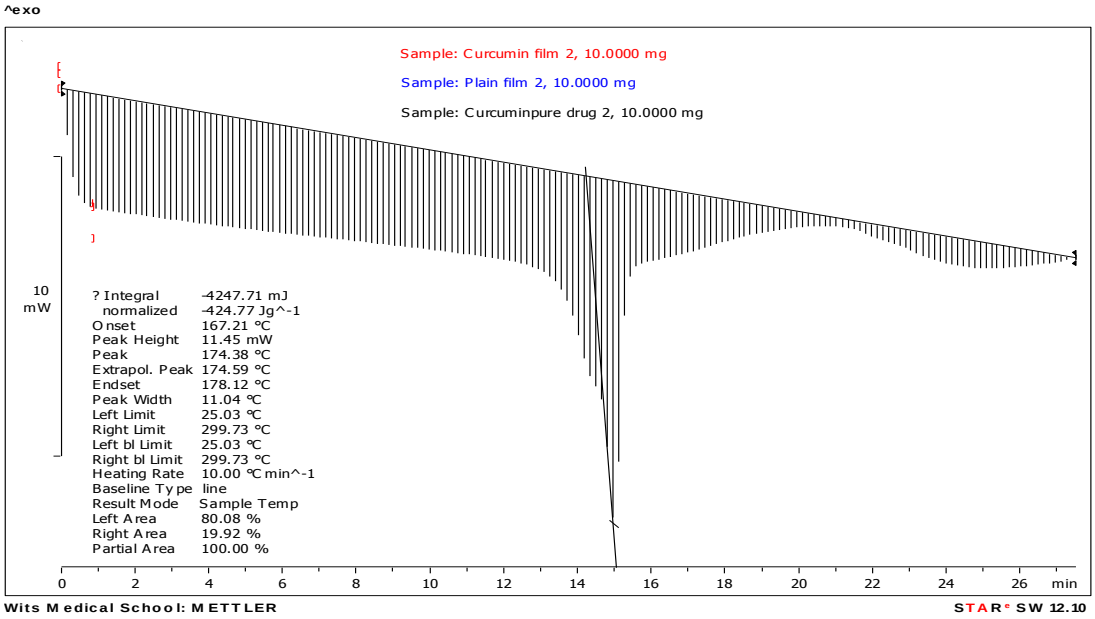


Figure 4.26: The super imposed DSC Thermogram for the Curcumin film with the plain and pure drug**.**

All these melting points are identified on our Resveratrol and Curcumin film DSC endotherms with almost similar melting points in the control (plain film) and only a slight shift in the melting point due to the homogeneous admixture in the film. This indicates no interaction between Resveratrol and the plain film ingredients.

4.4.4 Calculation of Resveratrol Drug Loading

The Karter Scientific Plastic Petri Dish of 10 cm Diameter

Internal Diameter with a Radius of 5 cm was used.

Area of a circle = πr^2

Area (A) = (22/7)*5*5 = 78.57 cm^2

Approximately 80 cm^2 will yield 20, 2cm^2 films

0.5 g of either Resveratrol or Curcumin are triturated on a white tile into 5ml of the thick suspension of rice starch suspension and spread onto the Petri dish.

One film is 1 cm x 2 cm in dimension

We expect 20 films from the Petri dish

i.e. 0.5g or 500mg are in 20 films

One film would contain 500mg/20 films*1 film = 25mg

Drug entrapment = Amount of drug in film /initial Amount*100 %

i.e. Drug entrapment = 20/25*100 = **80%**

Amount of the drug in the formulation = drug load = 80% of 25mg = **20mg.**

Table 4.9: Calibration Curve for Resveratrol Using the Avian Spectrophotometer for Franz Diffusion Cell in Formulated Simulated Saliva Saline Buffer pH 7.4 at 315 nm.

B/No. 1	Conc. (mg/ml)	Abs Rdg 1	Abs Rdg 2	Abs Rdg 3	Min	Max	AVG Abs at 420 nm	STDEV	CV%
0	0	0	0	0	0	0	0	0	#DIV/0!
1	0.0005	0.066	0.066	0.067	0.066	0.067	0.066	0.00015	0.23
2	0.0010	0.13	0.12	0.12	0.13	0.13	0.13	0.00046	0.37
3	0.0015	0.19	0.20	0.19	0.19	0.20	0.19	0.00055	0.28
4	0.0020	0.27	0.27	0.27	0.27	0.27	0.27	0.00036	0.13
5	0.0025	0.33	0.33	0.33	0.33	0.33	0.33	0.00020	0.061
6	0.0030	0.40	0.40	0.40	0.40	0.40	0.40	5.77E05	0.014

Table 4.10: Calibration Curve for Resveratrol Using the Avian Spectrophotometer for Franz Diffusion Cell in Formulated Simulated Saliva Saline Buffer pH 7.4 at 315 nm.

B/No.2	Conc. (mg/ml)	Abs Rdg 1	Abs Rdg 2	Abs Rdg 3	Min	Max	AVG Abs at 420 nm	STDEV	CV%
0	0	0	0	0	0	0	0	0	#DIV/0!
1	0.00050	0.04	0.040	0.040	0.040	0.040	0.040	0.00012	0.29
2	0.0010	0.11	0.11	0.11	0.11	0.11	0.11	1E-04	0.088
3	0.0015	0.16	0.16	0.16	0.16	0.16	0.16	0.00015	0.09
4	0.0020	0.23	0.23	0.23	0.23	0.23	0.23	0.00015	0.07
5	0.0025	0.28	0.28	0.29	0.28	0.28	0.28	0.00023	0.08
6	0.0030	0.36	0.36	0.36	0.36	0.36	0.36	0.00045	0.12

Table 4.11: Calibration Curve for Resveratrol Using the Avian Spectrophotometer for Franz Diffusion Cell in Formulated Simulated Saliva Saline Buffer pH 7.4 at 315 nm.

B/No. 3	Conc. (mg/ml)	Abs Rdg 1	Abs Rdg 2	Abs Rdg 3	Min	Max	AVG Abs at 420 nm	STDEV	CV%
0	0	0	0	0	0	0	0	0	#DIV/0!
1	0.0005	0.049	0.048	0.047	0.047	0.049	0.048	0.00078	1.63
2	0.0010	0.10	0.10	0.10	0.10	0.10	0.10	0.00040	0.394
3	0.0015	0.17	0.17	0.17	0.17	0.17	0.17	0.00015	0.091
4	0.0020	0.26	0.26	0.26	0.26	0.26	0.26	0.00017	0.066
5	0.0025	0.30	0.30	0.30	0.30	0.30	0.30	0.00078	0.26
6	0.0030	0.37	0.37	0.37	0.37	0.3662	0.37	0.00046	0.014

Table 4.12: Calibration Curve for Curcumin Using the Avian Spectrophotometer for Franz Diffusion Cell in Formulated Simulated Saliva Saline Buffer pH 7.4 at 420 nm.

Y AVG Abs at 420 nm	X Conc. (mg/ml)	Y Pred	y pred +delta y confidence max	ypred - delta y confidence min	
0	0	-0.00793	-9.28963E-05	-0.015770747	
0.0515	0.5	0.055431	0.063269854	0.047592003	
0.1142	1	0.118794	0.126632604	0.110954753	
0.1745	1.5	0.182156	0.189995354	0.174317503	
0.2555	2	0.245519	0.253358104	0.237680253	
0.3023	2.5	0.308882	0.316720854	0.301043003	
0.3771	3	0.372245	0.380083604	0.364405753	
Regression					
slope (m)	0.126726	-0.00793	b	tval	2.570582
sem	0.003049	0.005498	seb	delta m	0.007839
R2	0.997113	0.008068	sey	delta b	0.014132
	1726.942	5	degree freedom	delta y	0.007839
	0.112415	0.000325			
	#N/A	#N/A			
	#N/A	#N/A			

4.4.5 Resveratrol and Curcumin Calibration and Dissolution using the Avian Cary 50 Spectrophotometer

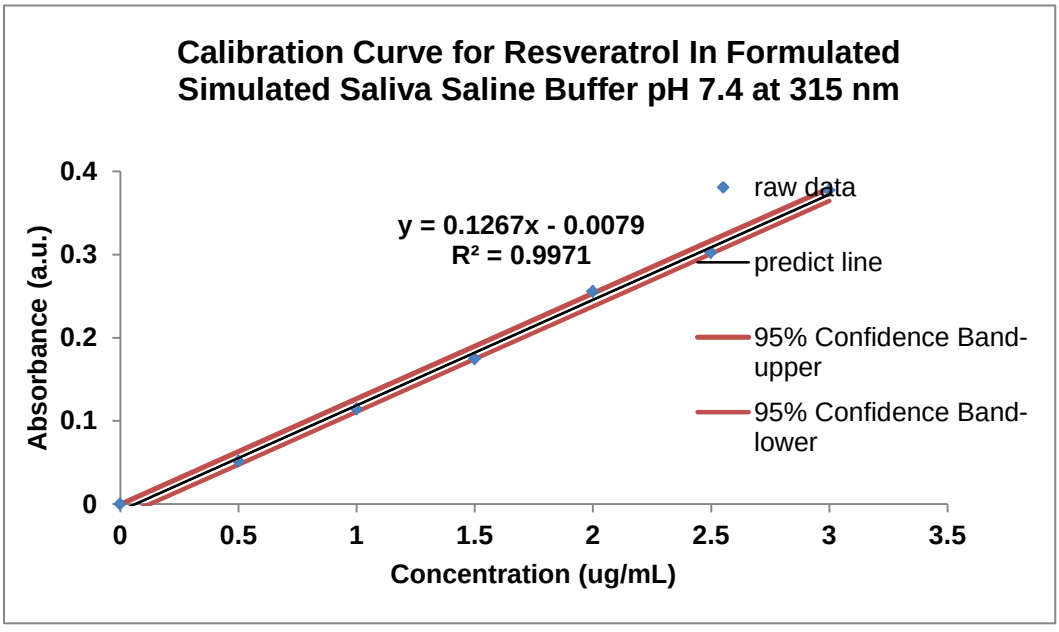


Figure 4.27: Resveratrol calibration curve.

Table 4.13: Dissolution of Resveratrol in Formulated Simulated Saliva Saline Buffer pH 7.4 at 315 nm

B/No. 1 Time (Hrs)	Abs Rdg 1	Abs Rdg 2	Abs Rdg 3	Min	Max	AVG Abs at 420 nm	STDEV	CV%
0	0	0	0	0	0	0	0	#DIV/0!
2	0.34	0.03	0.34	0.03	0.34	0.24	0.17	74.24
4	0.61	0.61	0.61	0.61	0.61	0.61	0.00034	0.06
6	0.83	0.83	0.83	0.83	0.83	0.83	0.00012	0.014
8	0.98	0.97	0.98	0.97	0.98	0.98	0.00025	0.026
10	1.08	1.08	1.08	1.08	1.08	1.08	0.00015	0.01
12	1.17	1.17	1.17	1.17	1.17	1.17	1E-04	0.0085

Table 4.14: Dissolution of Resveratrol in Formulated Simulated Saliva Saline Buffer pH 7.4 at 315 nm

B/No. 2 Time (Hrs)	Abs Rdg 1	Abs Rdg 2	Abs Rdg 3	Min	Max	AVG Abs at 420 nm	STDEV	CV%
0	0	0	0	0	0	0	0	#DIV/0!
2	0.32	0.32	0.32	0.32	0.32	0.32	0.00029	0.089
4	0.53	0.53	0.53	0.53	0.53	0.53	0.00029	0.054
6	0.73	0.73	0.73	0.73	0.73	0.73	0.00012	0.016
8	0.90	0.90	0.90	0.90	0.90	0.90	0.00021	0.023
10	1.01	1.01	1.01	1.012	1.01	1.01	0.00020	0.020
12	1.11	1.11	1.11	1.11	1.11	1.11	0.00017	0.016

Table 4.15: Dissolution of Resveratrol in Formulated Simulated Saliva Saline Buffer pH 7.4 at 315 nm

B/No. 3 Time (Hrs)	Abs Rdg 1	Abs Rdg 2	Abs Rdg 3	Min	Max	AVG Abs at 420 nm	STDEV	CV%
0	0	0	0	0	0	0	0	#DIV/0!
2	0.34	0.34	0.34	0.34	0.34	0.34	0.00047	0.14
4	0.60	0.60	0.60	0.60	0.60	0.60	0.00015	0.026
6	0.77	0.77	0.77	0.77	0.77	0.77	1E-04	0.013
8	0.95	0.95	0.95	0.95	0.95	0.95	0.00049	0.052
10	1.10	1.10	1.10	1.10	1.10	1.10	0.00012	0.01
12	1.22	1.22	1.22	1.22	1.22	1.22	0.00015	0.01

Table 4.16: Average Absorbance of the Three Batches of Resveratrol

Time(Hrs)	AVG Abs at 420 nm B/No. 1	AVG Abs At 420 nm B/No. 2	AVG Abs At 420nm B/No. 3	Min	Max	AVG Abs	STDEV	CV%
0	0	0	0	0	0	0	0	#DIV/0!
2	0.24	0.32	0.34	0.24	0.34	0.30	0.05	18.20
4	0.61	0.53	0.60	0.53	0.61	0.58	0.05	7.79
6	0.83	0.73	0.77	0.73	0.83	0.78	0.05	6.36
8	0.98	0.90	0.95	0.90	0.98	0.94	0.04	4.24
10	1.08	1.01	1.10	1.01	1.10	1.06	0.05	4.34
12	1.17	1.11	1.22	1.11	1.22	1.17	0.06	4.79

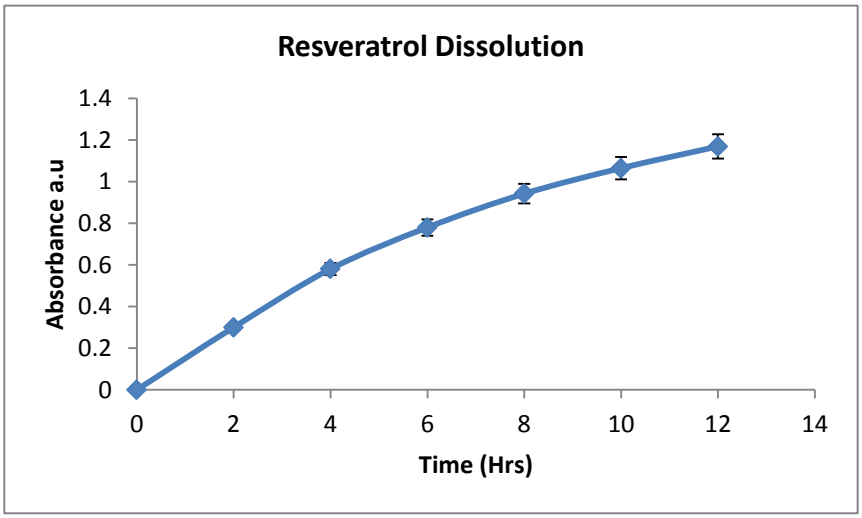


Figure 4.28: Dissolution of Resveratrol curve

Substituting in y for Absorbance;

Y = 0.126x -0,007

X = (y +0.007/0,126)

Table 4.17: Table of concentration. of Resveratrol in µg/ml

Time (Hrs)	AVG Abs	Concn. (mg/ml)	Concn. (µg/ml)	log Concn.
0	0	0	0	0
2	0.2999	0.0024	2.4357	0.3866
4	0.580067	0.0047	4.6593	0.6683
6	0.779689	0.0062	6.2436	0.7954
8	0.941667	0.0073	7.2591	0.8609
10	1.064278	0.0085	8.5022	0.9253
12	1.168678	0.0093	9.3308	0.9699

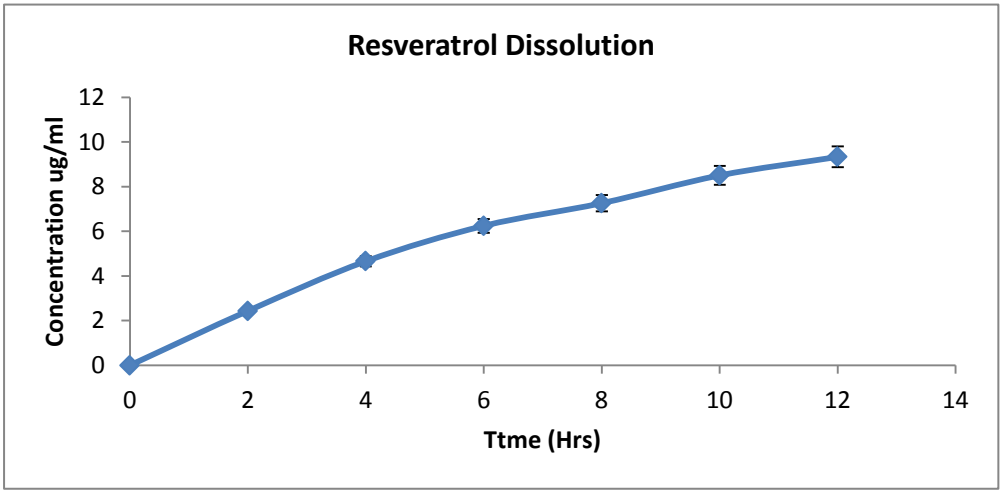


Figure 4.29: Dissolution of Resveratrol

y = o.126x -0.007 x =(y+0.007/0.126)
Substituting in y for
Absorbance;

Table 4.18: Cumulative percentage Dissolution (%) against time in seconds in a 12 hr. period

Time (Hrs)	AVG Abs	Conc. (mg/ml)	Conc. (µg/ml)	Cumm. Drug Release %
0	0	0	0	0
2	0.30	0.0024	2.44	0.014
4	0.58	0.0047	4.66	0.039
6	0.78	0.0062	6.24	0.074
8	0.94	0.0073	7.26	0.11
10	1.06	0.0085	8.50	0.16
12	1.17	0.0093	9.33	0.21

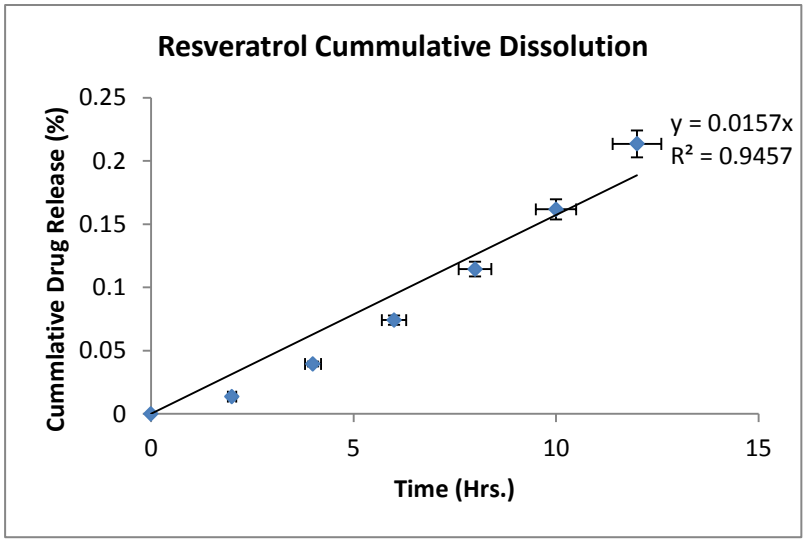


Figure 4.30: Resveratrol Cumulative dissolution curve

Slope of the Cumulative drug dissolution (%) = **0.015**
i.e., 0.015 g (%) are dissolved in one hour
Therefore 1.5 *1000 mg are dissolved in 3600 seconds

In 1 sec (0.015*1000)*100/3600 mg (%) are released = **0.42mg**
If 0.42 mg are dissolved in 1sec,
20 mg are dissolved in 20 / 0.42 sec = 47.6sec.

Table 4.19: Showing the amount of Drug (mg) dissolved in Time in seconds (s) at steady state.

Time (Sec)	Amount of Drug (mg)	Amount of Drug (µg)
0	0	0
1	**0.42**	**420**
5	2.1	2100
10	4.2	4200
20	8.4	8400
30	12.6	12600
40	16.8	16800
47.6	**20**	**20000**
50	20	20000
60	20	20000

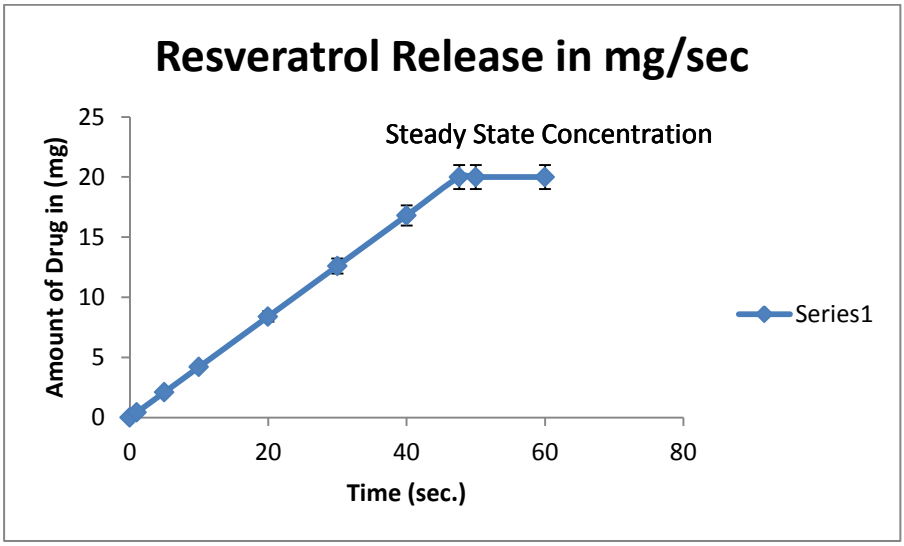


Figure 4.31: Resveratrol Dissolution in mg/sec.

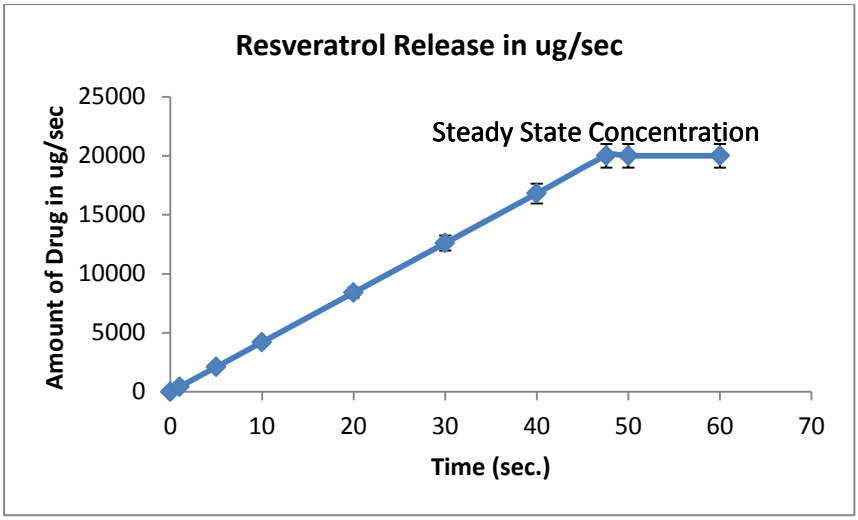


Figure 4.32: Resveratrol Dissolution in µg/sec.

In vitro release of five formulations of Verapamil Hydrochloride Fast dissolving strips showed similar release profile.

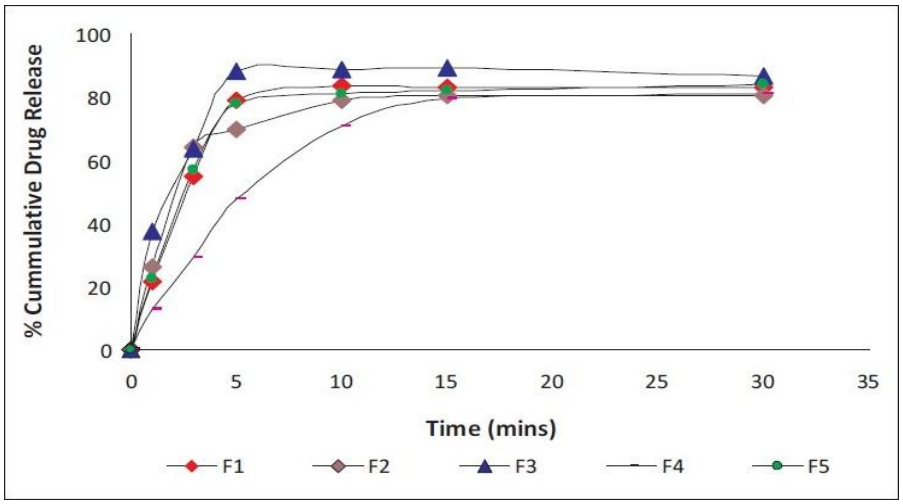


Figure 4.33: In vitro release profile of five formulations of Verapamil Hydrochloride (Kunte and Tandale, 2010).

4.4.6 Cumulative Drug Release

4.4.6.1 Formula for determination of percentage of dissolution of Resveratrol from in vitro dissolution testing

Concentration of drug (µg/ml)= [(Absorbance) ± intercept]/slope

Concentration of Drug **Equation 4.7**

Amount of drug released mg/ ml = Concentration × Dissolution bath volume × dilution factor/1000. **Equation 4.8**

Cumulative percentage dissolution (%) = Volume of sample withdrawn (ml)/bath volume (v) × P (t – 1) + Pt **Equation 4.9**

Where Pt = Percentage dissolution at time t Where P (t – 1) = Percentage release previous to 't' (Chandrasekaran et al., 2011).

Data:

y = 0.126x - 0.007 (R^2 = 0.997)

Concn. (x)µg/ml = [y (Absorbance)+ 0.007]/0.126

Dissolution basket volume = 900ml
Dilution factor = 0.0222 (i.e. 20mg / 900ml = 0.0222)

Amount of Drug released in mg/ml = Concn. X 900 x 0.0222 / 1000 for the 7 readings at Time (Hrs.) 0, 2, 4, 6, 8, 10, 12
We shall later express this in seconds since our film is a fast dissolving (IDF), and in the convectional units of µg/ml
Percentage of drug dissolved at time (t) = Amount of drug dissolved (mg/ml) /Original Amount of Drug loaded in the sample

Volume of sample withdrawn = 5 ml
Volume of methanol added 0.25 ml
Total volume of sample = 5.25 ml

Cumulative percentage dissolved (%) = Volume of sample withdrawn (ml)/bath volume (v) × P (t – 1) + Pt
Where Pt = Percentage dissolved at time t Where P (t – 1) = Percentage dissolution previous to 't
Cummulative percentage dissolved (%) = 5.25/900 X + Present percentage dissolution at time t

Cumulative percentage dissolved (%) = 5.25/900 X
Previous percentage dissolved (P(t-1)) + present percentage dissolution at time t
Plot Cummulative percentage Release (%) against time in seconds

Flux data were plotted as the cumulative amount of Resveratrol that diffused from the Mucosal to the serosal side of the epithelium versus time. The permeability coefficient, P, was calculated from the formula:

$P = (dQ/dt) / (C. A)$ (1)

In which dQ/dt 0.015(%) at steady-state slope of the cumulative flux curve 0.42mg/sec ,
C is the concentration difference across the buccal mucosa,
Flux Data was plotted as the cummulative amount of Curcumin that diffused from the Film into solution versus time.
The permeability Coefficient, (P) is calculated from the formula: P = (dQ/dt) / (C.A).
A is the effective cross- sectional area of the film available for diffusion = ($2cm^2$)
There is no concentration difference (C) since we did not put the dialysis membrane in the dissolution basket to separate the film, as is the case with the Franz Diffusion Cell, which has a separating buccal mucosal membrane.
The steady – state slope of the cumulative flux curve dQ/dt = 0.015
i.e. 0.015* 1000 mg (%) are released in one hour i.e. 3600 sec.
Flux
In 1 sec (0.015*1000)*100/3600 mg are dissolved = **0.42 mg**
If 0.42 mg are dissolve in 1 sec,
20 mg are released in 20 mg / 0.42 sec = **47.6 mg/sec**.
The cross- sectional area of the film dropped in the dissolution basket was $2cm^2$

Resveratrol Dissolution Flux (P) = $(dQ/dt) /(C. A)$ = 0.42mg/sec/$2cm^2$ =**0.21 mg/sec.cm^2**

4.4.6.2 Calculation of Curcumin Theoretical Drug Loading

The calculation can be done as follows;

"First % Total Drug Load (TDL) should be calculated. The theoretical percentage of drug loading (TDL) can be calculated using the following equation. TDL = (Weight of drug added (g)/Weight of polymers and drug added (g) x 100 For example, if you use 2 g of drug and 8 g of polymer, then the theoretical loading is 2/10 x 100 = 20, hence the theoretical loading is 20% w/w. (Note: dominator in the previous equation always should contains total amount of excipients and drug).

You should validate an analytical method to calculate the actual loading and this will depend on you what instruments do you have, etc..... However, assume that, during analysis each gram of SLN contains 100 mg of drug. Then actual loading is 100/1000 x100 = 10% w/w.

Therefore, the percentage of drug entrapment efficiency will be calculated according to the following equation: % DEE= (experimental drug loading/Theoretical drug loading (TDL)) x 100

As per the above example the DEE= 10/20 x100 = 50% w/w. Let's assume the yield of the microsphere is 9 g. Remaining one gram lost during the manufacture".

How to define and calculate Entrapment efficiency? - Research Gate. Available from: https://www.researchgate.net/post/How_to_define_and_calculate_Entrapment_efficiency [accessed Sep 30, 2016]; (Gattani et al., 2014).

Dimensions: 10 cm Internal Diameter with a Radius of 5 cm

Area = $\boldsymbol{A = \pi r^2}$ **Equation 4.10**

Area (A) = (22/7)*5*5 = 78.57 cm^2

Approximately 80 cm^2 will yield 20, 2cm^2 films

0.5 g of either Resveratrol or Curcumin are triturated on a white tile into 5ml of the thick suspension of rice starch suspension and spread onto the Petri dish.

One film is 1 cm x 2 cm in dimension

We expect 20 films from the Petri dish

i.e. 0.5g or 500mg are in 20 films

1 film would contain 500mg/20 films*1 film = 25mg

Drug entrapment = Amount of drug in film /initial Amount*100 %

i.e. Drug entrapment = 20/25*100 = 80%

Amount of the drug in the formulation = drug load = 80% of 25mg = 20mg

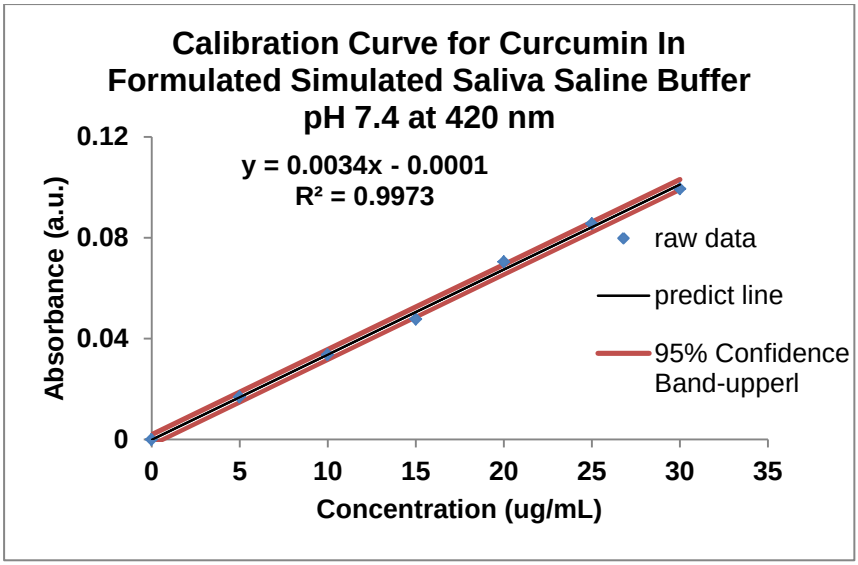


Figure 4.34: Curcumin Calibration Curve in µg/ml

Table 4.20: Dissolution of Curcumin
In Formulated Simulated Saliva Saline Buffer pH 7.4 at 420 nm

Time (Hrs)	AVG Abs at 420 nm B/No. 1	AVG Abs at 420 nm B/No. 2	AVG Abs at 420 nm B/No. 3	Min	Max	AVG Abs	STDEV	CV%
0	0	0	0	0	0	0	0	#DIV/0!
2	0.0047	0.0053	0.0051	0.0047	0.0053	0.0050	0.00030	6.02
4	0.010	0.012	0.012	0.010	0.012	0.012	0.00061	5.60
6	0.014	0.015	0.015	0.014	0.015	0.015	0.00052	3.57
8	0.018	0.017	0.018	0.017	0.018	0.018	0.00031	1.75
10	0.020	0.019	0.020	0.020	0.020	0.020	0.00047	2.41
12	0.021	0.020	0.021	0.02	0.021	0.021	0.00063	3.01

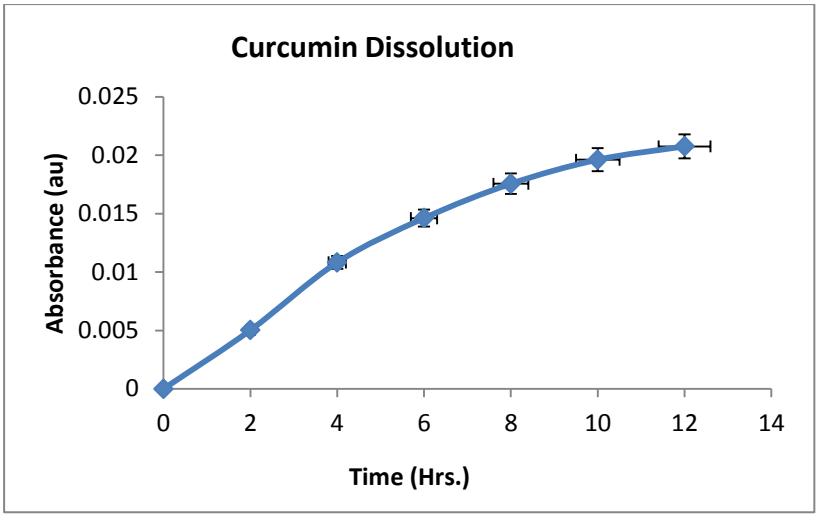


Figure 4.35: Curcumin Dissolution Curve.

4.4.7. *In vitro* Curcumin dissolution profile from the rice paper films

y = 3.37x -0.000

x = (y+0.000/3.37)
Substituting in Absorbance for y;

Table 4.21: Cumulative percentage Dissolution (%) against time in a 12 hr. period

Time (Hrs)	AVG Abs	Conc. (mg/ml)	Conc. (µg/ml)	Cum. Drug Release %
0	0	0	0	0
2	0.0050	0.0015	1.49	0.0082
4	0.011	0.0032	3.21	0.026
6	0.015	0.0043	4.34	0.05
8	0.018	0.0052	5.21	0.079
10	0.020	0.0058	5.82	0.11
12	0.021	0.0061	6.16	0.15

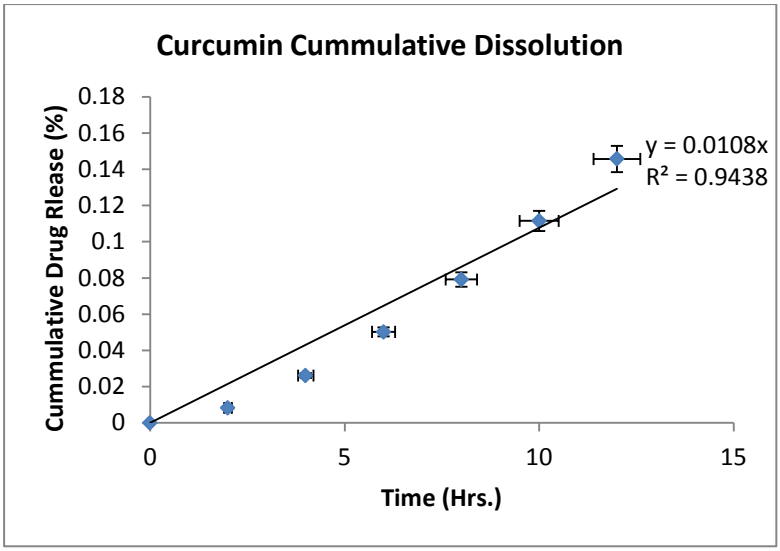


Figure 4.36: Curcumin Cumulative Dissolution Curve.

4.4.8. Cumulative Drug Release

4.4.8.1 Formula for determination of percentage of release of Curcumin from in vitro dissolution testing:

Concentration of drug (µg/ml)= [(Absorbance) ± intercept]/slope

Amount of drug released mg/ ml = Concentration × Dissolution bath volume × dilution factor/1000.

Cumulative percentage release (%) = Volume of sample withdrawn (ml)/bath volume (v) × P (t – 1) + Pt

Where Pt = Percentage release at time t Where P (t – 1) = Percentage release previous to 't'

(Chandrasekaran et al., 2011)

Data:

y = 0.0034x - 0.0001

Concn. (x) µg/ml = [y (Absorbance)+ 0.0001]/0.0034

Dissolution basket volume = 900ml

Dilution factor = 0.0222

Amount of Drug released in mg/ml = Concn. X 900 x 0.0222 / 1000
for the 7 readings at Time (Hrs.) 0, 2, 4, 6, 8, 10, 12

We shall later express this in seconds since our film is a fast dissolving (IDF)
Percentage of drug released at time (t) =
Amount of drug released (mg/ml) /Original Amount of Drug loaded in the sample

Volume of sample withdrawn = 5 ml

Volume of methanol added 0.25 ml

Total volume of sample = 5.25 ml

Cumulative percentage release (%) = Volume of sample withdrawn (ml)/bath volume (v) × P (t – 1) + Pt
Where Pt = Percentage release at time t Where P (t – 1) = Percentage release previous to ‘t

Cumulative percentage Release (%) = 5.25/900 X
Previous percentage release (P(t-1)) + present percentage release at time t
Plot Cummulative percentage Release (%) against time in seconds

Flux data was plotted as the cumulative amount of Curcumin that diffused from the film versus time. The permeability coefficient (P), was calculated from the formula;

Permeability coefficient P = (d*Q*/d*t*) / (*C. A*) **Equation 4.11**

In which d*Q*/d*t* 0.010 (%) at steady-state slope of the cumulative flux curve 0.28mg/sec , C is the concentration difference across the buccal mucosa, and A (2 cm^2) is effective cross-sectional area available for diffusion.

Slope of Cumulative drug release % = 0.010
i.e, 0.010 mg (%) are released in one hour
Therefore 0.010 *1000*100 mg are released in 3600 seconds
Flux

In 1 sec (0.010*1000)*100/3600 mg are released = **0.28mg**
If 0.28 mg are released in 1sec,
20 mg are dissolved in20/0.28 = **71.4 sec.**
The cross-sectional Area of the film dropped in the dissolution basket was 2cm^2

Curcumin Dissolution Flux (P) = dQ/dt /(C.A) = 0,28mg/sec/2cm^2 = **0.14 mg/sec/cm^2**

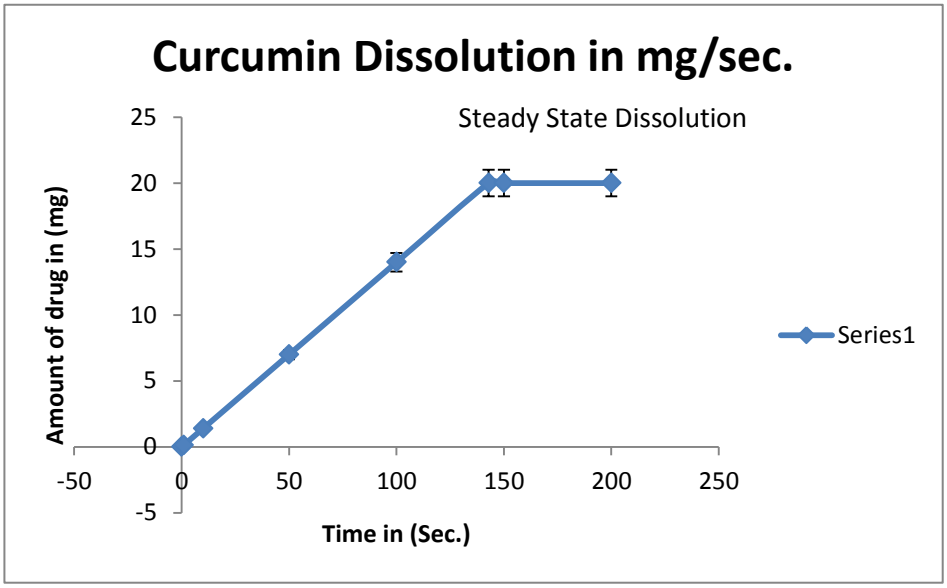


Figure 4.37: Cumulative dissolution of Curcumin in mg/sec

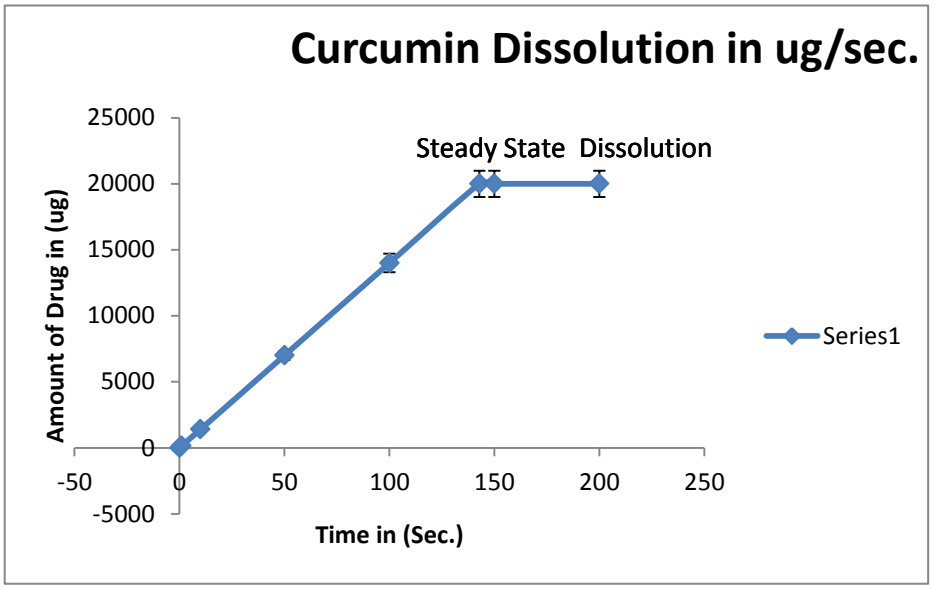


Figure 4.37: Cumulative dissolution of Curcumin in µg/sec

It is very important to note that, since the maximum drug there can be in the film is the drug load which is 20mg, the graph can be drawn in terms mg of the drug that is released per time in seconds using the rate. In this way, it can be easily seen that all the 20 mg could dissolved in about 47.6 seconds for Resveratrol (See Figure 4.31) and in about 142.9 seconds for Curcumin (See Figure 4.37). However, in a practical sense, the film must first of all be hydrated in a small amount of saliva entrapped between the tongue and the upper palate and the rice starch will swell, disintegrate/dissolve as a result of the crashing effect of the tongue, then release/dissolve the drug.

The swelling index study revealed the average swelling index to be 91.7 % for the plain films, 79.4 % for Resveratrol films and 73% for the Curcumin loaded films. This explains the difference in the time lag that may occur in the practical sense.

The experiment was done in a large volume of saliva (900 ml) and for a long time of 12 hrs due to practicality reasons. However, the rate of drug release is independent of the volume at Steady state saturation point or maximum drug release/dissolution and can be used the calculate the Flux in mg/ sec. cm^2 (Chou and Wollast, 1985); (Chandrasekaran et al., 2011); (Ritschel, 1989).

4.4.9 Method for Folding Endurance

To determine folding endurance, a strip of film was cut and repeatedly folded at the same place until it broke. The number of times the film could be folded at the same place without breaking gives the value of folding endurance.(Guo and Cooklock, 1995). The films were folded for not more than 4 times before they broke or a showed some sign of cracks. Formulation 16 broke with only one fold. Four (4) times was taken as maximum folding endurance that the film could be folded at the same place without breaking. The mean value and standard deviation was calculated and found to be 0 percent.

Table 4.22: Folding Endurance for the Plain films.

Form	B/N0.1			B/N0. 2			B/N0.3					
No	1	2	3	1	2	3	1	2	3	AVG	STDEV	CV %
1	1	1	1	1	1	1	1	1	1	1.0	0	0
2	7	8	8	8	7	8	8	7	8	7.7	0.58	7.53
3	15	12	13	20	24	25	20	24	25	19.8	2.65	13.38
4	3	3	4	3	5	4	3	5	4	3.8	1	26.47
5	2	3	2	7	6	7	7	6	7	5.2	0.58	11.06
6	1	1	1	1	1	1	1	1	1	1.0	0	0
7	28	30	27	22	25	20	22	25	20	24.3	2.52	10.34
8	20	19	20	14	12	15	14	12	15	15.7	1.53	9.75
9	8	9	6	12	11	15	12	11	15	11.0	2.08	18.92
10	3	5	5	3	3	3	3	3	3	3.4	0	0
11	5	4	5	16	14	15	16	14	15	11.6	1	8.65
12	2	3	3	4	8	6	4	8	6	4.9	2	40.90
13	20	19	21	13	12	15	13	12	15	15.6	1.53	9.82
14	5	5	5	1	2	2	1	2	2	2.8	0.58	20.78
15	2	3	3	1	3	3	1	3	3	2.4	1.15	47.24
16	3	3	3	2	5	2	2	5	2	3.0	1.73	57.74

Mode	1
Median	5.06
Mean	8.32
Min	1
Max	24.33
Range	1.00 - 24.33
SD	0.72

Table 4.23: Folding Endurance for the Resveratrol films.

Form No.	B/No.1(1)	2	3	B/No.2(1)	2	3	B/No.3(1)	2	3	AVG	CV %
1	1	1	1	1	1	1	1	1	1	1	0
2	1	1	1	1	1	1	2	2	2	1.33	0
3	2	2	2	2	2	2	2	2	2	2	0
4	1	1	1	2	1	1	2	2	2	1.44	0
5	1	1	1	1	1	1	3	2	2	1.44	39.97
6	1	1	1	1	1	1	1	1	1	1	0
7	2	2	2	2	2	2	2	2	2	2	0
8	1	1	1	1	1	1	1	1	1	1	0
9	1	1	1	1	1	1	2	2	2	1.33	0
10	3	3	3	1	3	4	2	2	2	2.56	0
11	1	1	1	1	1	1	2	2	2	1.33	0
12	1	1	1	1	1	1	1	1	1	1	0
13	2	2	2	2	2	2	1	1	1	1.67	0
14	1	1	1	1	1	1	1	1	1	1	0
15	1	1	1	1	1	1	1	1	1	1	0
16	2	2	1	2	2	2	2	2	2	1.89	0

Mode	1
Median	1.33
Mean	1.44
Min	1
Max	2.56
Range	1.00 - 2.55
SD	0.47

Table 4.24: Folding Endurance for the Curcumin films.

Form No.	B/No.1(1)	2	3	B/No.2(1)	2	3	B/No.3(1)	2	3	AVG	STDEV	CV %
1	1	1	1	1	1	1	1	1	1	1	0	0
2	2	2	2	2	2	2	1	1	1	1.67	0	0
3	2	2	2	2	2	2	1	1	1	1.67	0	0
4	2	2	2	2	2	2	3	3	3	2.33	0	0
5	1	1	1	1	1	1	2	2	2	1.33	0	0
6	1	1	1	1	1	1	1	1	1	1	0	0
7	2	2	2	2	2	2	2	2	2	2	0	0
8	1	1	1	1	1	1	1	1	1	1	0	0
9	1	1	1	2	2	2	1	1	1	1.33	0	0
10	4	3	4	2	2	2	4	4	4	3.22	0	0
11	1	1	1	1	1	1	2	2	2	1.33	0	0
12	1	1	1	1	1	1	1	1	1	1	0	0
13	2	2	2	2	2	2	3	3	3	2.33	0	0
14	1	1	1	1	1	1	1	1	1	1	0	0
15	1	1	1	2	1	1	1	1	1	1.11	0	0
16	2	2	2	2	2	2	1	1	1	1.67	0	0

Mode	1
Median	1.33
Mean	1.56
Min	1
Max	3.22
Range	1.00-3.22
SD	0.00

4.4.9 Method for Moisture content

In the Ideal situation we would use a moisture balance or a hot air oven and an analytical balance to determine the moisture content of the films. Alternatively, we dipped the films in a small volume of water and blotted the excess with filter paper (blotting paper) and determined the Wettability by measuring the initial and final weights and calculating the difference and expressing it as percentage water content. The optimum formulation 16 had 32.5 % ± 7.6 SD for the plain film, 30.3 % ± 6.5 SD for Resveratrol film and, 35.1 ± 8.4 SD for Curcumin film.

Table 4.25: Moisture content for the plain films.

AVG MOISTURE CONTENT

Plain **Form No.**	B/No 1 Moisture Content (%)	2 Moisture Content (%)	3 Moisture Content (%)	AVG Moisture Content (%)	STDEV	CV %
1	11.11	10.56	10.33	10.67	0.40	3.76
2	10.67	19	8.5	12.72	5.54	43.57
3	13.73	13.4	13.47	13.53	0.18	1.30
4	12.27	12.07	12	12.11	0.14	1.15
5	19.05	18.91	19.05	19	0.08	0.43
6	9.78	9.61	9.56	9.65	0.12	1.20
7	25.52	25.19	24.95	25.22	0.29	1.14
8	11	10.82	10.83	10.89	0.10	0.91
9	11.89	11.69	11.39	11.66	0.25	2.16
10	14.57	14.04	13.24	13.95	0.67	4.81
11	9.33	9.46	9.13	9.31	0.17	1.77
12	8.75	8.83	8.83	8.81	0.05	0.55
13	9.82	9.55	9.76	9.71	0.14	1.48
14	10	9.67	9.61	9.76	0.21	2.16
15	30.83	29.67	30.50	30.33	0.60	1.98
16	32.57	32.38	32.56	32.50	0.10	0.33
					Mode	#N/A
					Median	11.88
					Mean	14.99
					Min	8.81
					Max	32.50
					Range	8.80 - 32.50
					SD	0.77

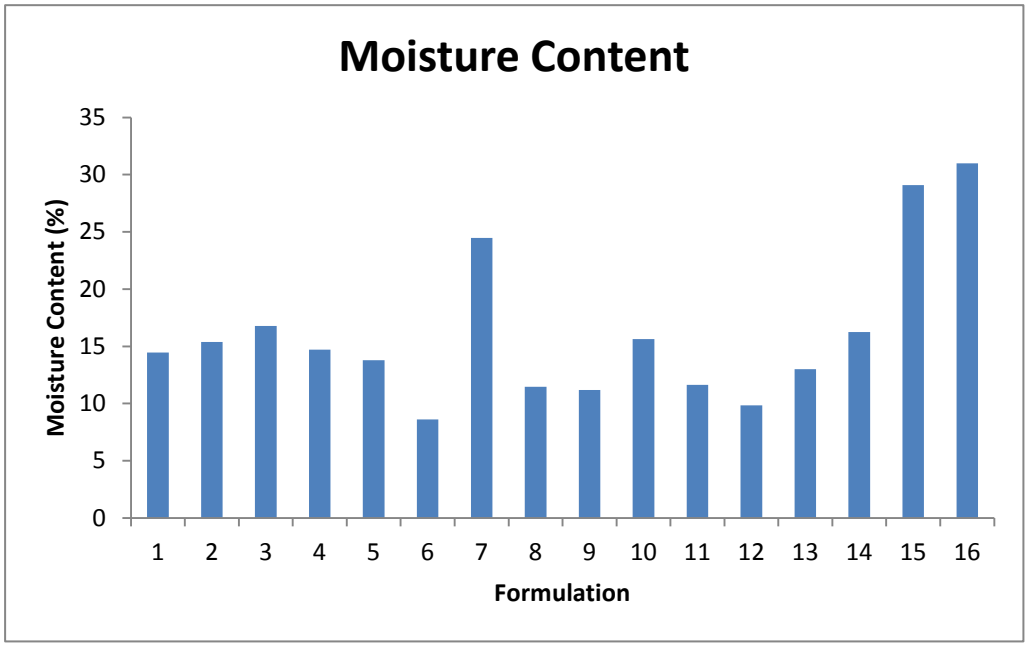


Figure 4.38: Moisture content for the plain films.

Table 4.26: Moisture content for the Resveratrol films.

AVG MOISTURE CONTENT						
Res	B/No 1	2	3	AVG	STDEV	CV %
Form No.	Moisture Content (%)	Moisture Content (%)	Moisture Content (%)	Moisture Content (%)		
1	14.47	17.11	13.83	15.14	1.74	11.48
2	15.39	15.28	15.72	15.46	0.23	1.50
3	16.77	16.56	16.92	16.75	0.18	1.08
4	14.72	14.29	14.70	14.57	0.24	1.66
5	13.80	13.25	13.73	13.60	0.30	2.19
6	8.62	8.43	8.45	8.51	0.10	1.22
7	24.48	24.22	24.37	24.36	0.13	0.53
8	11.46	10.63	11.25	11.12	0.43	3.88
9	11.19	10.92	10.93	11.02	0.15	1.38
10	15.64	14.25	14.09	14.66	0.85	5.82
11	11.62	11.25	11.39	11.42	0.19	1.65
12	9.86	9.76	9.88	9.83	0.06	0.65
13	13.01	11.79	11.60	12.13	0.76	6.30
14	16.25	13.60	15.17	15.01	1.33	8.87
15	29.083	28.14	28.70	28.64	0.48	1.66
16	31	29.18	30.76	30.31	0.99	3.26
					Mode	#N/A
					Median	14.61
					Mean	15.78
					Min	8.50
					Max	30.31
					Range	8.50 - 30.31
					SD	0.65

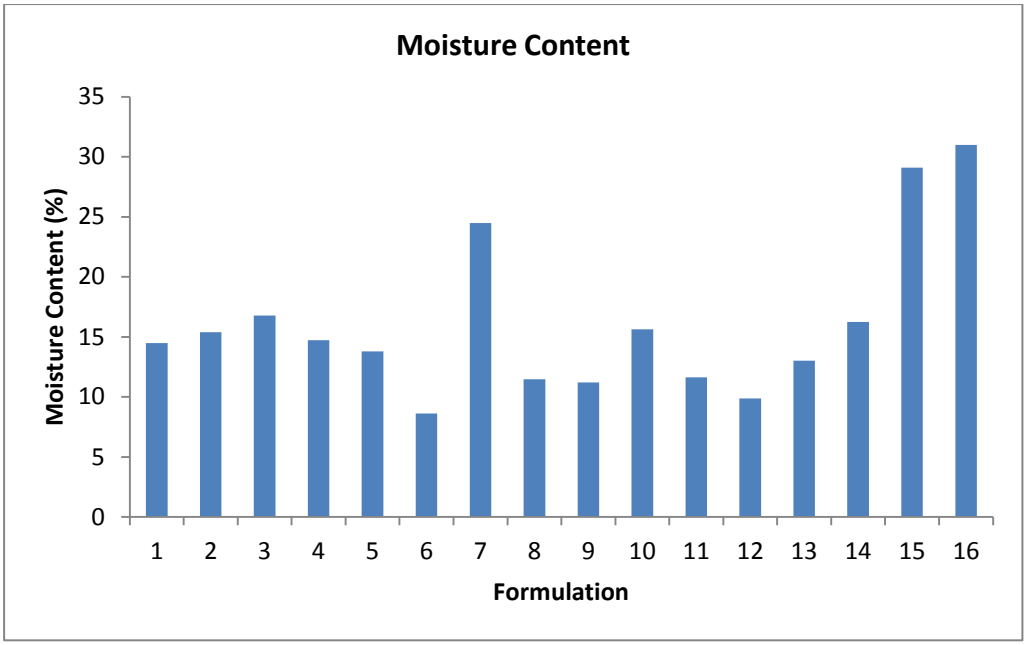


Figure 4.39: Moisture content for the resveratrol films.

Table 4.27: Moisture content for the Curcumin films.

AVG MOISTURE CONTENT

Curc. **Form. No**.	B/No 1 Moisture Content (%)	2 Moisture Content (%)	3 Moisture Content (%)	AVG Moisture Content (%)	STDEV	CV %
1	7.86	7.58	7.75	7.73	0.14	1.81
2	11.18	10.58	11.18	10.98	0.35	3.15
3	11.92	14.21	13.17	13.10	1.14	8.72
4	12.89	12.59	12.81	12.76	0.15	1.20
5	17.54	17.23	17.59	17.45	0.20	1.13
6	7.53	7.25	7.42	7.40	0.14	1.91
7	26.34	25.98	26.29	26.20	0.20	0.75
8	10.85	10.74	10.78	10.79	0.05	0.50
9	11.04	11.29	10.22	10.85	0.56	5.17
10	16.31	14.01	15.06	15.13	1.15	7.63
11	16.32	16.76	15.49	16.19	0.65	3.99
12	17.31	15.97	16.26	16.51	0.71	4.28
13	13.97	11.63	12.35	12.65	1.20	9.47
14	14.80	14.79	14.82	14.80	0.01	0.11
15	34.77	33.57	34.17	34.17	0.60	1.76
16	35.39	34.39	35.66	35.15	0.67	1.89

Mode	#N/A
Median	13.95
Mean	16.37
Min	7.40
Max	35.15
Range	7.39 - 35.14
SD	0.84

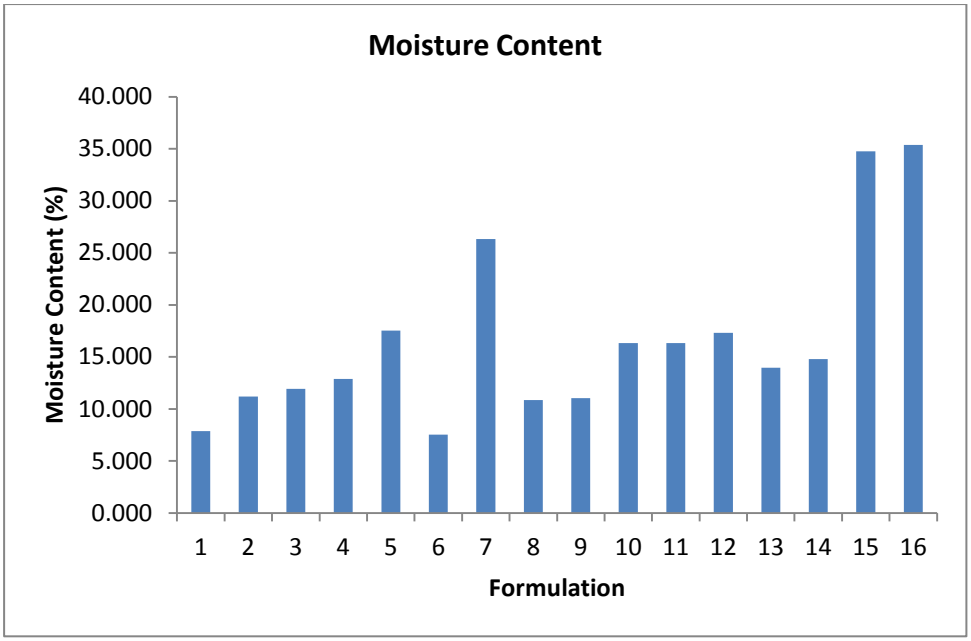


Figure 4.40: Moisture content for the resveratrol films**.**

4.4.10 Method for thickness variation

Oral film thickness determination and weight variation are routine tests. A typical film thickness ranges from 12 to 100 µm (Barnhart and Vondrak, 2008). The thickness of ten films that were selected at random from the centre and the four sides of a single Petri dish of every formulation was measured using a Digital Micrometer screw gauge (Besto, England). The thickness of the left, centre and right sections of the rectangular film were measured. The idea was to produce a film of even thickness and as thin as possible (Nafee et al., 2003).

Laying the Petri dishes on a perfectly levelled table during casting of the films was critical for this step.

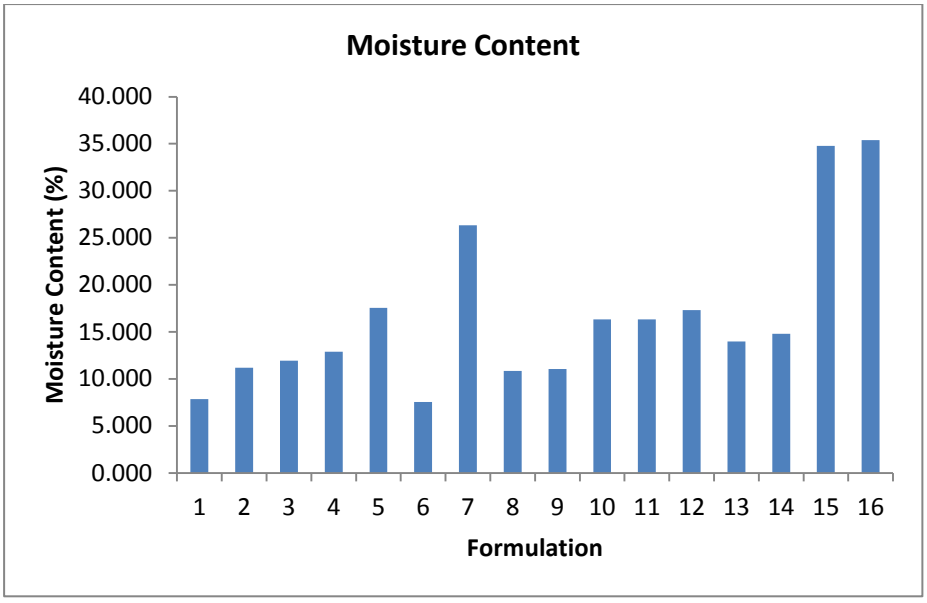


Figure 4.41: Moisture content for the resveratrol films**.**

Table 4.28: Thickness variation the plain film (A), Resveratrol film (B), and Curcumin film (C).

A -Plain

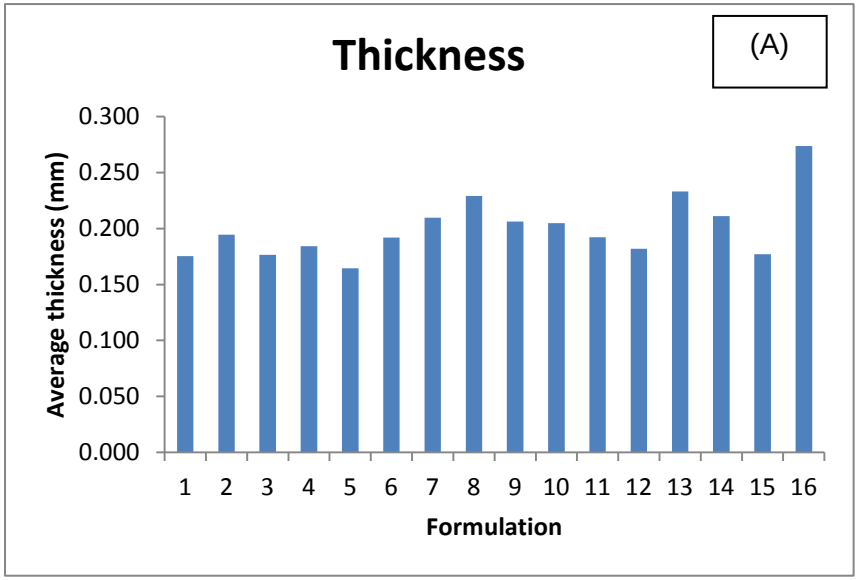
Formula No.	**Average** (mm)	**Average** (mm)	**Average** (mm)	**Average** (mm)	**Average** (mm)	**Average** (mm)	**Average** (mm)	**Average** (mm)	**Average** (mm)	**MEAN** (mm)	**STDEV**	**CV (%)**
1	0.167	0.213	0.197	0.217	0.137	0.180	0.170	0.150	0.147	0.175	0.029	16.51
2	0.227	0.197	0.197	0.197	0.187	0.183	0.197	0.183	0.183	0.194	0.014	7.02
3	0.190	0.147	0.157	0.227	0.177	0.180	0.183	0.163	0.163	0.176	0.02	13.28
4	0.213	0.157	0.153	0.197	0.203	0.200	0.187	0.173	0.173	0.184	0.02	11.48
5	0.157	0.147	0.143	0.167	0.167	0.170	0.183	0.173	0.173	0.164	0.013	7.98
6	0.167	0.163	0.160	0.190	0.170	0.170	0.227	0.237	0.243	0.192	0.034	17.77
7	0.223	0.230	0.223	0.220	0.220	0.213	0.230	0.163	0.163	0.210	0.027	12.76
8	0.220	0.207	0.207	0.267	0.260	0.250	0.220	0.213	0.217	0.229	0.023	10.22
9	0.290	0.237	0.243	0.207	0.197	0.197	0.163	0.160	0.163	0.206	0.044	21.21
10	0.247	0.200	0.067	0.287	0.247	0.237	0.197	0.183	0.180	0.205	0.063	30.62
11	0.230	0.217	0.213	0.170	0.160	0.160	0.187	0.193	0.200	0.192	0.025	13.18
12	0.247	0.180	0.190	0.190	0.173	0.177	0.153	0.163	0.163	0.182	0.027	14.98
13	0.380	0.283	0.270	0.200	0.167	0.170	0.220	0.207	0.200	0.233	0.068	29.16
14	0.200	0.207	0.213	0.240	0.173	0.173	0.240	0.230	0.223	0.211	0.025	12.05
15	0.227	0.160	0.173	0.170	0.173	0.160	0.220	0.153	0.157	0.177	0.027	15.40
16	0.313	0.273	0.287	0.280	0.303	0.297	0.257	0.227	0.227	0.274	0.031	11.48
									Mode	#N/A		
									Median	0.19		
									Mean	0.20		
									Min	0.16		
									Max	0.27		
									Range	0.16-0.27		
									SD	0.028		

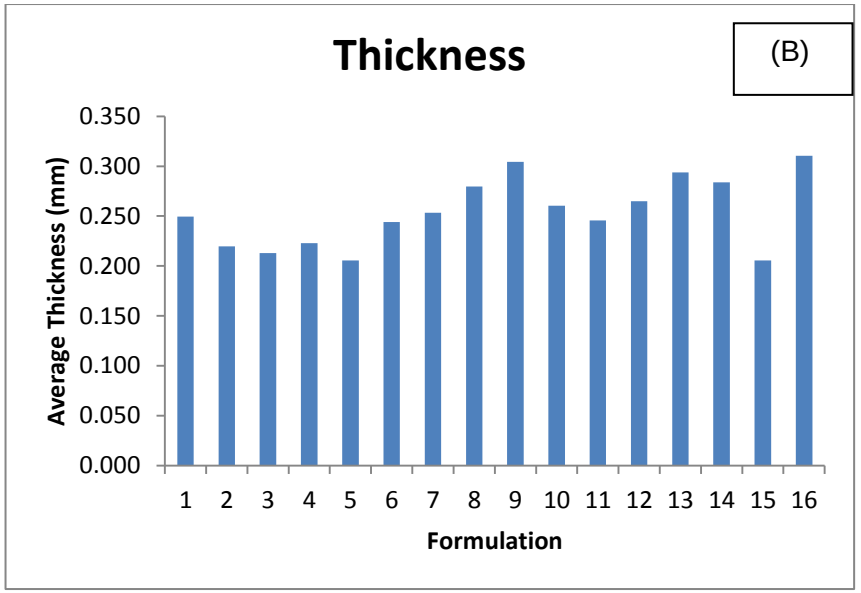
B-Resveratrol

Formula No.	Average (mm)	Average (mm)	Average (mm)	Average (mm)	Average (mm)	Average (mm)	Average (mm)	Average (mm)	Average (mm)	MEAN (mm)	STDEV	CV (%)
1	0.307	0.283	0.310	0.240	0.190	0.173	0.277	0.240	0.227	0.250	0.049	19.45
2	0.210	0.227	0.223	0.203	0.217	0.217	0.227	0.227	0.227	0.220	0.0086	3.90
3	0.207	0.180	0.177	0.217	0.227	0.233	0.237	0.217	0.223	0.213	0.022	10.16
4	0.230	0.197	0.200	0.253	0.210	0.210	0.230	0.237	0.240	0.223	0.020	8.76
5	0.203	0.190	0.190	0.260	0.197	0.203	0.193	0.207	0.207	0.206	0.022	10.45
6	0.230	0.220	0.223	0.263	0.227	0.227	0.273	0.263	0.270	0.244	0.023	9.26
7	0.260	0.267	0.257	0.233	0.257	0.257	0.270	0.237	0.243	0.253	0.013	5.05
8	0.257	0.247	0.240	0.343	0.330	0.317	0.277	0.257	0.250	0.280	0.040	14.17
9	0.297	0.303	0.307	0.343	0.227	0.223	0.360	0.340	0.340	0.304	0.050	16.35
10	0.260	0.260	0.263	0.253	0.260	0.253	0.293	0.250	0.250	0.260	0.013	5.099
11	0.250	0.227	0.220	0.220	0.267	0.267	0.277	0.240	0.243	0.246	0.021	8.61
12	0.327	0.277	0.277	0.260	0.270	0.273	0.237	0.230	0.233	0.265	0.030	11.35
13	0.350	0.347	0.347	0.290	0.230	0.233	0.263	0.290	0.293	0.294	0.047	15.89
14	0.290	0.263	0.277	0.350	0.307	0.310	0.253	0.250	0.253	0.284	0.034	11.89
15	0.223	0.187	0.197	0.207	0.230	0.227	0.200	0.190	0.190	0.206	0.017	8.27
16	0.270	0.317	0.320	0.347	0.323	0.323	0.300	0.297	0.297	0.310	0.022	7.11
									Mode	#N/A		
									Median	0.251		
									Mean	0.254		
									Min	0.206		
									Max	0.310		
									Range	0.20-0.31		
									SD	0.034		

C-Curcumin

Formula No.	**Average** (mm)	**Average** (mm)	**Average** (mm)	**Average** (mm)	**Average** (mm)	**Average** (mm)	**Average** (mm)	**Average** (mm)	**Average** (mm)	**MEAN** (mm)	**STDEV**	**CV (%)**
1	0.187	0.240	0.250	0.17	0.160	0.173	0.263	0.260	0.253	0.217	0.044	20.061
2	0.240	0.227	0.223	0.313	0.210	0.200	0.227	0.260	0.267	0.241	0.035	14.37
3	0.260	0.250	0.253	0.303	0.220	0.213	0.287	0.287	0.283	0.262	0.031	11.86
4	0.187	0.183	0.183	0.253	0.217	0.213	0.217	0.227	0.237	0.213	0.025	11.54
5	0.240	0.210	0.207	0.24	0.220	0.223	0.193	0.197	0.193	0.214	0.018	8.60
6	0.217	0.207	0.213	0.273	0.240	0.230	0.317	0.270	0.267	0.248	0.036	14.57
7	0.270	0.250	0.250	0.32	0.257	0.240	0.347	0.253	0.267	0.273	0.036	13.28
8	0.327	0.263	0.267	0.55	0.287	0.277	0.353	0.303	0.303	0.326	0.089	27.34
9	0.340	0.327	0.330	0.327	0.223	0.223	0.307	0.230	0.233	0.282	0.052	18.68
10	0.323	0.303	0.307	0.393	0.260	0.263	0.290	0.310	0.303	0.306	0.039	12.71
11	0.280	0.253	0.253	0.25	0.253	0.243	0.243	0.233	0.233	0.249	0.014	5.63
12	0.347	0.290	0.260	0.22	0.260	0.250	0.260	0.253	0.253	0.266	0.035	13.22
13	0.330	0.333	0.330	0.473	0.380	0.360	0.427	0.303	0.337	0.364	0.055	14.99
14	0.330	0.280	0.283	0.36	0.327	0.333	0.320	0.297	0.317	0.316	0.026	8.14
15	0.233	0.183	0.190	0.26	0.223	0.223	0.287	0.280	0.277	0.240	0.038	16.05
16	0.413	0.327	0.340	0.347	0.357	0.360	0.293	0.273	0.263	0.330	0.047	14.34
									Mode	#N/A		
									Median	0.26		
									Mean	0.27		
									Min	0.21		
									Max	0.36		
									Range	0.21-0.36		
									SD	0.045		


Thickness
(A)
Average thickness (mm)
0.300
0.250
0.200
0.150
0.100
0.050
0.000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Formulation


Thickness
(B)
Average Thickness (mm)
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Formulation

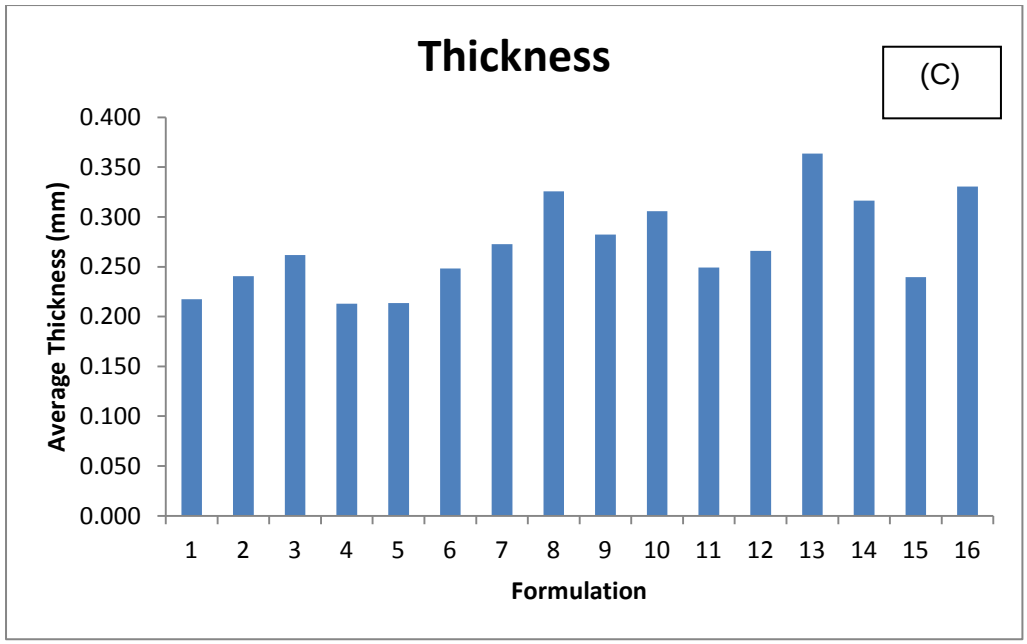


Figure 4.42: The Plain film (A), Resveratrol film (B) and, Curcumin(C) Thickness Variation

Tables A, B and C show the Thickness expressed as a mean and standard deviation for the Rice paper films.

The published specifications for an oral film are; 12 to 100 µm (Barnhart and Vondrak, 2008) using other polymers.

The thickness of Batch 3 of the optimal formulation 16 had maximum thickness. It was **0.274mm** for the plain film**, 0.310mm** for Resveratrol film and, **0.330mm** for Curcumin film. This lied close the specifications of the thickness of an intraoral film though not exactly, it yielded a robust film specifically for Rice starch polymer. Table 4.32 summarises all the film mechanical properties of the Rice paper films.

Table 4.29: Physical and Mechanical evaluation of Curcumin and Resveratrol IDF; data presented as medians and (range) of the 16 formulations.

	Specifications	**Plain**	**Curcumin**	**Resveratrol**
Thickness (mm)		0.19 (0.16- 0.27)	0.26 (0.21- 0.36)	0.25 (0.20 - 0.31)
Disintegration Time (sec)	*Published (5 - 30 sec)	12.75 (5 - 26.77)	13.98 (4 - 26.55)	14.76 (5.11 - 26.66)
Folding Endurance (No. of folds)	In house (1-4 folds)	8.31 (1 - 24.33)	1.56 (1 - 3.22)	1.43 (1 - 2.55)
Surface pH	In house (5 - 7)	6.38 (5.76 - 6.88)	6.37 (5.55 - 6.94)	6.31 (5.61 - 6.84)
Swelling Index (%)	In house (70-100 %)	46.07 (24.44 - 91.66)	47.64 (28.96 - 73.42)	49.58 (32.01 - 79.41)
Moisture Content (%)	In house (5 - 40%)	14.98 (8.80 - 32.50)	16.36 (7.39 - 35.14)	15.78 (8.50 - 30.31)
Tensile Strength (Ns/cm2)	*Published (1.8 ± 0.20 Mpa)	0.57 (0.18 - 1.63)	0.90 (0.13 - 2.0)	1.58 (0.13 - 7.64)
Elongation (%)	*Published (32.2.4 ± 63.3%)	51.42 (13.17 - 129.744)	30.80 (8.11 - 68.05)	47.70 (15.06 - 108.21)
Viscosity (Ns/m^2)	In house (Ns/m^2)	11.18 (2.16 - 84.66)		
Weight variation (g/2cm^2)	N/A		72.1 (71.73 - 72.9)	64.5 (63 - 65.43)
Dissolution (mg/ml)	N/A		0.0070 (0 - 0.017)	0.49 (0 - 0.72)
Casting time (Hrs)	N/A	18.15 (14 - 23.2)		

*See published parameter Specifications references in Chapter 4, (4.4.1).

Considering the drug dissolution studies of the rice paper films fully loaded with the model drugs Resveratrol and Curcumin in simulated saliva, the dissolution profiles of formulation 16 proved it to be optimal. Considering the statistical analysis, the type of plasticizer did not matter p – value was greater than 0.05 showing to be of no significant value from the statistical evaluation. The robustness of the film varied either way as the concentration of the any plasticizer increased or decreased rendering the type of plasticizer irrelevant in terms of significance.

The tensile strength studies of formulation 16 was moderate to allow the film to withstand all handling stages and finally could be handled comfortably by the patient as the human taste testing suggested (SeeTable 5.10) and (SeeTable 5.11). Rice starch films showed a reciprocal relationship because when the rice starch was significantly increased the films became brittle and this was not desirable. When the starch concentration was too low, the films were too soft to be viable therefore a balance in between had to be reached. Experimental Design was not all conclusive of the optimal formulation. None the less it gave an important guide. It generated the 16 variable formulations.

Many other factors had to be considered to select the optimum formulation and one parameter could not be based on for a judgment because of this reciprocal relationship of the rice starch and its poor mechanical properties. It was evident that the incorporation of the plasticizers. Filler, sweeteners and flavours within the rice starch resulted in a good drug Disintegration of a more controlled manner in a very short period of time of generally less than 30 seconds. In addition, they contributed to the acceptability as the result showed from the human taste testing.

4.4.9. Quality control data sheet

As a result of the characterisation of the rice paper films in this study, a quality control Data sheet containing some of the parameters to test for the attributes of a good film was generated. See table 4.7 below. This could be used as a quality control production document. Its limits are not binding as they may differ from product to product.

Table 4.30: Quality control data sheet

Quality Control Data

Certificate of Analysis			
Product : Rice paper film			
Batch / Control Number			
Element	Limit	Results	Comments
Mechanical properties			
Appearance			
Consistency			
Thickness			
Dryness / tack test			
Tensile Strength			
Percentage Elongation			
Tear Resistance			
Folding Endurance			
Young's Modulus			
Organoleptic tests			
Swelling test			
Surface Ph test			
Contact Angle			
Transparency			
Assay / Content Uniformity			
Disintegration test			
In – vivo Dissolution test			
Ex – vivo Permeation test			
Quality Control Pharmacist	Signature	Date	
Product Released by	Yes / No	Signature and Date	
Product Sheet prepared by	Checked by	Signature and Date	
Any Other Comments			

4.4.10 Concluding remarks

Good results were obtained for the Rice paper intraoral films from both *in vitro* and *ex vivo* studies. The present investigation shows that it is possible to formulate the Rice paper intraoral films for systemic drug delivery with the intention of obtaining better therapeutic efficiency of essential natural drugs by administering these drugs through the oral mucosa. These are accompanied by decreased dosing because therapeutic blood levels are reached in a short period. Fewer side effects follow the decreased dose. As an added advantage, hepatic first pass metabolism is avoided. It may be concluded that the Rice paper IDF formulation 16 showed good swelling, controlled release and dissolution though curcumin presented with some dissolution problems.

It is very important to note that, since the maximum drug there can be in the film is the drug load, which is 20mg; a graph could be drawn in terms of mg of the drug that were dissolved / released per time in seconds using the rate. Resveratrol dissolution rate was 0.42 mg/sec. and that of Curcumin was 0.14 mg/sec. Resveratrol Flux was 0.21 mg/sec./cm^2. Curcumin Flux was 0.14 mg/sec. / cm^2. Drug entrapment was 80% for both Resveratrol and Curcumin.
The 20 mg of Resveratrol and Curcumin then dissolved in 47.6seconds and 71.4 seconds respectively. However, in a practical sense, the film must first be hydrated in a small amount of saliva entrapped between the tongue and the upper palate. The rice starch will swell and disintegrate/dissolve because of the crashing effect of the tongue, then release the drug. The swelling index study revealed the average swelling index of the optimal formulation 16 to be 91.7 % for the plain films, 79.4 % for Resveratrol films and 73% for the Curcumin loaded films.

From the drug extraction studies (See chapter 5 page 242), the percentage recovery of Resveratrol was 84% and 59% for curcumin because the purity of curcumin was 77% and 98% for resveratrol from the manufactures' Certificate of Analysis (CA) (See Appendix H page 270 - 270). The percentage recovery was 16.07 mg for Resveratrol and 8.61mg for resveratrol out of 20 mg loaded in the 2cm^2 films .If the 77% impurity in the standard of Curcumin were accounted for, the percentage recovery would be higher. All the results are within experimental limits (See Table 4.41). This can explain the difference in the time lag and the actual amount of drug delivered into the blood stream letting alone the protein binding in the blood stream that may occur in the practical sense. Further *in vivo* studies need to be carried out to prove enhanced Bioavailability, but from this study, it can be verified that more than 50% 0f the drug loaded can be delivered to the blood stream.

It could also be appreciated that, if we got an intra oral film can be loaded with 20 mg of the drug and get it disintegrated in the oral cavity within 5-30 sec. or even after a minute 60 sec, this would qualify our drug delivery system to a fast dissolving intraoral film.
The dissolution experiment was done in a large volume of saliva (900 ml) and for a long time of 12 hrs due to practicality reasons. However, the rate of drug release is independent of the volume at Steady state saturation point or maximum drug release and can be used the calculate the flux in mg/ sec. cm^2 (Chandrasekaran et al., 2011); (Chou and Wollast, 1985); (Kunte and Tandale, 2010); (Ritschel, 1989).

Drug Dissolution revealed first order then later on zero order meaning that there was at first a steady linear release of the drugs and later a decline as the drugs get exhausted from the dosage form or drug delivery system. Again, formulation 16 had the highest moisture content of 30.3% for Resveratrol and 35.1% for Curcumin. It maintained a soft texture and was the most appealing (See) and (See). Taste tasting. Rice starch concentration played a significant role in the formulation of the film. The concentration of glycerine as plasticizer was also significant and must have contributed to the acceptable soft texture together with retention of the water content. It seems to be a potential candidate for the development of an intraoral fast dissolving (IDF) for the effective systemic delivery of the some essential natural drugs via the intraoral route.

Chapter 5. *Ex* vivo analysis of the drug delivery system

5.1 Introduction

5.1.1 Animal studies

5.1.1. 1. The swine animal model

The most important factors that are considered to select an appropriate animal for biomedical study include: the cost of the animal, ease of handling the animal, breeding time, and whether it meets size requirements (Swindle, 1992); (Swindle, 2015); (Pennington, 1992); (Sachs, 1992); (Bloor et al., 1992); (White et al., 1992). There is not even a single animal which perfectly meets all the requirements of an ideal animal model to substitute human studies (Pennington, 1992); (White et al., 1992). Though, in many cases, dogs, cats, rabbits, and rats have been used in the past, there is a growing difficulty in using these animals due to high cost and pressure from animal protective organizations (Swindle, 1992); (Swindle, 2015).

On the other hand, the use of pigs in *ex – vivo* studies has increased because of the similarities between the human and swine anatomy and physiology, and also meeting many of the requirements for the factors stated above (Sachs, 1992). None the less, it is very important that proper care and handling techniques of the pig is strictly adhered to (Bloor et al., 1992). With particular relevance to this study, white porcine buccal mucosal membranes have been used. The membranes have been surgically harvested and trimmed to the size of the Franz Diffusion Cell (0.9mm ± 0.1mm thick and about 20mm in diameter) from the heads of euthanized pigs obtained from the University animal house. The animals had already been sacrificed for other purposes. These membranes were used to simulate permeation and release characteristics of the rice paper films. Permission to use animal tissue was obtained from the University animal ethics committee (See Ethics permission letter Appendix D page 267).

Taste testing to determine the acceptability of the rice paper films was done using human volunteers at Charlotte Maxeke Emergency Unit. In a similar manner, Human ethics approval and the Distress protocol approval were obtained from the CEO of Charlotte Maxeke Academic Hospital and the Wits University Human ethics committee (See Distress protocol at Appendix E page 269 and Human ethics approval letter No. M140920 Appendix F. Extraction of the drugs from the formulation and the HPLC analysis of the recovered drugs were done and results reported.

5.2. Methods

5.2.1. Tissue isolation

The pig buccal membranes, which were harvested from the heads of the euthanized porcines obtained from the University of the Witwatersrand animal house, were separated from the underlying connective tissue by a surgical procedure. Upon collection, these membranes were placed in an icebox packed with ice packs at 4 °C to preserve them during transportation to the pharmacy department laboratory. The membranes were then transferred to a fridge at 4^0C for storage until the time of use. The membranes were used within 24 hours from the time of removal from the super deep freezer to prevent spoilage. They were mounted over the Franz diffusion cell.

The receptor chamber of the Franz Diffusion Cell was filled to the brim with 12 ml of simulated saline phosphate buffer at pH 7.4 to simulate the plasma since the drug will be absorbed into the jugular veins. Two Resveratrol film samples were cut to a size of 2cm x1cm and placed in a plastic conical centrifuge test tubes and 3ml of simulated saliva also pH 7.4 was added. The test tube was shaken for 5 minutes on a shaker to ensure dispersion of the films.3 ml was used to rinse the tube. A total of 6ml was used to disperse the film. 3ml was added to the donor chamber the remaining 3 ml were filtered through a 0.22µm filter and analyzed by UV/vis spectrophotometer to determine the initial concentration which was recorded. The donor chamber accommodated 3ml. Simulated saliva at pH 7.4 was used in the donor chamber for Resveratrol to maintain uniformity since curcumin only dissolved in simulated saliva at a pH of 7.4. The pig buccal membrane that was cut to a size 20mm diameter and a thickness of about 0.9mm± 0.1mm and previously stored at -80^0C was defrosted by adding purified water at room temperature and laid to fit on the receptor chambers. The Donor chamber was then placed over the membranes and clamped to the receptor chamber with a metallic clamp. The Franz Diffusion Cell was allowed to run for 15 min to equilibrate. Then the 3 ml of Resveratrol were poured into the donor chamber. The membranes of the porcines were mounted in triplicate on the three chambers of the Franz Diffusion cell. The Franz Diffusion Cell was maintained at 37^0C by circulating water from a warm water bath and the lower receptor chamber was stirred by a magnetic bead. The process was similarly repeated for Curcumin after proper cleaning of the Franz Diffusion Cell with purified water.

5.2.2 Administration of drug delivery system

Two rice paper films loaded with the model drugs and cut to a size of 2cm x 1 cm were dissolved in 6 ml of simulated saliva 3 of which were powered in the donor chamber on one side of the Franz diffusion cell. The samples were drawn from the 3 receptor chamber through the 3 arms of the of the Franz diffusion cell since the membranes were mounted in three's and withdrawn, filtered through a 0.22 m filter and stored in 10 ml polytopes pending analysis. 5 ml were drawn at 2 hour intervals of time.

5.2.3 Sampling of the permeated drug

The testing of the permeation was done in triplicate. The samples were drawn at 0, 2, 4, 6, 8, 10, and 12 hours from the receptor chamber. The same amount of 5 ml of fresh saliva was replaced. A sample of 3ml was initially drawn from the donor each chamber to determine the initial concentration on the serosal side of the membrane. At the end, the 3ml sample was drawn from the mucosal side across the membrane and the absorbances were measured to determine the initial and final concentration across the membrane. **A concentration difference was computed and later used to calculate the permeability coefficient detonated by the letter (P).** The testing span over a 12 hour period. Samples were filtered through a 0.22µm filter and stored in the deep fridge at 4°C until required for analysis. The Avian UV –Vis Spectrophotometer Avian Cary - 50 was used in this study.

5.2.4 ex vivo permeation studies

Drug release is defined as the amount of free or unreacted drug substance that passes from the film into the interstitial space per unit time under standardized conditions of liquid/solid interface, temperature and solvent concentration. The drugs first dissolved in the simulated saliva as the film swells before release and permeation. The dissolution medium was maintained at a temperature of 37 ± 0.5º C by a warm water jacket from heated water bath and stirred with a magnetic bead in built in the receptor cell which could simply be switched on. At pre-determined time intervals, samples were withdrawn and replaced with fresh dissolution medium. The samples were filtered through 0.22µm Millipore millex filter. Absorbance was measured using the Avian UV- VIS spectrophotometer. The permeability Coefficient, Drug release and the cumulative percentage of drug released were then calculated (Perioli et al., 2004); (Ilango et al., 1997).

Porcine (Pig) buccal mucosa was used as a barrier membrane. The pig buccal mucosa was harvested by a surgical procedure from euthanized pig heads from Wits University animal house The permeability across the buccal membrane was determined in order to evaluate permeation by using Franz diffusion cell (Patel et al., 2007); (Sekhar et al., 2008).

Simulated saliva was prepared at pH 7.4 using the Saline phosphate buffer tablets from Sigma Eldritch SA. The solution was adjusted to a pH of 7.4 by using a few drops of either 32% HCL or 10M NaOH. Each tablet was dissolved in 200ml of distilled water to yield the solution of 0.1M concentration and pH 7.4 to imitate that of blood plasma. Saliva could also be at the same pH as it varies from 6.5 - 7.5 (Gohel et al., 2009) (Jantratid and Dressman, 2009) (Marques et al., 2011).

5.2.4.1 *Ex Vivo* Release of the drugs

This parameter was determined by loading a single porcine membrane of dimensions (0.9 mm thick and approximately 20mm in diameter), in sets of 3s on modified Franz diffusion cell over a period of 12 hrs. This was repeated 3 times i.e. in triplicate. The film was dissolved in simulated saliva at pH 7.4 in the donor compartment. Saline phosphate buffer solution (simulated plasma) at pH 7.4 was filled in the receptor compartment and maintained at 37 ± 0.5°C and constantly stirred with the magnetic beads.

Franz Diffusion cell receptor compartment was filled with 12 ml of simulated plasma warmed to 37 ± 0.2º C with circulating water through the jacket from a heated warm water bath that is attached to the equipment. Initially the donor compartment was filled with simulated saliva at pH 6.8 (Adeleke, 2011), but later both chambers were filled with Simulated saliva pH 7.4 for uniformity since curcumin dissolved only in alkaline saliva pH 7.4 and it was suggested that the films would be administered at a fasting buccal pH of 7.4 for maximum absorption, i.e. One hour before or one hour after meal. Drug concentration was analysed, after filtration using the Avian UV/Vis Spectrophotometer loaded with Cary - 50 software and programmed at 315nm and 420nm. Wavelengths (λmax) for Resveratrol and Curcumin respectively. All samples were filtered using a 0.22µm Millipore Millex filter (Doijad et al., 2006). The Absorbance was measured in triplicate (Patel et al., 2012).

5.2.4.2 Permeation of the model drugs curcumin and resveratrol through porcine buccal mucosa

The study was done on the optimal batch 16 that was the most appealing having eliminated the others on grounds of robustness. Concentration was measured by using the Avian Spectrophotometer and employing Beer Lambert Law. Absorbance Readings were taken using the Avian - Cary 50 software loaded in the Spectrophotometer and the concentration was calculated employing Beer Lambert's law.

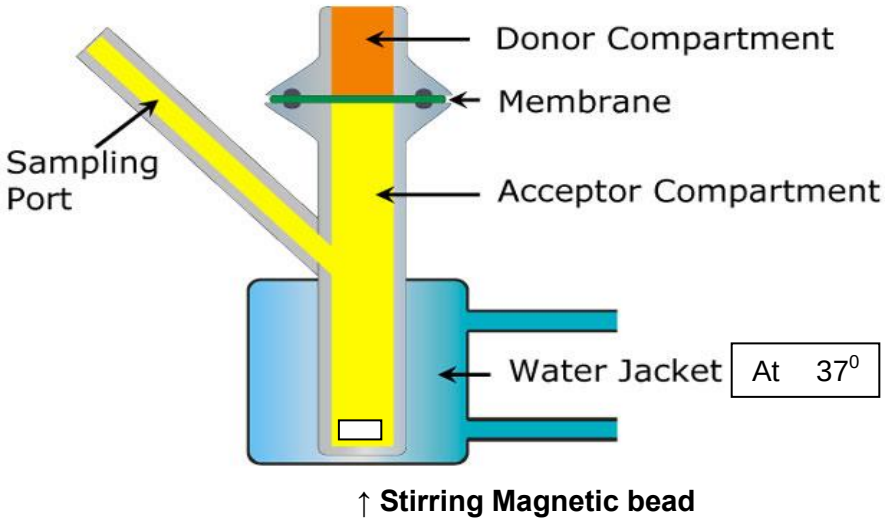


Figure 5.1: The Franz Diffusion Cell.
www.google.co.za/search?q=images+of+franz+diffusion+cell&biw (Accessed on 09/01/16).

It should be noted that simulated saliva buffered at pH 7.4 was used to dissolve the film in the donor chamber of the Franz Diffusion Cell and the same simulated saliva buffer at pH 7.4 was used in the receiving chamber of the Franz Diffusion Cell, as this was also the pH of blood plasma (See Figure 5.1). This is now so because both drugs dissolve in saliva at the pH of 7.4 and would eventually be absorbed intraorally into the intraoral veins. This leads to a suggestion that the films will have to be administered at the staving buccal pH of 7.4 one hour either before meal or one hour after meal. The calibration curve for the Franz Diffusion Cell concentrations was drawn using the Avian Spectrophotometer for both Resveratrol and Curcumin respectively.

5.2.5 HPLC analysis / UV-Vis Spectrophotometry

Concentrations per ml of the extracted samples in saliva fluid were determined using HPLC analysis and concentration of the permeation of the samples were determined using the UV-Vis. Spectrophotometer as outlined in the procedure (See Figure 5.2) below. Three (3) samples of each drug were used in this study.

Below is a schematic flow diagram of the permeation and the extraction study on the sample films;

Mounting of the porcine membrane onto the Franz diffusion cell in simulated saliva

↓

Administration of drug delivery system

↓

Sampling of permeated drug / Extraction of the drugs

↓

HPLC / UV - Vis Spectrophotometry analysis

Figure 5.2: The flow diagram for the HPLC and spectrophotometry Analysis

5.2.5.1. Extraction and HPLC analysis of the rice paper film loaded with resveratrol

5.2.5.2. Reagents

Trans-Resveratrol was purchased from Sigma (St. Louis, MO, USA). Acetonitrile (ACN) Ethyl Acetate and methanol (MeOH) 99.9 % were purchased from Romil Ltd Cambridge, UK and were HPLC grade. Distilled water was freshly demonized and filtered on0.22 µm Millipore filter water purification system (Milli-Q gradient, Mass, USA).

5.2.5.3 Preparation of calibration standard solutions and determination of the limit of Quantification for Resveratrol.

Trans-Resveratrol was dissolved in 98.0% v/v Acetonitrile (ACN) to make a stock solution of 1000µg/ l^{-1}. This was stable for at least 12 weeks at 4°C when protected from light. Carbamazepine (5 Hydroxy-5 benzapine-5 Carboxamide) Internal standard was purchased from sigma and was stable when stored in a fridge in dark brown glass containers and Simulate saliva (pH 6.8) was prepared as phosphate buffer solution. The pH of saliva is usually in the range of 6.5 **-** 7.5 (See Human Digestive Tract pH Range Chart (See Figure 4.5) and, (See Table 5.10:) and (See Table 5.11:) (Gohel et al., 2009) (Jantratid and Dressman, 2009) (Marques et al., 2011).

Working solutions were prepared in 98% v/v ACN. A stock solution of 1000g/l was prepared in the following manner. 50 mg of Resveratrol were accurately weighed on an analytical balance and dissolved in 500 ml of ACN 98 % v/v in a 500 ml volumetric flask to yield a solution of 0.1mg/ml. Then, 1 ml contained 0.1 mg of Resveratrol. 1ml, 2ml, 3ml, 4ml, and 5ml were accurately measured from it using a pipette and placed in a 15 ml conical plastic centrifuge test tube. 4ml, 3ml, 2ml, 1ml, and 0 ml of ACN mobile phase were added to the test tubes respectively. Finally, 5ml of the internal standard (IS) i.e., Carbamazepine for Resveratrol corresponding to a concentration of 0.5mg/ml which yielded to about half the height of the peak of that of the least concentration of the Resveratrol peak were added to each test tube to make a total volume of 10ml of the standard solutions. A constant volume of the (IS), i.e., 5ml were added to each test tube. The corresponding concentrations were 0.2mg/ ml, 0.4 mg/ ml, 0.6 mg/ml, 0.8mg/ ml and 1.0 mg/ml in ascending order.

20 µl of each sample were injected in the Perkin Elmer HPLC for qualitative analysis of Resveratrol and the peaks for Resveratrol and Carbamazepine were obtained. The area under

the curve of Resveratrol was divided by the Area under the curve (AUC) of Carbamazepine to get the (y - ratio). This was plotted on the y- axis against the concentrations on the x- axis to construct the linear calibration graph. The (y-value) of the extracted drug sample was obtained in a similar way on the HPLC.

This was substituted in the linear equation:

$$y = mx + c. \qquad \textbf{Equation 5.1}$$

From the linear calibration graph, the concentration of the drug (x) was Calculated from the substitution equation:

$$x = (y - c) / m \qquad \textbf{Equation 5.2}$$

Where x = concentration of the drug, y = AUC ratio of the drug: (IS), c = constant of the linear calibration graph, and m = the gradient of the linear calibration graph. The mobile phase was filtered and degassed through a 0.45µ filter. First the organic phase then the water. The working solutions were filtered through a 0.22µm filter before injection into the HPLC column to prevent any blockage in the column. The injection needle was also flashed with Acetone after each use. The set up time was 30 minutes and the washout period was 1 hour (Katsagonis et al., 2005); (Zhu et al., 1999).

The HPLC was used later only for the Extraction methods for both Curcumin and Resveratrol where the samples were sprinkled with the internal standards 4-Hydroxybezophenone and Carbamazepine respectively. The means of the least square was plotted and the linearity equation and correlation coefficient (R^2) were obtained. R^2 for the calibration curve using the HPLC method was 0.9986 for Resveratrol and for curcumin calibration curve R^2 was 0.9972.

As mentioned before, in chapter four, the calibration curve for the permeation studies was dawn using the Avian UV-Vis Spectrophotometer due to the long time break down of the HPLC equipment. The UV / Vis Spectrophotometer was used for the permeation study to save the cells of the buccal tissue membrane from dying as these cells would not be viable after a month even when stored at -80^0 C. The UV/Vis Spectrophotometer concentrations were calculated from the absorbance to draw the calibration curve to get the linear equation (See tables 5.3). The limit of quantification (LOQ) was determined by calculating as the lowest detectable concentration of Resveratrol and was found to be 7.95 ng/ml with a correlation coefficient (R^2) = 0.9916. The limit of quantification (LOQ) for Curcumin was 7.377 and R^2 was 0.9916.

5.2.5.4 Sample preparation

A Binary HPLC (pump Perkin Elmer SA) coupled to a manual injection sampler, and on a Pheomenex C18 (250mm × 4.6 mm, 5µm) column was set in the Reverse-phase mode. The mobile phase was Acetonitrile: Phosphate buffer pH 4.2 (30: 70, v/v) and the flow-rate (isocratic) was set at 1 ml/min. Dissolved Resveratrol IDF samples were previously stored in a deep freezer at (-80°C). They were removed from the freezer and thawed. A 150µL aliquot of each sample was transferred to a centrifuge tube. ACN (150µL) and deionised water (50µL) were added to each vial and vortexed. Each tube was subsequently centrifuged at 3000 rpm for 15 minutes (Optima® LE-80K, Beckman, USA) (Katsagonis et al., 2005); (Zhu et al., 1999).

The supernatant was removed and subjected to the extraction procedure and spiked with (50µL) of the Carbamazepine internal standard. The samples were then placed in the certified vials for analysis. The samples of (20 µl) were drawn from the vial and directly injected into the HPLC through the manual sampling loop and the absorbance monitored at a wavelength of 315 nm using a 2489 variable-wavelength UV detector (Perkin Elmer SA).

Purity checks were performed by measuring the absorbance at 3 points on the spectrum (upswing, downswing, and peak) and the ratios for unidentified peaks were compared with those of the pure compound using Chromera software provided by the manufacturer, which calculated a quantitative estimate of spectral identity. The Gradient and the flow rate were set at the following parameters: Flow rate (1.00 ml/min), the mobile phase Acetonitrile (A 30%): Phosphate Buffer pH4.2 (B70 %)

5.2.5.5. Method and operation of the HPLC

Chromera software was used for the HPLC analysis. Set up time was run for 30 min. The mobile phase was filtered through a 0.45µm filter. First, the organic mobile phase (B) then the Aqueous phase (A). The pump was switch on. Then pump A and B were degassed or purged by using a 10ml syringe manually fitted onto the degassing loop on the HLPC. About 5ml of each of the mobile phase were drawn to make sure all entrapped air bubbles were eliminated from the tubbings. Wash out time was run for one hour.
The Chromera software was started. The password was entered as – (Elective Mukasa Group A). The HPLC parameters were set as specified above and proceeded to put the Detector on and set Auto zero to obtain a straight baseline.

Choose the wavelength at 315 nm, saved the Date, profile as - Mukasa 27 11 14, and saved the sequence. Equilibrated the system to monitor the baseline and choose Mukasa 27 11 14 to equilibrate the HPLC. The pumps started automatically on the control panel, the system indicated green on the Toolbar. Choose Monitor flow and pressure; and set the mobile phase percentage ratios and system in sequence mode sampling run for 30 minutes on the Control panel. Then clicked Run.

5.2.5.6. Extraction of Resveratrol from rice paper film samples

The rice paper film of dimensions 2cm x 1 cm (cm^2) and containing 20 mg of Resveratrol was cut and placed in a plastic, graduated (10ml), transparent, , and capped, conical test tube, 3ml of simulated plasma were added. 100 µl of internal standard, Carbamazepine, (50 µg / ml) were added to the tube and mixed on a magnetic shaker for 15 seconds. 0.4 ml of the Phosphate buffer for extraction at a pH 6 was added and mixed for another 15 seconds. 3ml of Ethyl Acetate was added to each tube to extract the Resveratrol then the mixture was centrifuged for 5 minutes at 3000 rpm. The top organic layer was carefully removed and placed in a 10 ml plastic jar. The extraction process was repeated three (3) times to exhaust the organic layer and the extracts were added to the 10 ml plastic jar. The whole extract was evaporated to dryness in the vacuum oven set at negative (-ve) 6 psi and 40^0C. The dry residue crystals were reconstituted in 6 ml of the mobile phase (ACN) and filtered through a 0.22 µm filter. One (1 ml) of this was diluted to 100ml, as the concentration of the expected drug was high for the HPLC to measure i.e. beyond the absorbance of 3000nm as could be seen by a ceiling in the chromatogram. An aliquot of 20 µl was directly injected into the HPLC to retrieve the results (Katsagonis et al., 2005). The recovery of Resveratrol in this assay was 84.16 %

5.2.5.7. HPLC analysis of the rice paper film loaded with curcumin

5.2.5.8 Reagent

Curcumin 77%.w/w was obtained from Sigma Aldrich China. 4-Hydroxybezophenone 98% internal standard (IS) was obtained from Sigma Aldrich UK. Methanol 99.9 % v/v and Acetonitrile (chromatographic grade) was purchased from Romil Ltd Cambridge UK, and was HPLC grade. Citric acid was analytical grade was purchased from Rochelle Chemicals (Johannesburg, South Africa) KH_2PO4 was purchased from Rochelle Chemicals (Johannesburg, South Africa). Demonized (18.2 MΩ/cm) water was generated in-house using Milli-Q System from Millipore (Bedford, MA, USA).

5.2.5.9 Preparation of calibration standard solutions and determination of the limit of quantification.

Curcumin was dissolved in 99.9% v/v Methanol to make a stock solution of 1000µg/ l. This was stable for at least 12 weeks at 4°C when protected from light. 4- Hydroxybenzophenone, Internal standard (IS) was purchased from sigma and was stable when stored in a fridge in dark brown glass containers. Simulate saliva (pH 6.8) was prepared as phosphate buffer solution. The pH of saliva is usually in the range of 6.5 **-** 7.5 (See Human Digestive Tract pH Range Chart (See Figure 4.5) (Marques et al., 2011); (Jantratid and Dressman, 2009); (Gohel et al., 2009).

Working solutions were prepared in 99.9% v/v Methanol. A stock solution of 1000g/l was prepared using the following ways: 50 mg of Curcumin were accurately weighed on an analytical balance and dissolved in 500 ml of Methanol 99.9 % v/v in a 500 ml volumetric flask to yield a solution of 0.1mg/ml. Then, 1 ml contained 0.1 mg of Curcumin. 1ml, 2ml, 3ml, 4ml, and 5ml were accurately measured from it using a pipette and placed in a 15 ml conical plastic centrifuge test tube. 4ml, 3ml, 2ml, 1ml, and 0ml of Acetonitrile (ACN) mobile phase were added to the test tubes respectively. Finally, 5ml of the internal standard (IS) i.e., 4-Hydroxybenzophenone for Curcumin corresponding to a concentration of 0.5mg/ml which yielded to about half the height of the peak of that of the least concentration of the Curcumin peak were added to each test tube to make a total volume of 10ml of the standard solutions. A constant volume of the (IS), i.e., 5ml were added to each test tube. The corresponding concentrations were 0.2mg/ ml, 0.4 mg/ ml, 0.6 mg/ml, 0.8mg/ ml and 1.0 mg/ml in ascending order.

20 µl of each sample were injected in the Perkin Elmer HPLC for qualitative analysis of Curcumin and the peaks for Curcumin and 4-Hydroxybenzophenone were obtained. The area under the curve on curcumin was divided by the Area under the curve (AUC) of 4-Hydroxybenzophenone to get the (y - ratio). This was plotted on the y- axis against the concentrations on the x- axis to construct the linear calibration graph. The (y-value) of the extracted drug sample was obtained in a similar way on the HPLC.
Where x = concentration of the drug, y = AUC ratio of the drug: (IS), c = constant of the linear calibration graph, and m = the gradient of the linear calibration graph.

The mobile phase was filtered and degassed through a 0.45µ filter. First the organic phase then the water. The working solutions were filtered through a 0.22µm filter before injection into the HPLC column to prevent any blockage in the column. The injection needle was also flashed with Acetone after each use. The set up time was 30 minutes and the washout period was 1 hour (Katsagonis et al., 2005); (Zhu et al., 1999).

The HPLC was used later only to measure the concentration of the drug extracted from the sample films. The HPLC was used later only for the Extraction methods for both Curcumin and Resveratrol where the samples were sprinkled with the internal standards 4-Hydroxybezophenone and Carbamazepine respectively. The means of the least square was plotted and the linearity equation and correlation coefficient would be obtained.

As mentioned before, in chapter four, the calibration curve for the permeation studies was dawn using the Avian UV-Vis Spectrophotometer due to the long time break down of the HPLC equipment. The UV / Vis Spectrophotometer was used for the permeation study to save the cells of the buccal tissue membrane from dying as these cells would not be viable after a month even when stored at -80^0 C. The UV/Vis Spectrophotometer concentrations were calculated from the absorbance to draw the calibration curve to get the linear equation (See Figure 4.27) and, (See Figure 4.34). The limit of quantification (LOQ) was determined by calculating as the lowest detectable concentration of Resveratrol and was found to be (n) = 7.377 µg/ml with a correlation coefficient (R^2) = 0.9916.

5.2.5.10 Sample preparation

A Binary HPLC (pump Perkin Elmer SA) coupled to a manual injection sampler, and on a Pheomenex C18 (250mm × 4.6 mm, 5µm) column was set in the Reverse-phase mode. The mobile phase was water-acetonitrile: Citric Acid 1 % at pH 3 (55:45, % v/v) and the flow-rate (isocratic) was set at 1 ml/min. Dissolved Curcumin IDF samples previously stored in a deep freezer were removed from the freezer at (-80°C) and thawed. A 150µL aliquot of each sample was transferred to a centrifuge tube. Methanol (150µL) and demonized water (50µL) was added to each vial and vortexed. Each tube was subsequently centrifuged at 3000 rpm for 15 minutes (Optima® LE-80K, Beckman, USA) (Ma et al., 2007).

The supernatant was removed and subjected to the extraction procedure and spiked with (50µL) of 4-Hydroxybenophenone internal standard. The samples were then placed in the certified vials for analysis. The samples of (20 µl) were drawn from the vial and directly injected into the HPLC and the absorbance was monitored at a wavelength of 428 nm using a 2489 variable-wavelength UV detector (Perkin Elmer SA). Purity checks were performed by measuring the absorbance at 3 points on the spectrum (upswing, downswing, and peak) and the ratios for unidentified peaks were compared with those of the pure compound using Chromera software provided by the manufacturer, which calculated a quantitative estimate of spectral identity. The Gradient and flow rate were set in the gradient mode at the following parameters: Flow rate at (1.00 ml/min), Mobile phase A (Acetonitrile 55%): B (1% Citric acid Buffer pH 3, 45 %). The wavelength was changed from 300 nm for the 4 – Hydroxybenzophenone to 428 nm for Curcumin. Therefore, a gradient mode was set.

5.2.5.11 Procedure for operating the HPLC

Chromera software was used for the HPLC analysis. Set up time was run for 30 min. The mobile phase was filtered through a 0.45µm filter. First the organic mobile phase (B) then the Aqueous phase (A). The pump was switch on then pump A and B were degassed by using a 10ml syringe manually fitted onto the degassing loop on the HLPC. About 5ml of each of the mobile phase were drawn to make sure all entrapped air bubbles were eliminated from the tubbings. Wash out time was run for one hour.

The Chromera software was started. The password was entered as – (Elective Mukasa Group A). The HPLC parameters were set as specified above and proceeded to put the Detector on and set Auto zero to obtain a straight baseline. Chose the wavelength at 420 nm, saved the Date, profile as- Mukasa 28 01 16, and saved the sequence. Equilibrated the system to monitor the baseline and chose Mukasa 28 11 14 to equilibrate the HPLC. The pumps started automatically on the control panel, the system indicated green and proceeded to view and selected Toolbar. Chose Monitor flow and pressure; and set the mobile phase percentage ratios of the Mobile phase (A) Acetonitrile 55% and (B) 1 % citric acid Buffer pH 3, 45% and run the system in the saved sequence mode sampling run for 30 minutes and Flow rate 1.0 (ml/min) on the Control panel. Then clicked Run and started the Detector and chose the wavelength 428 nm, dated the profile - Mukasa 28 01 16 and saved. Equilibrated the system to monitor the baseline. Chose Mukasa 28 01 16 to equilibrate the HPLC. The pumps started automatically. Went to control panel, as the system will indicate green. Choose Monitor flow and pressure; set the mobile phase and system set at run for 30 minutes. Run at wavelength 428 nm and saved the sequence. Inject and run the sample set under profile Mukasa 28 01 16.

5.2.5.12. Extraction procedure of Curcumin from the rice paper film samples. Analysis is then perfumed by HPLC

The rice paper film of dimensions 2cm x 1cm ($2cm^2$) and containing 20mg of curcumin was cut and placed in a 10 ml conical, transparent, graduated, and capped plastic test tube. 3ml of 10% simulated Plasma were added. The tube was put on a shaker to dissolve the film. The suspension was acidified to pH 3.0 with 6N HCI and 6ml of ethyl acetate-propanol (9:1, v/v) was added to the test tube to extract the Curcumin. The suspension was then centrifuged at 3000 rpm for 15 minutes to separate the organic layer. This was placed in a 10 ml plastic jar and 100µl of 4 - Hydroxybenzophenone internal standard (IS) 50 µg/ml was added. The organic layer was dried in a vacuum oven set at 40^0C under a pressure of – 0.6 psi. The dried residue was then reconstituted with 6ml of ACN mobile phase. This was filtered through a 0.22µm filter and stored in approved vials in a freezer at -80^0C ready for analysis with the HPLC.

One (1 ml) of this was diluted to 100ml as the concentration of the expected drug was high for the HPLC to measure i.e. beyond the absorbance of 3000nm as could be seen by a ceiling in the chromatogram. 20µl were drawn and injected into the HPLC for analysis following the procedure for the analysis of curcumin described above. The wavelengths of the detection

were set at 300nm 4 - Hydroxybenzophenone internal standard and changed manually or set in a sequence (gradient mode) to change to 428 nm after the peak of 4 - Hydroxybenzophenone internal standard and for Curcumin (Wang et al., 1997); (Ma et al., 2007). The recovery of curcumin in this assay was 55.95 %.

5.2.6 Taste testing

Palatability study is conducted on the basis of taste, after bitterness, and physical appearance. The Taste testing study was done at Charlotte Maxeke Academic Hospital Surgical Unit to score the degree of acceptability. Prior to the test, the study was presented to the doctors to inform them about the film. The poster is shown in (See Figure 6.1 Poster presentations Appendix K). Then the panelist of volunteers was formed from the volunteer doctors. The ethical protocol was strictly followed. A questionnaire was administered to help score the sweetness taste of the rice paper film. The questionnaire is attached in appendix K. The rice paper films did not leave any unpleasant residues in the mouth and was rated innovative and desirable.

5.2.7 In vivo Dissolution of the Rice paper films

5.2.7.1 Procedure to follow to establish the time the film takes to dissolve in the mouth

1 A healthy volunteer not on any medication must be used as a exclusion criteria

2 The volunteer must be fully informed of the safety of the formulation and the purpose of the study.

3 The volunteer must sign the informed consent form and will be given a simple step by step instruction to be followed exactly.

4. The instructions are as follows;

(a) Rinse your mouth three times with purified water and spit the risings into the container provided.

(b) Collect some saliva between the tongue and the upper jaw. Open the mouth and put the small piece of the film on the tongue and hold for three minutes for the film to soak. Please **DO NOT SWALLOW.**

(c) Start to rub the film between the tongue and the upper palate until you feel the film is dissolved and open the mouth.

(d) You may swallow the excess saliva but not the one trapped between the tongue and the upper jaw.

(e) The time that the film has taken to dissolve will be recorded.

(i) Resveratrol…....seconds. (ii) Curcumin…...seconds.

(f) Rinse your mouth three times and wait for 5 minutes and repeat the test for the second film following the same procedure from (a to f).

5. The procedure will be witnessed by three witnesses who must sign on this form

(i)	Name	Signature	Date
(ii)	Name	Signature	Date
(iii)	Name	Signature	Date

	Name	Signature	Date
Developed by	……	……..	……
Approved by	……	…….	…….

,

The Resveratrol and Curcumin films were inserted on the tongue and the actual time the films took to dissolve in the mouth was recorded on three Healthy volunteers. Again the Human ethics procedures and the protocol were strictly followed.

5.3 Results and Discussion

The calibration was calculated using only the least recorded absorbance to get the LOQ and in the concentration that ranged from 10 µg/ml – 100 µg/ml. The Regression Coefficient was good at 0.9916. The lower limit of quantification (LOQ) in simulated plasma for Resveratrol in this study was determined to be (n) = 7.377 µg/ml. The same pH as that of plasma as has already been noted that the pH of saliva ranges from 6.5 – 7.5. This is because the Curcumin standard sample that we used was not the soluble curcumin. It dissolves at pH 7.4 alkaline media.

5.3.1 *Ex vivo* Resveratrol release profile from the rice paper films

According to the Certificate of analysis (C/A), Resveratrol original sample from Sigma Aldrich, the original supplier, curcumin was only 98%. The remaining 2 % was an unknown impurity (See Appendix G). The assumption is that did not the composition of the film but only the yield, i.e. percentage Recovery.

Table 5.1: Permeation of Resveratrol through porcine buccal mucosa over a 12 hour period in simulated saliva buffer pH 7.4 and in simulated plasma at pH 7.4.

Time(Hr.)	AVG Abs B/No. 1	AVG Abs B/No. 2	AVG Abs B/No. 3	AVG	STDEV	CV %	Concn. mg/ml	Cumm Drug Release %
0	0	0	0	0	0	#DIV/0!	0.056	0.023
2	0.84	0.85	0.84	0.84	0.0049	0.59	**6.73**	2.83
4	0.72	0.70	0.70	0.71	0.0099	1.40	5.66	5.19
6	0.60	0.592267	0.59	0.59	0.0083	1.40	4.77	7.17
8	0.50	0.50	0.49	0.50	0.0065	1.30	3.98	8.83
10	0.41	0.42	0.41	0.41	0.0065	1.57	3.35	10.22
12	0.38	0.37	0.38	0.38	0.0053	1.41	3.046	11.50

$y = mx + c$

$y = 0.126X - 0.007$ $R^2 = 0.997$

Concn = $X = (Y + 0.007)/0.126$

Y = Absorbance

Substituting in Absorbance

Cumm Drug Release % = Sample volume/Bath volume * Concn. At time t +Concn. Previous to time t

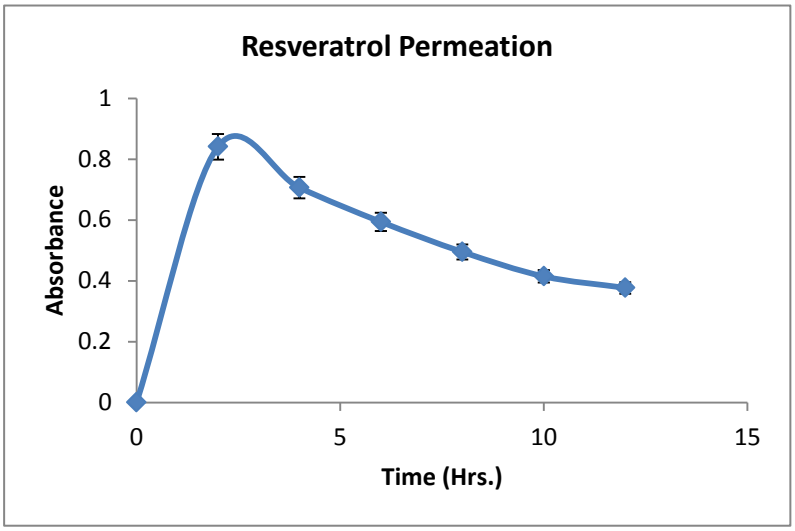


Figure 5.3: The *ex vivo* profile for Resveratrol permeation from the rice paper intraoral Drug Delivery system.

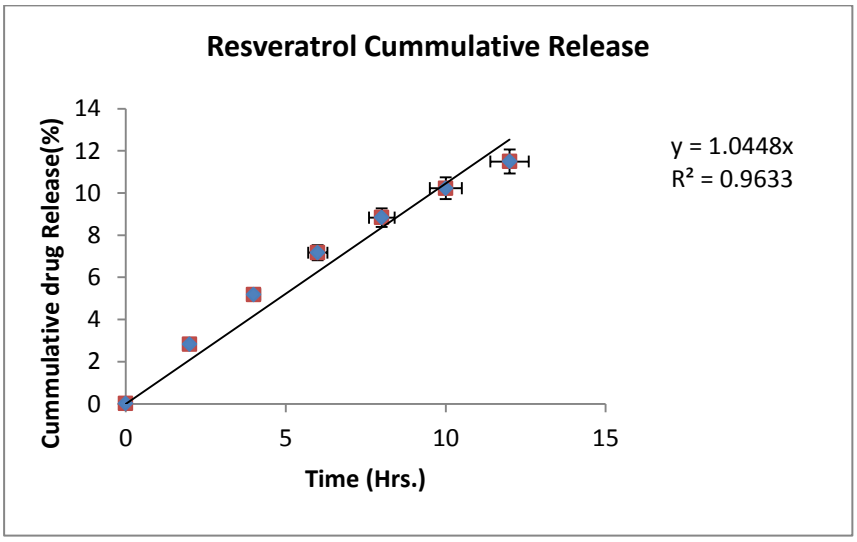


Figure 5.3: The Cumulative Drug Release profile of Resveratrol from the Rice paper films.

Table 5.2: Drug (mg) Released in Time in seconds (s).

Time (Sec)	Amount of Drug (mg)	Amount of Drug (µg)
0	0	0
1	**2.41**	**2410**
2.07	5	5000
4.14	10	10000
6.22	15	15000
8.3	**20**	**20000**
10	20	20000
20	20	20000
30	20	20000
40	20	20000
50	20	20000
60	20	20000

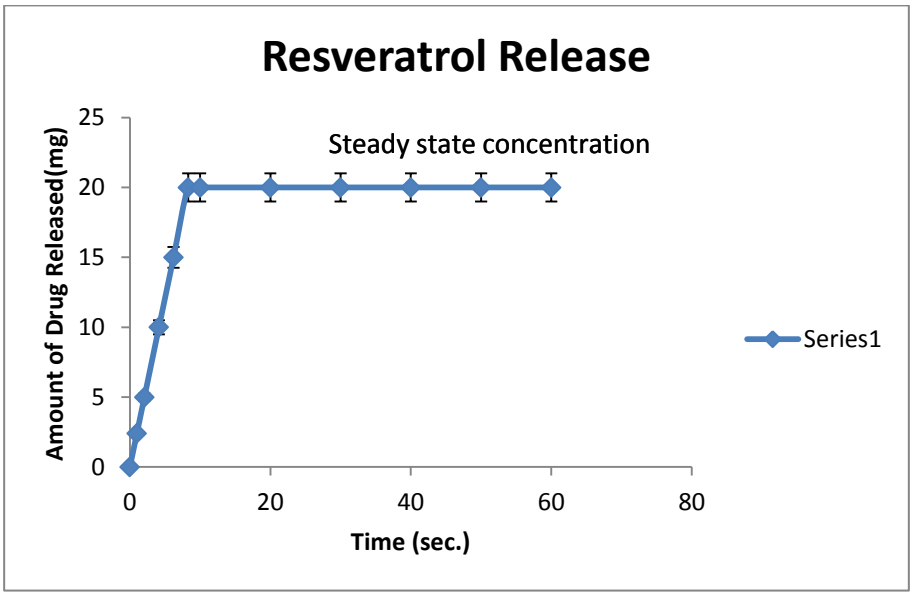


Figure 5.4: Drug Release profile of Resveratrol from the Rice paper films in mg/sec.

The release is burst release. Almost all the drug is released.

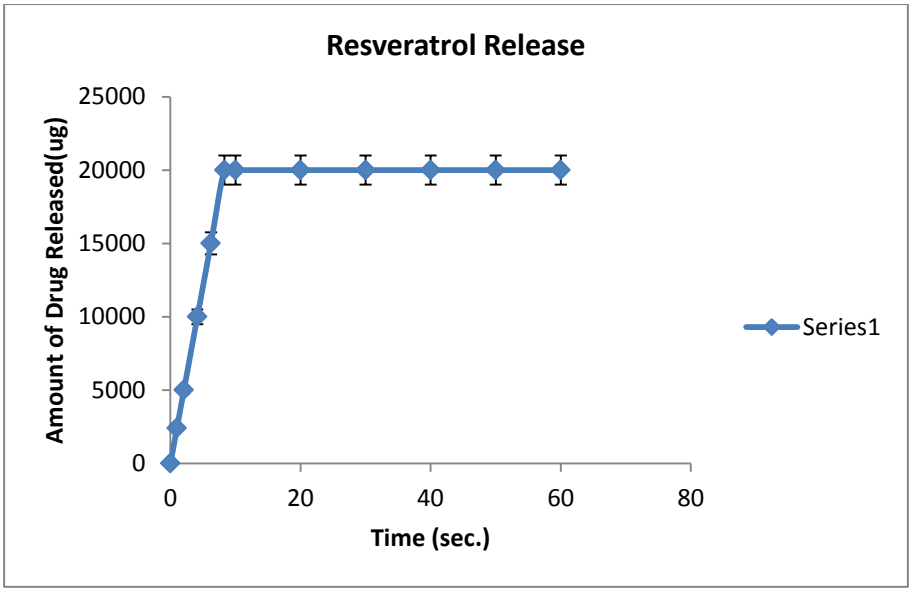


Figure 5.5: Drug Release profile of Resveratrol from the Rice paper films in µg/sec.

Cumulative Drug Release

(1) Formula for determination of percentage of release of drug from in vitro dissolution testing:

Concentration of drug (µg/ml)= [(Absorbance) ± intercept]/slope

Amount of drug released mg/ ml = Concentration × Dissolution bath volume × dilution factor/1000.

Cumulative percentage release (%) = Volume of sample withdrawn (ml)/bath volume (v) × P (t – 1) + Pt

Where Pt = Percentage release at time t Where P (t – 1) = Percentage release previous to 't'

(2) **Data**:

$y = 0.126x - 0.007$ $(R^2 = 0.997)$

Concn. (x) µg/ml = [y (Absorbance)+ 0.007]/0.126

Volume of the receptor chamber of the Franz Diffusion Cell = 12ml

Dilution factor = 20mg / 12ml = 1.67

Amount of Drug released in mg/ml = Concn. X 12 x 1.67 / 1000 for the 7 readings at Time (Hrs.) 0, 2, 4, 6, 8, 10, 12

We shall later express this in seconds since our film is a fast dissolving (IDF), and in the conventional units of µg/ml

Percentage of drug released at time (t) = Amount of drug released (mg/ml) /Original Amount of Drug loaded in the sample

Volume of sample withdrawn = 5 ml

Volume of methanol added 0.25 ml

Total volume of sample = 5.25 ml

Cumulative percentage release (%) = Volume of sample withdrawn (ml)/bath volume (v) × P (t – 1) + Pt

Where Pt = Percentage release at time t Where P (t – 1) = Percentage release previous to ‘t

Cummulative percentage Release (%) = 5.25/12 X Previous percentage release (P(t-1)) + Present percentage release at time t

$y = mx + c$ **Equation 5.1**

$y = 0.126X - 0.007$ $R^2 = 0.997$

Concn = $X = (Y + 0.007)/0.126$

Y = Absorbance

Table 5.3: Resveratrol Concentration Difference in the donor chamber

Resveratrol	1	2	3	AVG Abs	Concn. mg/ml	Concn. Difference(C)
Initial Abs	0.92	0.92	0.92	0.92	0.0074	**0.0042**
Final Abs	0.39	0.39	0.39	0.39	0.0031	

Flux data were plotted as the cumulative amount of Resveratrol that diffused from the mucosal to the serosal side of the epithelium versus time.

The permeability coefficient, P, was calculated from the formula:

Permeability coefficient P = (d*Q*/d*t*) / (*C. A*)

In which d*Q*/d*t* = 1.044 (%) at steady-state slope of the cumulative flux curve

C is the concentration difference from the mucosal side to the serosal side across the buccal membrane = 0.004212 mg/ml

and A (πr^2) is effective cross-sectional area available for diffusion.

Diameter = 15 mm Radius = 7.5 mm = 0.075cm

$= 22/7*(0.075)^2 = 0.0176785 = 0.0177 cm^2$

Slope of the cummulative drug release (%) = **1.044**

i.e., 1.044 g (%) are released in one hour

Therefore 1.044 *1000 mg(%) are released in 3600 seconds

Flux

In 1 sec (1.044*1000)*100/3600 mg are released = **29mg**

P = (d*Q*/d*t*) / (*C. A*)

C, the Concn. Difference across the pig buccal mucosal membrane from the mucosal side to the serosal side = 0.004212mg/ml

The Flux (dQ/dt) in mg/sec. = 29 as seen above.

The flux Coefficient P = (dQ/dt) / (C. A) =29/0.004212*0.0177 = **389261 µg/sec.cm^2**

= **389 mg/sec/cm^2.**

5.3.2 *Ex vivo* Curcumin release profile from the rice paper films

According to the Certificate of analysis (C/A), Curcumin original sample from Sigma Aldrich, the original supplier, curcumin was only 77%. The remaining 33% was an unknown impurity (See Appendix F). The assumption is that this did not affect the composition of the film but only the yield, i.e. percentage Recovery.

Table 5.4: Permeation of Curcumin through porcine buccal mucosa in simulated saliva buffer pH 7.4 and in simulated plasma at pH 7.4

Time(Hr.)	AVG Abs B/No. 1	AVG Abs B/No. 2	AVG Abs B/No. 3	AVG	STDEV	CV %	Concn. mg/ml	Cum Drug Release %
0	0	0	0	0	0	#DIV/0!	0	0
2	0.20	0.21	0.22	0.21	0.011	5.26	**0.061**	0.026
4	0.21	0.23	0.24	0.23	0.016	7.40	0.067	0.037
6	0.18	0.18	0.21	0.19	0.02	8.16	0.056	0.06
8	0.17	0.16	0.19	0.17	0.016	9.51	0.05	0.08
10	0.10	0.09	0.11	0.11	0.014	13.03	0.032	0.10
12	0.11	0.089	0.10	0.10	0.010	10.37	0.03	0.11

y = mx +c

y = 3.374X - 0.000 R^2 = 0.997

Concn = X = (Y +0)/3.374

Y = Absorbance

Substituting in Absorbance

Cumm Drug Release % = Sample volume/Bath volume * Concn. At time t +Concn. Previous to time t

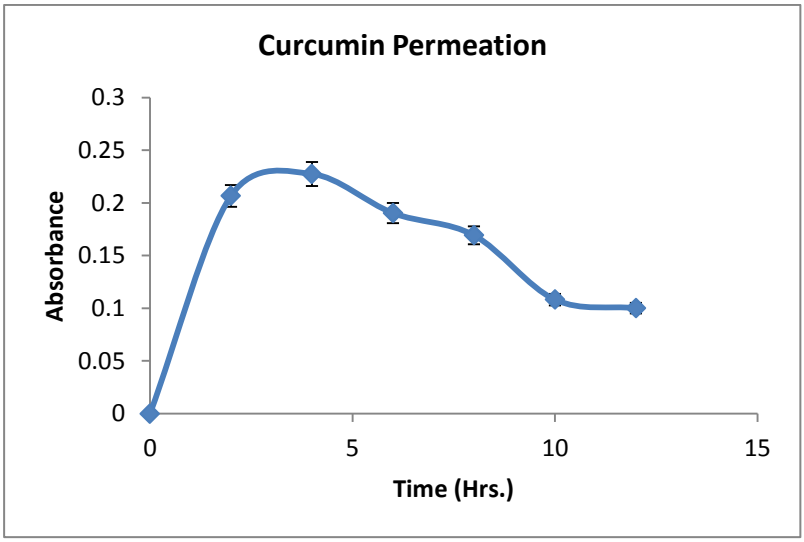


Figure 5.6: The ex vivo profile for curcumin permeation from the rice paper intraoral Drug Delivery system

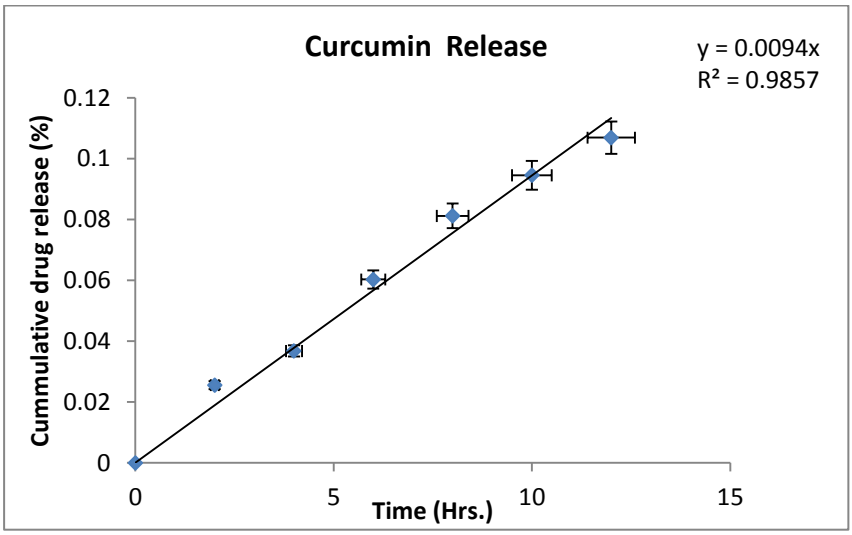


Figure 5.7: The Cummulative Drug Release profile of Curcumin from the Rice paper films

Table 5.5: Curcumin (mg) Released in Time in [seconds (s)] at steady state.

Time (Sec)	Amount of Drug (mg)	Amount of Drug (µg)
0	0	0
1	**1.65**	**1650**
3	5	5000
6	10	10000
9	15	15000
12.12	**20**	**20000**
15	20	20000
20	20	20000
30	20	20000
40	20	20000
50	20	20000
60	20	20000

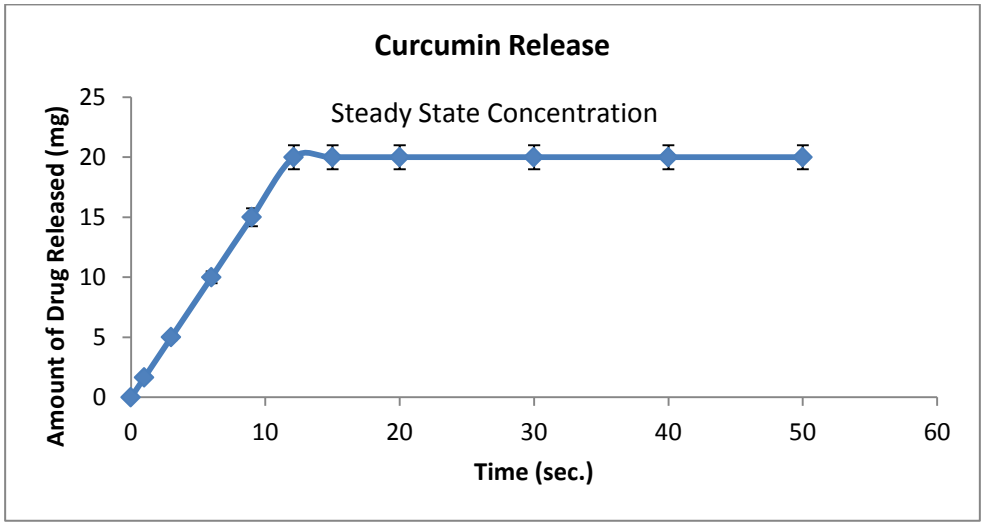


Figure 5.8: Drug Release profile of Curcumin from the Rice paper films in mg/sec.

The release is burst release. Almost all the drug is released.

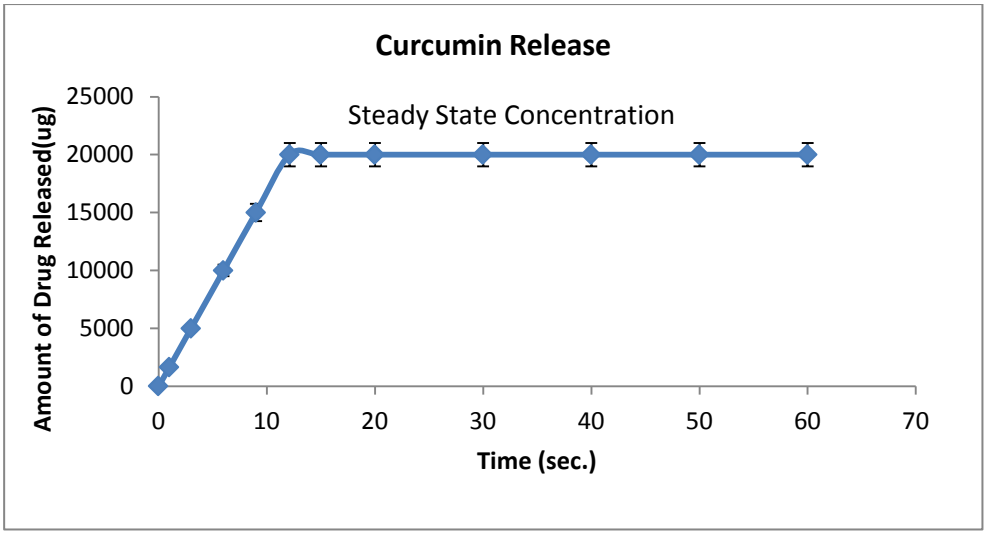


Figure 5.9: Drug Release profile of Curcumin from the Rice paper films in µg/sec.

y = mx +c
y = 3.374X - 0.000 R^2 = 0.997
Concn = X = (Y +0.000)/3.374
Y = Absorbance

Table 5.6: Curcumin Concentration Difference in the donor chamber

Curcumin	1	2	3	AVG Abs	Concn. mg/ml	Concn. Difference(C)
Initial Abs	0.21711	0.22832	0.21612	0.220517	0.065358	**0.033072**
Final Abs	0.10891	0.10897	0.10892	0.108933	0.032286	

Cumulative Drug Release Profile

(1) Formula for determination of percentage of release of drug from in vitro dissolution testing:
Concentration of drug (µg/ml)= [(Absorbance) ± intercept]/slope
Amount of drug released mg/ ml = Concentration × Dissolution bath volume × dilution factor/1000.
Cumulative percentage release (%) = Volume of sample withdrawn (ml)/bath volume (v) × P (t – 1) + Pt
Where Pt = Percentage release at time t Where P (t – 1) = Percentage release previous to 't'

(2)
Data:

y = 0.009x (R^2 = 0.985)

Concn. (x) µg/ml = [y (Absorbance)]/0.009

Dissolution basket volume = 900ml i.e. 20mg / 900ml = 0.0222
Dilution factor = 0.0222

Amount of Drug released in mg/ml = Concn. X 900 x 0.0222 / 1000 for the 7 readings at Time (Hrs.) 0, 2, 4, 6, 8, 10, 12
We shall later express this in seconds since our film is a fast dissolving (IDF)
Percentage of drug released at time (t) = Amount of drug released (mg/ml) /Original Amount of Drug loaded in the sample

Volume of sample withdrawn = 5 ml
Volume of methanol added 0.25 ml
Total volume of sample =
5.25 ml

Cumulative percentage release (%) = Volume of sample withdrawn (ml)/bath volume (v) × P (t – 1) + Pt
Where Pt = Percentage release at time t Where P (t – 1) = Percentage release previous to 't

Cummulative percentage Release (%) = 5/900 X Previous percentage release(P(t-1)) + Present percentage release at time t
Plot Cummulative percentage Release (%) against time in seconds.

Flux data

The cumulative amounts of Curcumin that dissolved from the film into the basket were plotted versus time

The permeability coefficient, P, was calculated from the formula:

$$P = (dQ/dt) / (C. A) \quad (1)$$

In which dQ/dt 0.009 (%) at steady-state slope of the cumulative flux curve 0.02 mg/sec. C is the concentration difference across the buccal mucosa, and A (2 cm^2) is the effective cross-sectional area available for diffusion.

Slope of the cummulative drug release (%) = 0.009
i.e. 0.009 g (%) are released in one hour
Therefore 0.09 *1000 mg(%) are released in 3600 seconds
Flux
In 1 sec (0.009*1000)*100/3600 mg are released = **0.25mg**

$P = (dQ/dt) / (C. A)$
C, the concn. Difference across the pig buccal mucosal membrane from the mucosal side to the serosal side = 0.033072mg/ml

Permeability coefficient P = (d*Q*/d*t*) / (*C. A*)

In which d*Q*/d*t* = 1.044 (%) at steady-state slope of the cumulative flux curve

C is the concentration difference from the mucosal side to the serosal side across the buccal membrane = 0.033072 mg/ml

and A (πr^2) is effective cross-sectional area available for diffusion.

Diameter = 15 mm Radius = 7.5 mm = 0.075cm

= 22/7*$(0.075)^2$ = 0.0176785 = 0.0177cm^2

Slope of the cumulative drug release (%) = 0.009
i.e. 0.009 g (%) are released in one hour
Therefore 0.09 *1000 mg(%) are released in 3600 seconds

Slope of the cumulative drug release (%) = **0.009**

i.e., 0.009g (%) are released in one hour

Therefore 0.09 *1000 mg(%) are released in 3600 seconds

Flux

In 1 sec (0.009*1000)*100/3600 mg are released = **0.25mg**

The flux Coefficient P = (dQ/dt) / (C. A) = 0.25/0.033072*0.0177 = **42.71 µg/sec.cm^2**

= **0.04271mg/sec/cm^2.**

Permeation was described in detail in chapters three and four. *Ex- vivo* permeation of Resveratrol and Curcumin through porcine buccal mucosa was carried out on Batch 16 over a period of 12 hours (Concentration in (mg/ml). The **Flux**, (i.e, the dissolution rate at steady state concentrations) of Resveratrol at steady state was 29mg per second and the Permeability coefficient was **389 mg/sec.cm^2**. The Flux of Curcumin at steady state was 0.25mg per second and the Permeability coefficient was **42.71 mg/sec.cm^2**. **These results can be interpreted as follows; A higher flux and permeation coefficient means higher solubility and permeation. A lower flux and permeation coefficient means lower solubility and permeation. The permeation of Resveratrol through the buccal mucosa was 9.1, about 10 times that of Curcumin. Definitely, Resveratrol is very soluble compared to Curcumin.** The flux of the drugs and the permeation coefficients indicate the release of the drugs is burst as all the drugs are released immediately.

Results could be predicted to be higher in the oral cavity because of the presence of enzymes like amylase that would quickly digest the rice starch and expose the drug and the great mechanical effect of the tongue that could not perfectly be simulated in the *in vitro* experiments. However, in *ex vivo* experiment, after permeation, high concentration of Resveratrol was 6.73 mg/ ml and 0.061 mg/ml of Curcumin after 2 hours; this meant that Resveratrol is 100 times more permeable than Curcumin. Perhaps this is a phenomenon that will be considered later in the dosing of the two products but this is beyond the scope of this study. However, concurrent administration of both drugs could enhance the permeability of curcumin through the buccal membrane (Rubin and Rubin, 2009).

5.3.3. HPLC Calibration Curve for Resveratrol

The calibration of the Resveratrol peak. The y Ratio A/B is calculated from the mean AUC of the drug divided by AUC of the internal standard to get the concentration of the extracted drug. Concentration (x) mg/ml = (y-c)/m

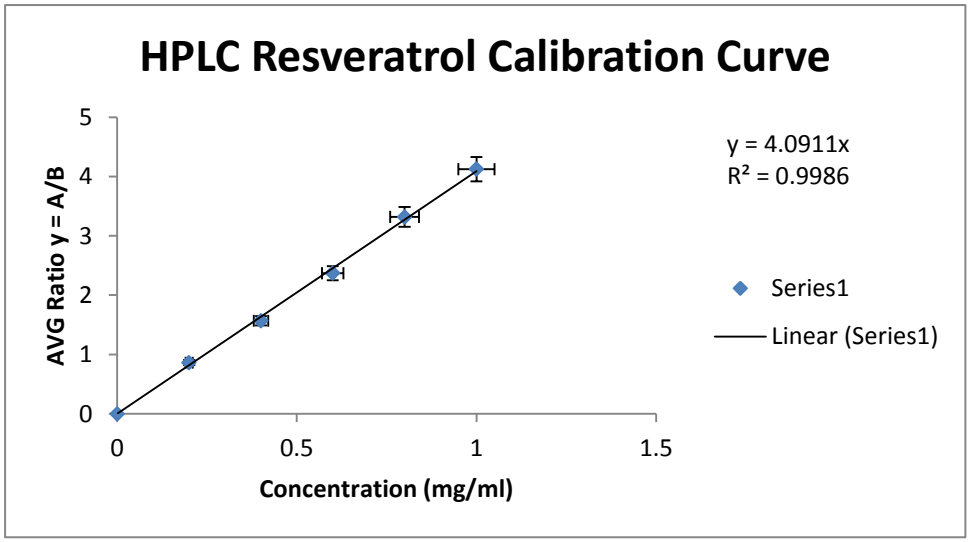


Figure 5.10: The linear Calibration curve constructed for the determination of Resveratrol concentrations from the Resveratrol extracted sample for the HPLC Method. Good linearity was achieved (R^2=0.9986).

5.3.4. HPLC Calibration Curve for Curcumin

Similarly the concentration of curcumin will be calculated as for Resveratrol above.

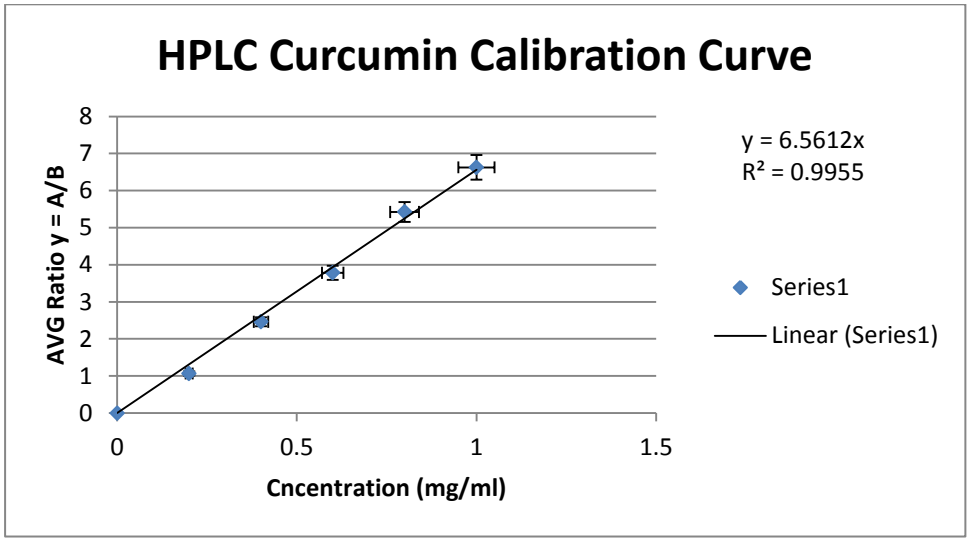


Figure 5.11: The linear Calibration curve constructed for the determination of Curcumin concentrations from the Resveratrol extracted sample for the HPLC Method. Good linearity was achieved (R^2=0.9955).

5.3.5. Chromatograms for Resveratrol and Curcumin

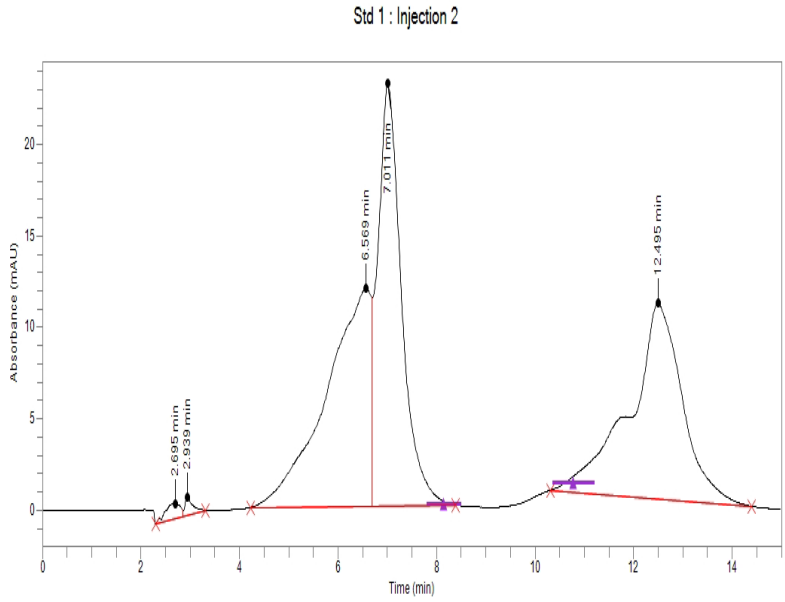


Figure 5.12: A typical chromatograms of standard solution of Resveratrol

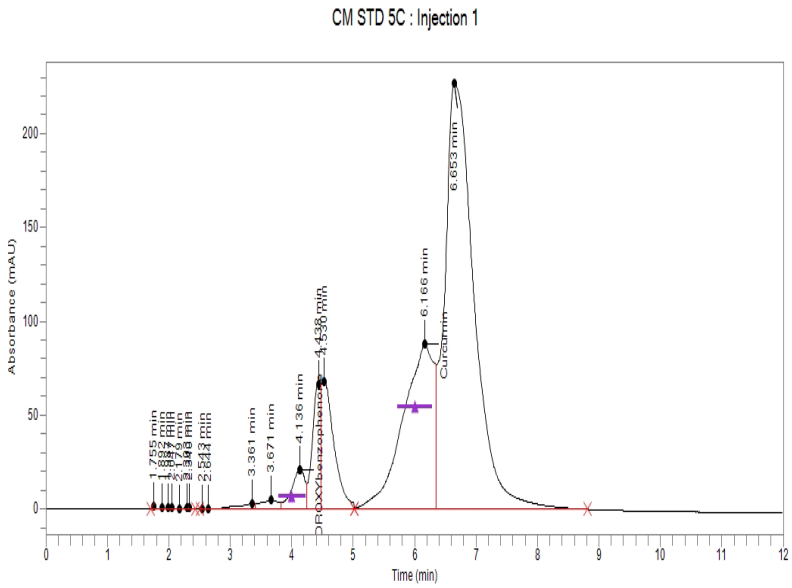


Figure 5.13: A typical chromatograms of standard solution of Curcumin.

Typical chromatogram of the extracted sample of Resveratrol with Carbamazepine Internal standard and that of Curcumin with 4 – Hydroxybenzophenone are given below (See Figure 5.14) (See Figure 5.15). These chromatograms prove that the HPLC methods employed for the extracted Resveratrol was successful. The peak for Resveratrol and Carbamazepine Internal standard (IS) were obtained using HPLC at 315 nm in Acetonitrile (CAN): Phosphate buffer pH 4.2 30%: 70% mobile phase as extracted from the rice paper film (Retention Time (Rt = 6.946. sec and 12.429 sec respectively (See Figure 5.15). The 2nd peak could have been as a result of the trans and cis isomers of Resveratrol with time.

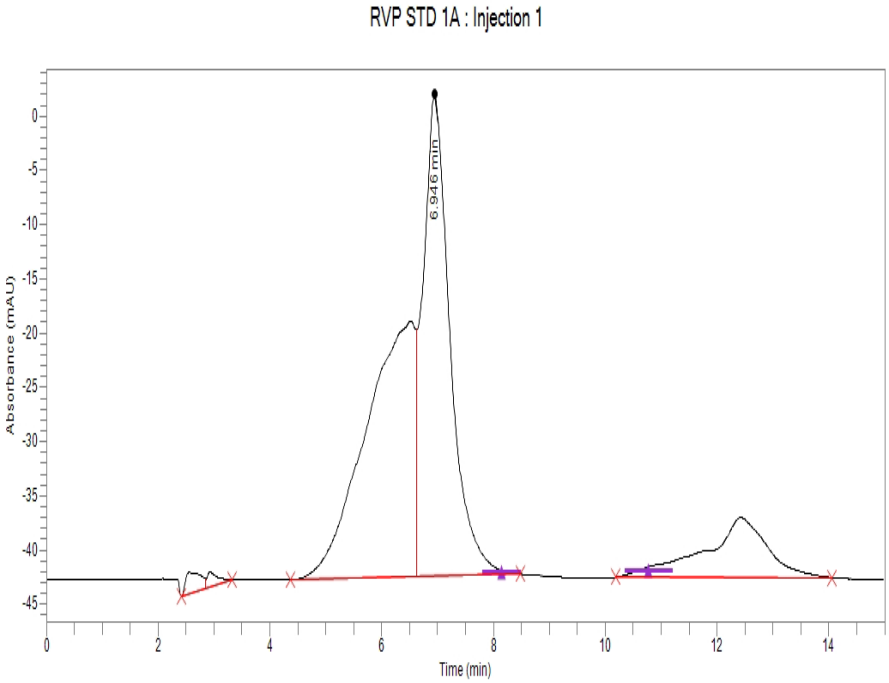


Figure 5.14: A typical chromatogram depicting the peak for extracted Resveratrol and Carbamazepine Internal standard (IS) from the film using HPLC.

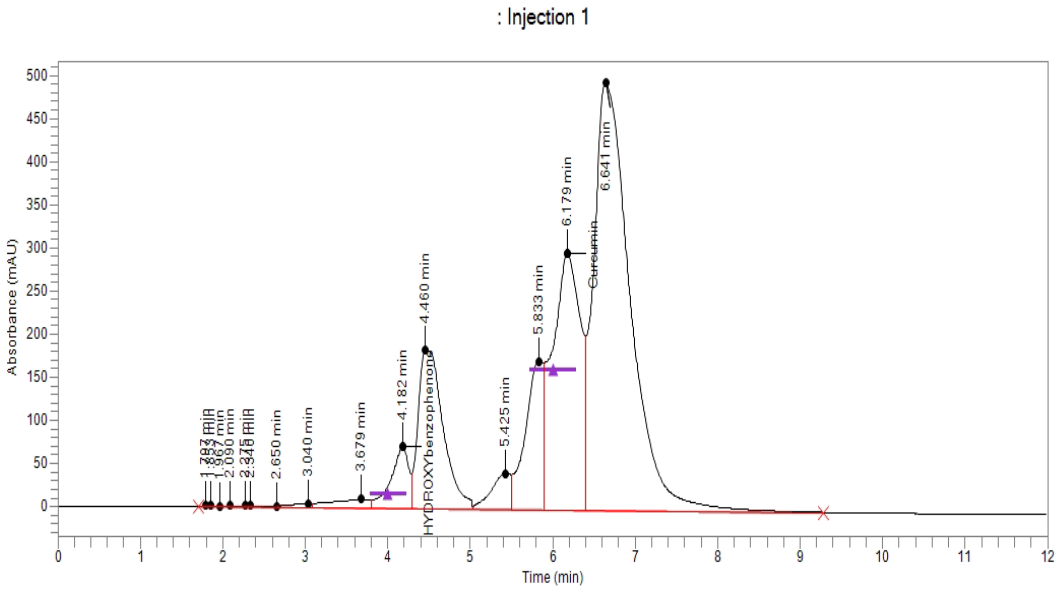


Figure 5.15: A typical HPLC chromatogram depicting the distinct separation of Curcumin at 428 nm from that of 4-Hydroxybenzophenone internal standard at 300nm appearing as a small peak as extracted from the rice paper film. Retention time (Rt) was 6.641 min for curcumin and for the internal standard (IS), 4-Hydroxybenzophenone (Rt) was 4.460 min. The second peak could have been as a result of the impurities

5.3.5. Curcumin Calculations for the percentage drug recovery:

y = mx + c x = (y-c)/m

y= 6.7888x +0.1669 **y = A/B** =15402198.7/ 3884116.0 = 3.965432

x = (3.965432 – 0.1669) / 6.7888 =0.8416 mg %, 55.95292 % (or dilution was x 100)

(55.95292/100) x 20 = 11.19 mg

But Curcumin is 77 %

Therefore, (77/100) x 11.19 = 8.61 mg.

Mean stdev = **± 0.795**

Table 5.7: The mean standard deviation for Curcumin

STDEV-A	**STDEV-B**	**A/B**
1.64	1.473	
1.1	1.040	
0.61	0.55	
0.57	1	
0.57	1.59	
Mean STDEV		
0.90	1.13	0.80.

5.3.6. Resveratrol Calculations for the percentage drug recovery:

y = mx + c x = (y-c)/m

y= 4.1138x +0.0167 y + A/B =1.506.665.3 / 433.113.3 = 3.479

x = (3.479 – 0.0167) / 4.1138 =0.8416 mg %, 84.16 % (or dilution was x 100)

(84.16/100) x 20 = 16.4 mg

But Resveratrol is 98 %

Therefore, (98/100) x 16.4 = 16.07 mg.

Mean stdev = **± 1.257**

Table 5.8: The mean standard deviation for Resveratrol

STDEV-A	STDEV-B	A/B
1.64	1.47	
1.1	1.041	
0.61	0.55	
0.57	1	
0.57	1.59	
Mean STDEV		
0.90	1.13	1.26

Table 5.9: The mean Recovery % for both curcumin and Resveratrol from the rice paper films

	Formulation amount of drug (mg) per film of 2cm x1 cm	Mean of % Recovery	Amount of drug Recovered	± Mean STDEV
Resveratrol (98%) in 1 film	20mg	**84.16 %**	**16.07mg**	± 1.26
Curcumin (77%) in 1 film	20mg	**55.95 %**	**8.61 mg**	± 0.80

It can be seen from table 5.10 that the percentage recovery of Resveratrol from the films was good **84.16 %,** but that of Curcumin was only **55.95 %.** The low percentage recovery for curcumin could have been due to the presence of **33%** impurities in the standard sample that was used.

5.4 Taste Testing Results

Table 5.10: Evaluation of Taste testing in Human volunteers summarized as percentage

Taste Testing in Human volunteers	Acceptable/Most Acceptable	Moderately Acceptable
Visual Appearance	60%	30%
Aroma	30%	60%
Texture	70%	20%
Overall	50%	40%
	Likely/Very Likely	Moderately Likely
Recommend	50%	40%
	Slightly/Much more than sucrose solution	Same as sucrose solution
Sweetness	10%	70%
Flavour	10%	70%

Table 5.11: Evaluation of Taste testing in Human volunteers summarized as Mean values with ± Standard Deviation.

Taste Testing in Human volunteers	Mean	STDEV
Visual Appearance	3.6	0.84
Aroma	3.3	0.82
Sweetness	2.9	0.57
Flavour	2.9	0.57
Texture	3.8	0.92
Recommend to someone else	2.6	0.70
Overall	3.5	0.85

A summary of the taste testing results are shown in (See Table 5.11) and (See Table 5.11:). From the statistical evaluation of the optimal formulation of the rice paper IDF, 60% of the volunteers judged the product most acceptable. 10 % rated the sweetness of the product to be equal to that of sugar while 40% said it was a little less sweet than sugar. 60 % liked the visual appearance and 70 % liked its soft texture.50 % said they would highly recommend the IDF to others. Overall, 50 % scored the product most acceptable and 40 %moderately accepted the product. From these scores, it can be taken that the Rice paper IDF would be acceptable to the patients. Hence Rice paper IDFs could be a suitable drug delivery system for some essential natural drugs associated with poor oral bioavailability.

5.5 *In vivo* Dissolution of the Rice paper films

Table 5.12: Shows the *in vivo* dissolution test to establish the time the film takes to dissolve in the mouth of three volunteers.

	Time in Seconds			AVG Time(Sec)	STDEV	CV (%)
Volunteer	1	2	3			
Resveratrol	35	21	15	**23.67**	10.26	43.37
Curcumin	50	19	43	**37.33**	16.26	43.55

The size of the film administered to the volunteers was small compared to the $2cm^2$

The idea was not to be anywhere near the dose so the film would only show the dissolving time and have no effect to the volunteer. The results indicate that Resveratrol dissolved in a relatively short time of 24 seconds and Curcumin in 37 seconds (See Table 5.12). This is only an indication of the relative short time the films take to dissolve in the mouth when the full dose is administered to the patient. As mentioned before, full human studies with patients need to be done to be very accurate.

5.6 Concluding remarks

This study addressed the *ex vivo* release of Resveratrol and Curcumin using HPLC techniques. HPLC analysis was done only for drug extraction to determine the percentage recovery of Resveratrol and Curcumin from the films with Carbamazepine and 4- Hydroxybenzophenone as internal standards respectively. **The mean drug Recovery for Resveratrol was 84.16 % and 55.95 % for curcumin.** The Avian UV/Vis Spectrophotometer was used to determine the calibration curve and concentrations of the drugs during the permeation and dissolution studies. The concentrations of Resveratrol and Curcumin after the drugs permeated through the pig buccal membrane mounted on the Franz Diffusion cell depicted the release characteristics of the drug delivery system that was designed and optimized in chapters 3 and 4.

The Flux of Resveratrol at steady state was 29mg per second and the Permeability coefficient was **389 mg/sec.cm^2**. The Flux of Curcumin at steady state was 0.25mg per second and the Permeability coefficient was **3.8 mg/sec.cm^2**. Drug release profiles illustrated that the rice paper film would yield superior plasma concentrations for both Resveratrol and Curcumin in comparison to the documented oral products currently on the market.

For instance, Boocock (Boocock et al., 2007) reported C_{max} for two separate mono-glucuronide metabolites, for a total glucuronide metabolite concentration ~7.5 µM following a single 5.0 g oral dosage of resveratrol. Piperine, a polyphenol found in black pepper, has been shown to substantially increase serum C_{max} and AUC of resveratrol in rats (Johnson et al., 2011). In coadministration in an oral gavage of 100 mg/kg and 10 mg/kg piperine, Johnson *et al.* showed a 1000% increase of peak plasma levels for resveratrol while delaying a major glucuronide resveratrol metabolite (Johnson et al., 2011). These findings lacked the backing of studies using human subjects.

In vivo **dissolution / Disintegration results of the rice paper films using human volunteers indicated that Resveratrol dissolved in a relatively short time of 24 seconds and Curcumin in 37 seconds** (See Table 5.18).

For the first time, Wahlstrom and Blennow in 1978 reported that, after oral administration of 1 g/kg of curcumin in Sprague-Dawley rats, negligible amounts of curcumin in blood plasma of rats was observed. This could have been due to its poor absorption from the GIT. Later, several studies were conducted on bioavailability of curcumin and found that certain amount of

curcumin was bioavailable in serum of animals. In another study, when curcumin was given orally at a dose of 2 g/kg to rats, a maximum serum concentration of 1.35±0.23 µg/ml was observed at time 0.83 hours, whereas in humans the same dose of curcumin resulted in either undetectable or extremely low (0.006±0.005 µg/ml at 1 hour) serum levels (Siebenand, 2010). Also, the *in vitro* release profile showed up to 95% release of curcumin from the developed nano-microparticulate systems (Guzman-Villanueva et al., 2013).

This delivery system if adopted with a little more further study, i.e. verification using studies in human subjects would be proved to increase bioavailability of these essential natural drugs, reduce the dosing intervals and ultimately improve patient compliance and therapeutic outcome.

1.6 Conclusion

Extensive *in vitro and ex vivo* testing has resulted in the development a novel Rice paper intraoral Dispersible film (IDF). A drug delivery system suitable for systemic delivery of some essential natural drugs that exhibit low oral bioavailability. The formulation was optimized by employing the Box-Behnken experimental design. 16 formulations were investigated for the best yield. Mean Dissolution Time (MDT_{12}) and Central Face-Centred designs were utilized to optimize the IDF formulation as well. Extensive tests including swelling, surface pH, FTIR, DSC, Tensile Strength, pH, Folding Endurance, Dissolution, Thickness, disintegration time.

Taste using a test panel of Human subjects and animal tissue study were undertaken in order to determine the physical mechanical and organoleptic characteristics of the developed IDF drug delivery system. The delivery system displayed effective drug release in an *in vitro* environment. The delivery system was tested *ex vivo* in the pig model in order to prove effective drug release. The development of effective HPLC and Spectrophotometric techniques allowed for the testing of *ex vivo* porcine permeated samples.

According to this study, *ex vivo* release, profiles of the most robust formulation revealed first order release and later zero order. **Formulation 16 proved to be optimal considering its mechanical properties, Appealingness, and Robustness. The Flux of Resveratrol at steady state was 29mg per second and the Permeability coefficient was 389 mg/sec.cm^2. The Flux of Curcumin at steady state was 0.25mg per second and the Permeability coefficient was 3.8 mg/sec.cm^2.** The permeation of Resveratrol through the buccal mucosa was about 100times that of Curcumin. However, after permeation, curcumin concentration was 0.029665 mg/ ml compared to 3.045944 mg/ml of resveratrol suggesting that curcumin is less permeable than Resveratrol.

Resveratrol dissolution rate was **0.42 mg/sec.** and that of Curcumin **was 0.14 mg/sec.** Resveratrol **Flux** was **0.21 mg/sec./cm^2.** Curcumin Flux was **0.14 mg/sec./cm^2.** Drug entrapment was 80% for both Resveratrol and Curcumin. **The 20 mg of Resveratrol and Curcumin would then dissolved in 47.6seconds and 71.4 seconds respectively. In this study, after permeation, a concentration of 6.73mg/ml of Resveratrol and 0.061mg/ml of Curcumin were detected after 2 hours of the experiment on administering only 20 mg of**

each of the drugs (See Table 5.1) and (See Table 5.4). The key finding is the drug permeation exhibited first and later zero order. *In vivo* dissolution / Disintegration results in human subjects indicated that Resveratrol dissolved in a relatively short time of 24 seconds and Curcumin in 37 seconds. Results of the drug delivery systems provided evidence that confirmed that effective intraoral fast rate of drug release and systemic drug delivery has been achieved.

6.2. Recommendations

With the development of the Rice paper intraoral Dispersible Film as a novel drug delivery system, we have been able to find a way to effectively deliver some of the essential natural drugs that exhibit low oral bioavailability into the systemic circulation. A drug delivery platform has therefore been set which would ultimately have the ability to deliver many drugs possessing low oral bioavailability. This is accompanied with single daily dose regimens with fewer side effects, improved patient compliance and a greater overall rate of therapeutic success. In addition to cancer and cardiovascular conditions, the same platform may also be adapted for the treatment of specific disease states like Epilepsy in children (Petit mal) by loading the rice film with Midazolam. This will tremendously add to the rare paediatric formulations. The elderly who have swallowing difficulty may also have a relief as the delivery system does not require swallowing.

None compliant patients like the psychiatrics and the disabled critically ill could also benefit from this drug delivery system. Local anesthesia with Lignocaine for smooth dental surgery may follow the same line by administering the film to the gum. If the natural drug Allicin from Garlic is added to the rice paper intraoral drug carrier, it would go a long way in treating chronic cardiac conditions in the elderly who find a problem in swallowing oral tablets. Indeed this drug delivery system would be a breakthrough for patients who would prefer to use natural medicines to the traditional orthodox medicines. While laboratory models indicate these approaches all have potential to improve bioavailability of resveratrol and Curcumin and optimize its clinical utility, there is surprisingly very little data regarding the bioavailability of resveratrol and Curcumin in humans (Smoliga and Blanchard, 2014).Further research is still called for to prove enhanced bioavailability by doing full animal studies and clinical trials in human subjects.

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Appendix A

Abstracts of papers published/submitted from this dissertation (None yet) but draft paper submitted to the supervisor for review to be published in the international journal of pharmaceutics

Intraoral dispersible rice paper films as a natural drug delivery system

ABSTRACT

At present, pharmaceutical researchers are focusing on instantaneous intraoral dispersible technologies as novel drug delivery systems because; they have outstanding advantages over the traditional oral and parenteral routes of drug administration. Some essential natural drugs have low oral bioavailability due to extensive first pass metabolism and pre systemic degradation in the gastrointestinal tract. This research, addresses these problems by formulating a cheap rice paper Intraoral Dispersible Film (IDF). IDFs are effective and could improve the bioavailability of some natural drugs. In this study, formulation was optimized using the experimental factorial design. The IDFs were loaded with model, natural, anti-cancer drugs, Resveratrol and Curcumin with low oral bioavailability. They were evaluated for thickness, folding endurance, swelling behaviour, among others. These related to drug release properties. Permeation was evaluated using the pig mucosal membrane mounted on a Franz diffusion cell, and taste testing was done to determine acceptability using a taste panel. Sixteen formulations showed variations in disintegration time, thickness, Tensile strength and permeation profiles. The key finding is, *ex vivo* release profiles of the optimized formulation revealed first order release and later zero order. Published results were that When curcumin was given orally at a dose of 2 g/kg to rats, a maximum serum concentration of 1.35±0.23 µg/ml was observed at time 0.83 hours, whereas in humans the same dose of curcumin resulted in either undetectable or extremely low (0.006±0.005 µg/ml at 1 hour) serum levels (Siebenand, 2010). Boocock (Boocock et al., 2007) reported C_{max} for two separate mono-glucuronide metabolites, for a total glucuronide metabolite concentration ~7.5 µM following a single 5.0 g oral dosage of resveratrol. In this study, after permeation, a concentration of 6.73mg/ml of Resveratrol and 0.061mg/ml of Curcumin were detected after 2 hours of the experiment after administration of 20 mg of each of the drugs (See Table 5.7 page 186 and Table 5.11 page 192).Therefore, it is evident that rice paper IDF could efficiently deliver natural drugs into the blood.

Keywords: Curcumin; Resveratrol; Instantaneous Intraoral Dispersible films; Rice paper films; *ex vivo* release profile.

conferenceseries.com SciTechnol

Mukasa Eliphaz, J Pharm Drug Deliv Res 2016, 5:3(Suppl) http://dx.doi.org/10.4172/2325-9604.C1.009
http://www.scitechnol.com/conference-abstracts-files/2325-9604.C1.009_008.pdf

9th World Drug Delivery Summit

June 30-July 02, 2016 New Orleans, USA

Formulation of a natural intraoral dispersible film (IDF) for intraoral delivery of various natural drugs using edible rice paper film as the carrier vehicle

Mukasa Eliphaz
University of the Witwatersrand, South Africa

Background: At present, pharmaceutical researchers are focusing on instantaneous intraoral dispersible technologies as novel drug delivery systems because of their outstanding advantages over the traditional oral and parenteral routes of drug administration. Most essential natural drugs have low oral bioavailability due to extensive first pass metabolism and pre systemic degradation in the GIT.

Purpose of Study: This research intends to addresses these problems by formulating a cheap, intraoral rice paper Intraoral Dispersible Film (IDF). IDFs are potent and could improve bioavailability of natural drugs.

Methods: In this study, formulation was optimized using the experimental factorial design. The IDFs were loaded with model natural anticancer drugs Resveratrol and Curcumin with low oral bioavailability. They were evaluated for thickness, folding endurance, swelling behaviour because these relate to drug release properties. Permeation was also evaluated using the pig mucosal membrane mounted on a Franz diffusion cell, and taste testing was done to determine acceptability using a taste panel.

Results: Sixteen formulations showed variations in disintegration time, thickness, tensile strength and permeation profiles. My key finding is, ex vivo release profiles of the optimized formulation revealed first order release and later zero order.

Conclusions: Therefore, it is evident that rice paper IDF could efficiently deliver natural drugs into the blood.

Biography

Mukasa Eliphaz has worked at Medipharm Industries EA Ltd. Uganda factory for 15 years. He is specialized in cGMP and ORS Manufacture. He has studied at Mulago Hospital School of Dispensing for a Diploma Pharm in 1988. He taught at Mulago Paramedical School for 2 years. He has attended a Clinical Instructor's course at Mbale Health Manpower Development Centre in 1999 and worked as an Assistant Drugs Inspector at Uganda National Drug Authority for 7 years. He has also attended NIPER Chandigarh India for Assessment of quality of Pharmaceuticals. He did his BPharm in 2012 at NMMU Port Elizabeth South Africa. He has worked at Johannesburg General Hospital Charlotte Maxeke for his Pharmacist Internship in 2013. Presently he is an MPharm student at the University of the Witwatersrand, SA 2013 to 2015.

Eliphaz.mukasa@yahoo.com

APPEDIX B

GRAPHICAL ABSTRACT

Graphical Abstract

The illustrated figure 1.1 below summarises the study concept.

GRAPHICAL ABSTRACT

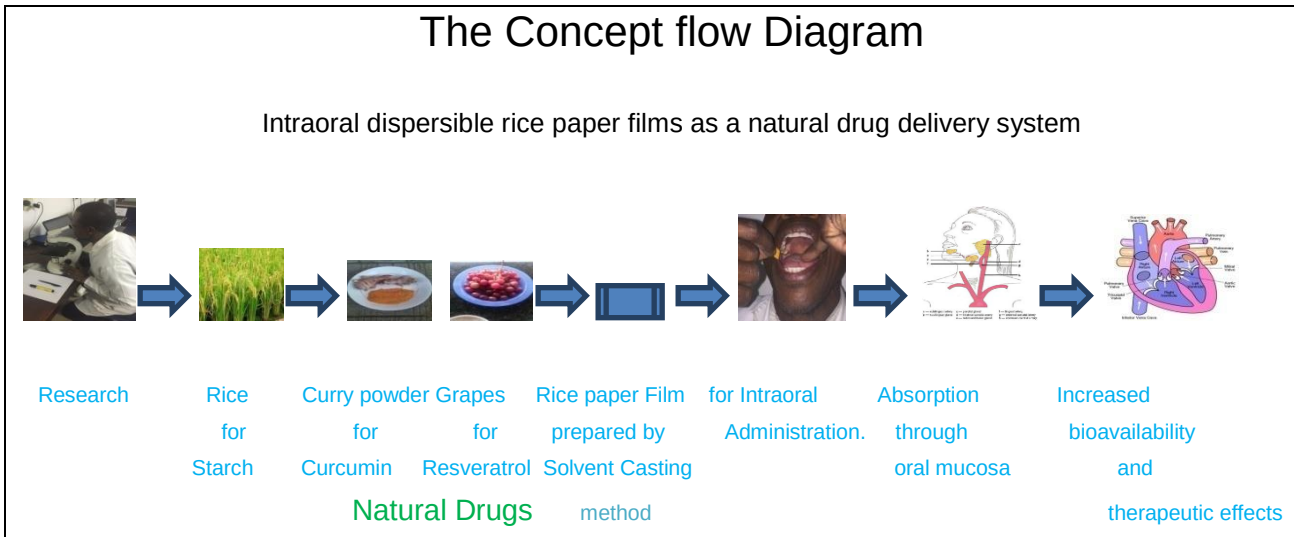


Figure: 1.1 Conceptual diagram of the rice paper intraoral dispersible film as a natural drug delivery system to carry natural drugs via the intraoral veins to the systemic circulation.

APPENDIX C:
CONFERENCE PRESENTATION

Appendix C

Abstracts of conference proceedings

FORMULATION OF A NATURAL INTRAORAL DISPERSIBLE FILM (IDF) FOR INTRAORAL DELIVERY OF VARIOUS NATURAL DRUGS USING EDIBLE RICE PAPER FILM AS THE CARRIER VEHICLE

ABSTRACT

Back ground and the purpose of study: Delivery of drugs using the intraoral drug delivery systems has been the subject of interest since the last 3 decades. The intraoral route has been advocated by many authors on the subject of drug delivery systems as an alternative route of administration for drugs, which undergo extensive hepatic first pass metabolism or which are susceptible to degradation and presystemic metabolism in the gastrointestinal tract.
Various advantages associated with the system made the intraoral drug delivery a novel route of drug administration. These include, by pass of first pass metabolism, rapid onset of action, due to high permeability, high blood flow, convenience and portability of the formulation, and that patients themselves can apply the delivery system at discrete times without water. Drug delivery systems that encourage patient compliance in a most practical way where, safety, efficacy, availability, and affordability is stressed in the existing environment of meagre recourses for the majority of the world's population to improve their quality of life are still called for. In this study, a safe and effective intraoral drug delivery systems even suitable for paediatric and geriatric use and with a proven permeation through the buccal membrane of the natural model drugs using edible rice paper was developed. The intraoral delivery of the anticancer phytochemicals, Curcumin and Resveratrol were investigated as a model.
Aim: The aim of the study is to develop an affordable IDF drug delivery system, which will enhance the bioavailabity of natural drugs, which are normally associated with poor oral bioavailability.
Objectives: To develop a feasible method for the formulation of an edible rice paper IDF. To investigate the addition of a natural sweetener like Honey and/or Stevia. To determine the physicochemical characteristics of the IDF. To develop an effective extraction and HPLC assay technique for the model drugs to assess the in vitro release of each model drug into simulated

saliva. To assess the *ex vivo* drug release characteristics of the optimum IDF through excised oral pig mucosa using a Franz diffusion cell.

Methods: Only natural ingredients were used to formulate the rice paper films. Compatibility with simulated saliva and buccal environment with the vehicle and active ingredients were studied during the formulation development of the products by performing the mechanical and organoleptic tests which included Tensile strength, DSC, FTIR, Disintegration, Taste testing in human volunteers among others. Porcine mucosal membrane was used during the permeation experiments. *In vitro* and *ex vivo* permeation and dissolution studies were done to determine the efficiency of rice paper as a natural drug delivery system to deliver the drugs into the blood. Statistical analysis of the collected data was performed using a Microsoft Excel add in program called Essential Experimental Design (Yeaton, Steppin and Werner) to establish that rice paper is suitable to deliver natural drugs.

Results: Box-Behnken design or Essential Factorial Experimental design was employed to elucidate the efficiency of rice paper as a drug delivery system for natural drugs. A p-value of less than 0.05 was established for the percentage of rice starch that could significantly affect disintegration time. Disintegration of a more controlled manner in a very short period of time of 5 - 26.77 seconds generally less than 30 seconds. We observed that the optimized formulation using the Essential experimental factorial design and its solver yielded the formulation with the best disintegration time but not necessarily the most robust formulation probably due to the reciprocal relationship between the poor mechanical properties of rice starch and disintegration time. According to this study, *ex vivo* release, profiles of the most robust formulation revealed first order release and later zero order. Formulation 16 proved to be optimal considering its mechanical properties, Appealingness, and Robustness. The Flux of Resveratrol at steady state was 29mg per second and the Permeability coefficient was 389 mg/sec.cm^2. The Flux of Curcumin at steady state was 0.25mg per second and the Permeability coefficient was 3.8 mg/sec.cm^2. The permeation of Resveratrol through the buccal mucosa was about 100times that of Curcumin. However, after permeation, curcumin concentration was 0.029665 mg/ ml compared to 3.045944 mg/ml of resveratrol suggesting that curcumin is less permeable than Resveratrol. Resveratrol dissolution rate was 0.42 mg/sec. and that of Curcumin was 0.14 mg/sec. Resveratrol Flux was 0.21 mg/sec./cm^2. Curcumin Flux was 0.14 mg/sec. / cm^2. Drug entrapment was 80% for both Resveratrol and Curcumin. The 20 mg of Resveratrol and Curcumin then dissolved in 47.6seconds and 71.4 seconds respectively. The key finding is the drug permeation exhibited first and later zero order. When curcumin was given orally at a dose of 2 g/kg to rats, a maximum serum concentration of 1.35±0.23 μg/ml was observed at time 0.83 hours, whereas in humans the same dose of curcumin resulted in either undetectable or extremely low (0.006±0.005 μg/ml at 1 hour) serum levels (Siebenand, 2010). Boocock (Boocock et al., 2007) reported C_{max} for two separate mono-glucuronide metabolites, for a total

glucuronide metabolite concentration ~7.5 µM following a single 5.0 g oral dosage of resveratrol. In this study, after permeation, a concentration of 6.73mg/ml of Resveratrol and 0.061mg/ml of Curcumin were detected after 2 hours of the experiment after administration of 20 mg of each of the drugs (See Table 5.1) page 185 and (See Table 5.4) page 190). ***In vivo* dissolution / Disintegration results of the rice paper films using human volunteers indicated that Resveratrol film dissolved / disintegrated in a relatively short time of 24 seconds and Curcumin film in 37 seconds. The release profile is a burst release** (See Table 5.12).

Conclusions: A Conclusion can now be drawn after analysing the data in support of the hypothesis that, Rice paper could be a suitable natural drug delivery system for some essential natural drugs. Therefore, it is evident that rice paper IDF could efficiently deliver natural drugs into the systemic circulation. Further studies to prove increased bioavailability will have to be performed in human subjects at a later stage.

***Keywords*:** Curcumin; Resveratrol; Instantaneous Intraoral Dispersible films; Edible Rice paper films; *ex vivo* release profile.

APPENDIX D

Animal ethics clearance certificate

Approval of Exemption from full animal ethics study.

Chairman and Head of Department Pharmacy and Pharmacology



UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

7 York Road, Parktown, 2193 South Africa * E-mail

physiology@health.wits.ac.za * Telephone (011)717-2363 * Fax (011) 643-2765

8 May 2014

To: Whom it may concern,

Re: approval for the use of animal tissue samples collected from pigs

Euthanized for other purposes in *in vitro* permeation studies

This letter is to confirm that Prof MP Danckwerts does not require full animal ethics clearance to collect porcine buccal mucosa. Prof Danckwerts will be using tissue from already euthanized animals, hence full animal ethics clearance is not required, as these animals have been euthanized for other purposes. The buccal mucosal tissue from pigs for the *in vitro* permeation studies will be collected with permission from the Central Animal Service Unit at the University of the Witwatersrand. A request for permission to the Animal ethics committee has been initiated.

The following Master of Pharmacy in Pharmaceutics student will be involved in these studies as of 2014: Mukasa Eliphaz (Student Number 772722). The title of his project is: "*Formulation of a natural intraoroal dispersible film (idf) for intraoral delivery of various natural drugs using edible rice paper film as the carrier vehicle"*.

These studies will be performed in the Department of Pharmacy and Pharmacology.

If you would like any further or more specific information in this regard, do not hesitate to contact me.

Yours sincerely,

Kennedy Erlwanger

(Chairman: Animal Ethics Screening Committee, University of the Witwatersrand)

Assoc Prof Kennedy H. Erlwanger

School of Physiology Faculty of Health Sciences, University of the Witwatersrand

7 York Road, Parktown, 2193

SOUTH AFRICA

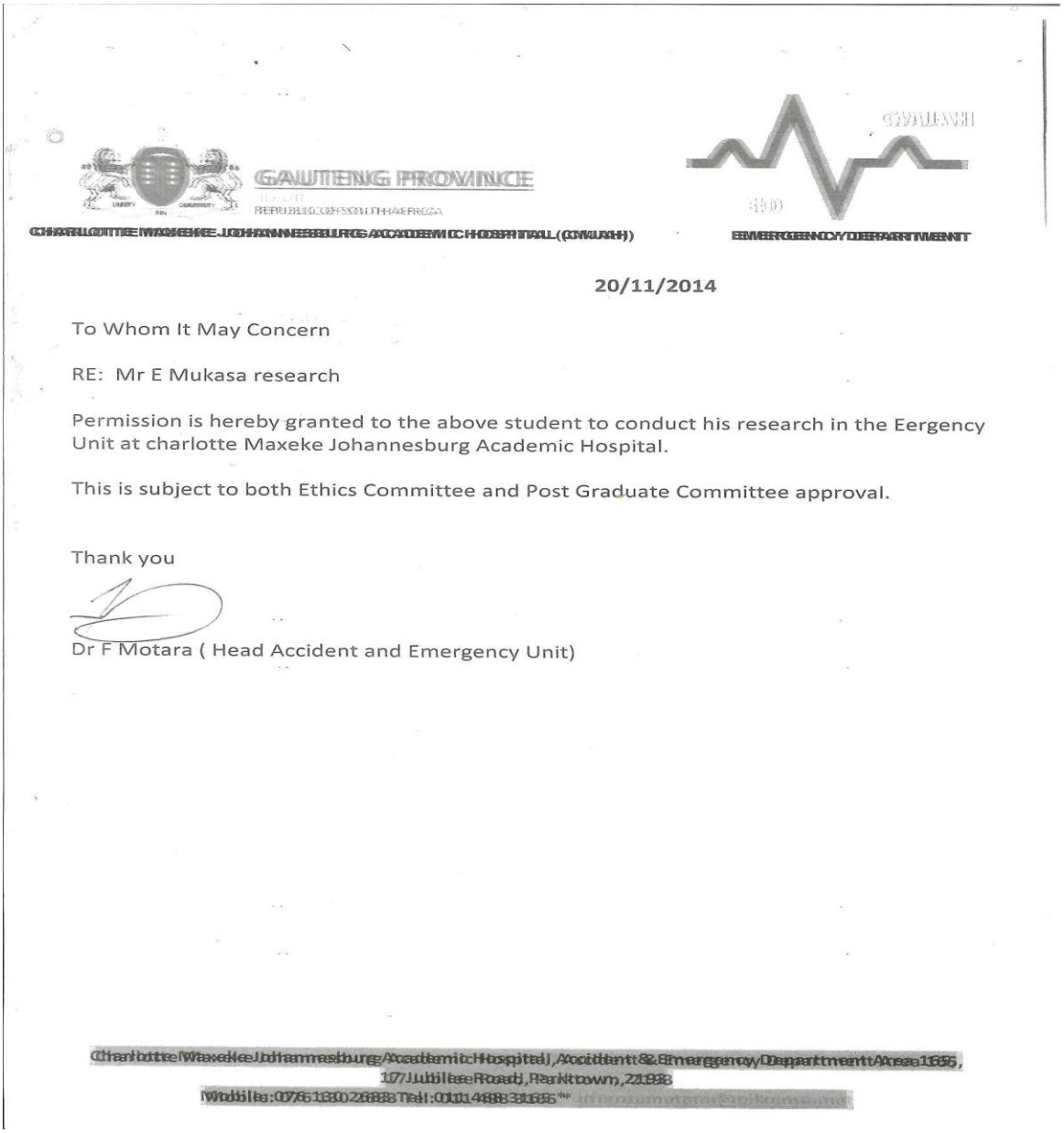
Private bag 3, Wits, 2050, South Africa.

Tel: +27 (0)11 717 2454 Fax: + 27 (0)11 643 2765

Email: Kennedy.Erlwanger@wits.ac.za

APPENDIX E

Distress protocol approval Additional



GAUTENG PROVINCE

REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL (CMJAH)

EMERGENCY DEPARTMENT

20/11/2014

To Whom It May Concern

RE: Mr E Mukasa research

Permission is hereby granted to the above student to conduct his research in the Eergency Unit at charlotte Maxeke Johannesburg Academic Hospital.

This is subject to both Ethics Committee and Post Graduate Committee approval.

Thank you

Dr F Motara (Head Accident and Emergency Unit)

Charlotte Maxeke Johannesburg Academic Hospital, Accident & Emergency Department Area 1655,
17 Jubilee Road, Parktown, 2193
Mobile: 0766130026883 Tell: 0111488331655

APPENDIX F

Human ethics clearance certificate

Human ethics permission letter

Clearance Certificate M140920 Mr Mukasa Eliphaz

- LangutaniMasingi
- Dec 8 at 9:37 AM

Tome

CC Prof. Danckwerts, Prof. Sandra Benn, Zanele Ndlovu

Dear Mr Eliphaz

Your ethics application has been approved and your certificate is attached.

Kind regards,

LangutaniMasingi| Research Ethics Committees: HREC (Medical), Biobank Ethics Committee (BEC) and Institutional Biosafety Committee (IBC)

Administrative Officer

011 717 2656/1234 – Langutani.Masingi@wits.ac.za

Faculty of Health Sciences, Phillip Tobias Building, 2nd Floor, Cnr York Road

and Princess of Wales Terrace

or

East/Main Campus

Braamfontein, 1 Jorrissen Street, JHB, Senate House, Room 10004,

10th Floor Senate House

Office Hours: Mon-Fri, 08h30-17h00 *Lunch 13h00-14h00*

University of the Witwatersrand, Johannesburg

University Research & PG Affairs Office

R14/49 Mr Mukasa Eliphaz

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140920

NAME: (Principal Investigator) Mr Mukasa Eliphaz

DEPARTMENT: Pharmacy and Pharmacology
University of the Witwatersrand

PROJECT TITLE: Formulation of a Natural Intraoroal Dispersible Film (IDF) for Intraoral Delivery of various Natural Drugs using Edible Rice Paper Film as the Carrier Vehicle

DATE CONSIDERED: 03/10/2014

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Prof MP Danckwerts

APPROVED BY:
Professor PE Cleaton-Jones Chairperson, HREC (Medical)

DATE OF APPROVAL: 05/12/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature

Date 5/12/14

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX G certificate of analysis Curcumin and Resveratrol

Certificate of Analysis Curcumin

SIGMA-ALDRICH®

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3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
Curcumin – from Curcuma longa (Turmeric), powder



Product Number:	**C1386**
Batch Number:	**SLBM5931V**
Brand:	SIGMA
CAS Number:	458-37-7
MDL Number:	MFCD00008365
Formula:	C21H20O6
Formula Weight:	368.38 g/mol
Storage Temperature:	Store at -20 °C
Quality Release Date:	18 FEB 2015

Test	Specification	Result
Appearance (Color)	Yellow to Orange	Orange
Appearance (Form)	Powder	Powder
Solubility (Color)	Yellow to Orange	Orange
Solubility (Turbidity) 10 mg/mL, EtOH	Clear to Hazy	Hazy
UV/VIS Absorbance	420 - 430 nm	424 nm
Purity (HPLC)	≥ 65 %	77 %

Rodney Burbach

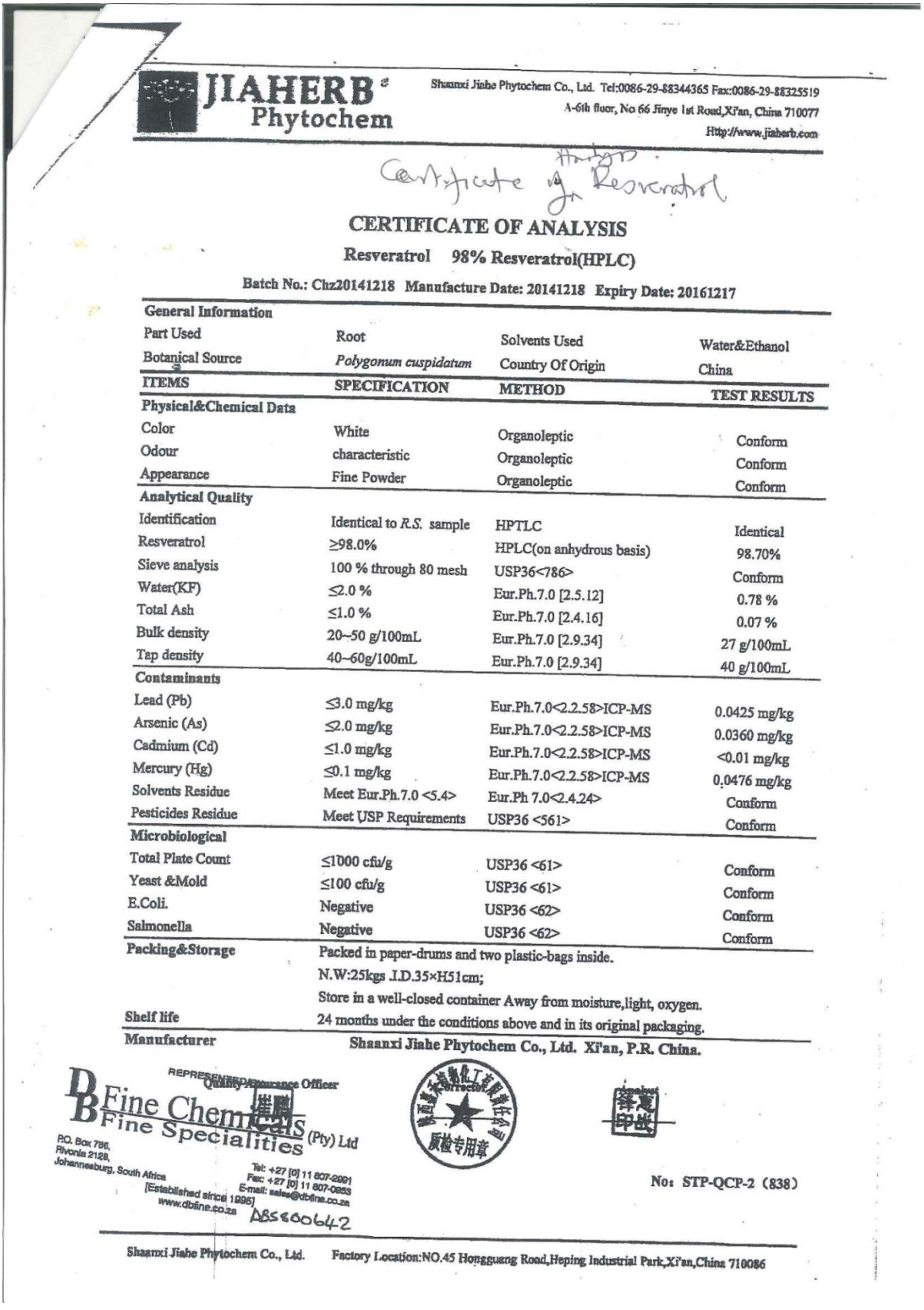
Rodney Burbach, Manager
Analytical Services
St. Louis, Missouri US

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Version Number: 1

Page 1 of 1

APPENDIX H Resveratrol certificate of analysis



JIAHERB® Phytochem

Shaanxi Jiahe Phytochem Co., Ltd. Tel:0086-29-88344365 Fax:0086-29-88325519
A-6th fluor, No 66 Jinye 1st Road,Xi'an, China 710077
Http://www.jiaherb.com

Certificate of Resveratrol

CERTIFICATE OF ANALYSIS

Resveratrol 98% Resveratrol(HPLC)

Batch No.: Chz20141218 Manufacture Date: 20141218 Expiry Date: 20161217

General Information			
Part Used	Root	Solvents Used	Water&Ethanol
Botanical Source	*Polygonum cuspidatum*	Country Of Origin	China
ITEMS	**SPECIFICATION**	**METHOD**	**TEST RESULTS**
Physical&Chemical Data			
Color	White	Organoleptic	Conform
Odour	characteristic	Organoleptic	Conform
Appearance	Fine Powder	Organoleptic	Conform
Analytical Quality			
Identification	Identical to *R.S.* sample	HPTLC	Identical
Resveratrol	≥98.0%	HPLC(on anhydrous basis)	98.70%
Sieve analysis	100 % through 80 mesh	USP36<786>	Conform
Water(KF)	≤2.0 %	Eur.Ph.7.0 [2.5.12]	0.78 %
Total Ash	≤1.0 %	Eur.Ph.7.0 [2.4.16]	0.07 %
Bulk density	20~50 g/100mL	Eur.Ph.7.0 [2.9.34]	27 g/100mL
Tap density	40~60g/100mL	Eur.Ph.7.0 [2.9.34]	40 g/100mL
Contaminants			
Lead (Pb)	≤3.0 mg/kg	Eur.Ph.7.0<2.2.58>ICP-MS	0.0425 mg/kg
Arsenic (As)	≤2.0 mg/kg	Eur.Ph.7.0<2.2.58>ICP-MS	0.0360 mg/kg
Cadmium (Cd)	≤1.0 mg/kg	Eur.Ph.7.0<2.2.58>ICP-MS	<0.01 mg/kg
Mercury (Hg)	≤0.1 mg/kg	Eur.Ph.7.0<2.2.58>ICP-MS	0.0476 mg/kg
Solvents Residue	Meet Eur.Ph.7.0 <5.4>	Eur.Ph 7.0<2.4.24>	Conform
Pesticides Residue	Meet USP Requirements	USP36 <561>	Conform
Microbiological			
Total Plate Count	≤1000 cfu/g	USP36 <61>	Conform
Yeast &Mold	≤100 cfu/g	USP36 <61>	Conform
E.Coli.	Negative	USP36 <62>	Conform
Salmonella	Negative	USP36 <62>	Conform
Packing&Storage	Packed in paper-drums and two plastic-bags inside. N.W:25kgs I.D.35×H51cm; Store in a well-closed container Away from moisture,light, oxygen.		
Shelf life	24 months under the conditions above and in its original packaging.		
Manufacturer	**Shaanxi Jiahe Phytochem Co., Ltd. Xi'an, P.R. China.**		

REPRESENTED Quality Assurance Officer

DB Fine Chemicals Fine Specialities (Pty) Ltd
P.O. Box 786,
Rivonia 2128,
Johannesburg, South Africa
[Established since 1995]
Tel: +27 (0) 11 807-2991
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E-mail: sales@dbfine.co.za
www.dbfine.co.za
ABS600642

No: STP-QCP-2 (838)

APPENDIX I Experimental factorial design rice paper film

Experimental factorial design

Box Behnken Design
3 Factors
4 Centerpoints

Equation 1 represents Quadratic Model with 10 items of the rice paper film experimental factorial design employed in generating the 16 formulations as depicted in Table 3.10.2

Response = b0 + b1 x Rice, %w/v + b2 x Plast, %w/v + b3 x Type + b4 x Rice, %w/v x Rice, %w/v + b5 Plast, %w/v x Plast, %w/v + b6 x Type x b7 x Rice, %w/v x Plast, %8b x Rice, %w/v x Type + b9 x Plast, % w/v x Type

Equation (1) Where b0 is the constant, b is number of response in percentage, Plastic the plasticizer, and Type is the type of plasticizer.

The Box-Behnken design template with randomly generated formulations is showed in table 1 below

Table 3.10.2. Rice paper film box Behnken design with 3 factors and 4 Centerpoints experimental factorial design (Two factors and three levels (2X3 or 2^3FACTORIAL))

Formula Codes			Exp. #	Rice grain %	Plasticizer %	Type of Plasticizer
Plain	**Curcumin**	**Resveratrol**				
P1	C1	R1	1	10	5	0
P2	C2	R2	- ◊ 2	15	10	0
P3	C3	R3	3	10	10	-1
P4	C4	R4	- ◊ 4	15	10	0
P5	C5	R5	- ◊ 5	15	10	0
P6	C6	R6	6	20	5	0
P7	C7	R7	7	15	15	-1
P8	C8	R8	8	20	10	1
P9	C9	R9	9	20	10	-1
P10	C10	R10	10	10	15	0
P11	C11	R11	- ◊11	15	10	0
P12	C12	R12	12	15	5	1
P13	C13	R13	13	20	15	0
P14	C14	R14	14	15	5	-1
P15	C15	R15	15	10	10	1
P16	C16	R16	16	15	15	1

Type of Plasticizer: 1 Glycerine
0 Honey
-1 Sorbitol
- ◊ The 4 Centerpoints

P Plain formulation
C Loaded with Curcumin
R Loaded with Resveratrol

APPENDIX J

Taste Testing Questionnaire

Questionnaire for the oral and sensory acceptability of Intraoral Dispersible films each loaded with, Resveratrol and Curcumin model drugs panellists

Intraoral dispersible film code number: …….

For each question below, please rate each Intraoral dispersible film on a scale from 1 -5, with 1 being the least acceptable and 5 being the most acceptable. Please mark your rating with an x in the relevant box**.**

Unipolar comparative scale (5 point) to score the acceptability of the rice paper film

1. Personal Information

Name **Initials only**

2. Visual Appeal

How would you rate the visual appearance of the product as a thin film? Would you be willing to place it on your tongue and to dose yourself with the medication?

Scale	1	2	3	4	5
Rating					

What does it look like? Does it look like something you would like to taste?

3. Aroma

Please remove the oral dispersible film from the packaging and rate it according the aroma of the product on the scale below.

Scale	1	2	3	4	5
Rating					

What is the smell of the product once removed from its packaging? Does it smell appealing? Does the smell make you want to taste it?

4. Sweetness

Take a sip of the 10% sucrose solution provided. Take note of the sweetness. Spit out the solution of the sucrose into container A supplied. Rinse your mouth with pure water in container B supplied. Tear off a small strip of the product and place it on your tongue for about 10 seconds. Then compare the sweetness of the oral dispersible film to that of the sucrose solution on the scale below.

Score in the Scale	much less than the sucrose solution	slightly less than the sucrose solution	the same than the sucrose solution	slightly more than the sucrose solution	much more than the sucrose solution
Rating Film 1					
Rating Film 2					
Rating Film 3					
Rating Film 4					
Rating Film 5					
Rating Film 6					
Rating Film 7					
Rating Film 8					
Rating Film 9					
Rating Film 10					

What is the sweetness of the product once removed from its packaging? Is the sweetness appealing? Does the sweetness make you want to taste the product?

5. Flavour

Take a sip of a Sweet Pepsi or Strepsils lozenges provided. Take note of the flavour. Spit out the solution of the Sweet Pepsi or Strepsils lozenges into container A supplied. Rinse your mouth with pure water in container B supplied. Tear off a small strip of the product and place it on your tongue for about 10 seconds. Then compare the flavour of the oral dispersible film to that of the Sweet Pepsi or Strepsils lozenges on the scale below. Etc. again I would add three difference amounts of flavours.

Score in the Scale	much less than the sucrose solution	slightly less than the sucrose solution	the same than the sucrose solution	slightly more than the sucrose solution	much more than the sucrose solution
Rating Film 1					
Rating Film 2					
Rating Film 3					
Rating Film 4					
Rating Film 5					
Rating Film 6					
Rating Film 7					
Rating Film 8					
Rating Film 9					
Rating Film 10					

Does it taste good? Is it fresh? Something you would like to eat again? Sweet? Fruity?

Do you like the taste? Is it fresh? Something you would like to eat again?

Score in the Scale	much less than the sucrose solution	slightly less than the sucrose solution	the same than the sucrose solution	slightly more than the sucrose solution	much more than the sucrose solution
Rating Film 1					
Rating Film 2					
Rating Film 3					
Rating Film 4					
Rating Film 5					
Rating Film 6					
Rating Film 7					
Rating Film 8					
Rating Film 9					
Rating Film 10					

Does it taste good? Is it fresh? Something you would like to eat again? Sweet? Fruity?

Do you like the taste? Is it fresh? Something you would like to eat again?

Score in the Scale	much less than the sucrose solution	slightly less than the sucrose solution	the same than the sucrose solution	slightly more than the sucrose solution	much more than the sucrose solution
Rating Film 1					
Rating Film 2					
Rating Film 3					
Rating Film 4					
Rating Film 5					
Rating Film 6					
Rating Film 7					
Rating Film 8					
Rating Film 9					
Rating Film 10					

Does it taste good? Is it fresh? Something you would like to eat again? Sweet? Fruity?

Do you like the taste? Is it fresh? Something you would like to eat again?

6. Texture

Does the texture of the Orodispersible film feel smooth on your tongue as it dissolves?

Scale	1	2	3	4	5
Rating					

Is the texture what you want from a product like this? Is it too gritty? Sticky? Is it soft? Chewy? Rubbery? Plastic? Does it have a nice texture?

5. How likely are you to recommend Product to others?

Scale	How likely are you to recommend Product to others? Extremely likely	Very likely	Moderately likely	Slightly likely	Slightly likely	Undecided
Rating						

8. Overall

What is your overall impression of the product as a means to take your medicine?

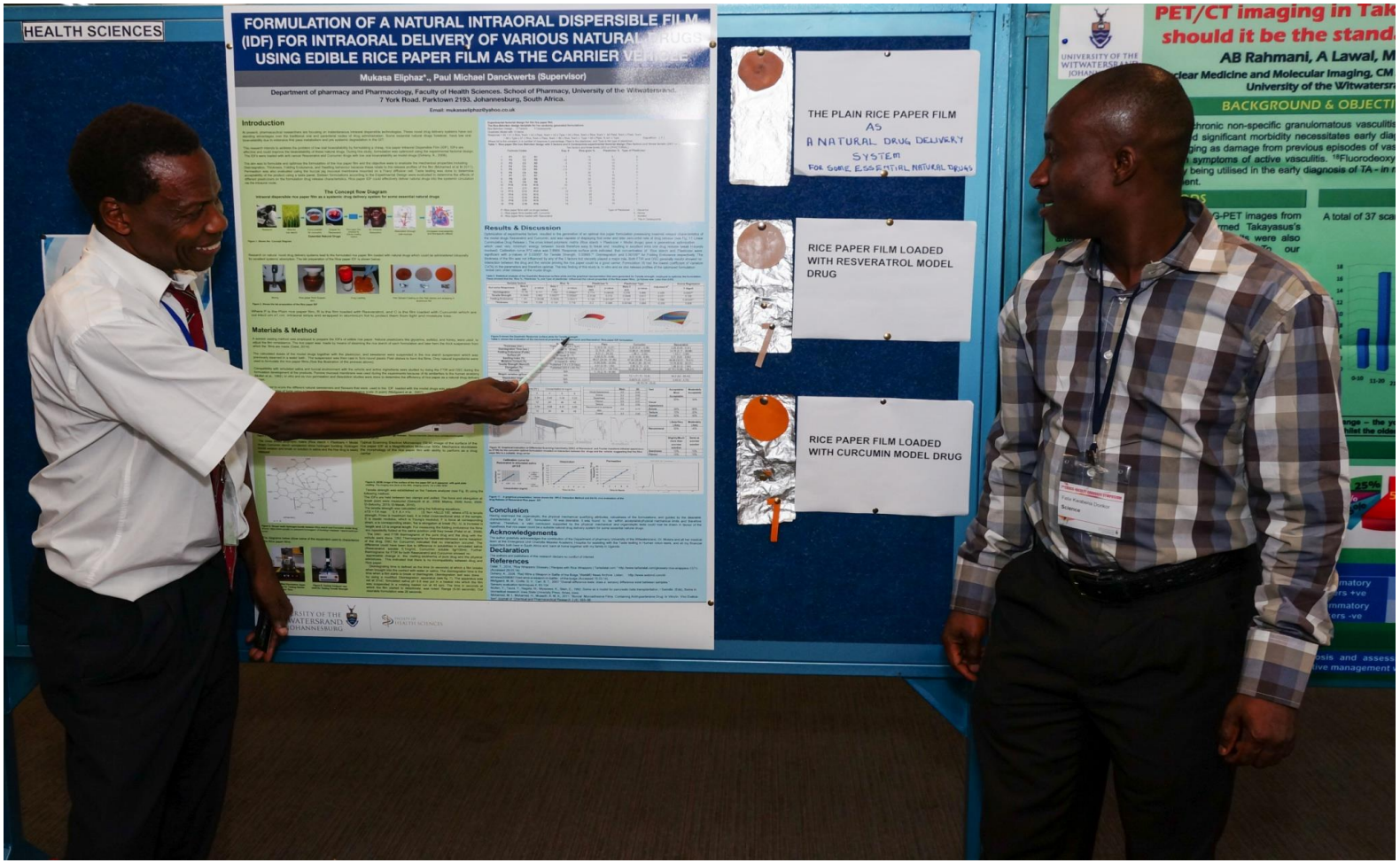
Scale	1	2	3	4	5
Rating					

Taking everything above into account.

Comments/Feedback.

APPENDIX K

Figure 6.1 Poster presentations



Poster presentation at the 7th. The postgraduate Cross faculty symposium March 2016



The research poster presented to the doctors at Charlotte Maxeke academic Hospital Emergency Unit before the taste testing with their informed consent.

Have you conducted patent and literature searches on your invention or do you know of any existing/ competing inventions? (*If yes) Please attach the report/documents when submitting form*	Yes	√	No	

Please indicate what you found;

Figure 6.2 : **Espacenet**

Espacenet
Patent search

- My patents list (0) Query history Refine search Results
-

Result list

0 results found in the Worldwide database for:

RICE PAPER AS AN INTRAORAL CARRIER VEHICLE FOR NATURAL DRUGS in the title

- Sitemap
- Accessibility
- Legal notice
- Terms of use
- Last updated: 09.03.2016
- Worldwide Database
- 6.0.12; 93p

Was this work done in the course and scope of your employment or studies at WITS?	Yes	√	No	

conferenceseries.com SciTechnol

Mukasa Eliphaz, J Pharm Drug Deliv Res 2016, 5:3(Suppl) http://dx.doi.org/10.4172/2325-9604.C1.009
http://www.scitechnol.com/conference-abstracts-files/2325-9604.C1.009_008.pdf

9th. World Drug Delivery Summit

June 30-July 02, 2016 New Orleans, USA

Formulation of a natural intraoral dispersible film (IDF) for intraoral delivery of various natural drugs using edible rice paper film as the carrier vehicle

Mukasa Eliphaz
University of the Witwatersrand, South Africa

Background: At present, pharmaceutical researchers are focusing on instantaneous intraoral dispersible technologies as novel drug delivery systems because of their outstanding advantages over the traditional oral and parenteral routes of drug administration. Most essential natural drugs have low oral bioavailability due to extensive first pass metabolism and pre systemic degradation in the GIT.

Purpose of Study: This research intends to addresses these problems by formulating a cheap, intraoral rice paper Intraoral Dispersible Film (IDF). IDFs are potent and could improve bioavailability of natural drugs.

Methods: In this study, formulation was optimized using the experimental factorial design. The IDFs were loaded with model natural anticancer drugs Resveratrol and Curcumin with low oral bioavailability. They were evaluated for thickness, folding endurance, swelling behavior because these relate to drug release properties. Permeation was also evaluated using the pig mucosal membrane mounted on a Franz diffusion cell, and taste testing was done to determine acceptability using a taste panel.

Results: Sixteen formulations showed variations in disintegration time, thickness, tensile strength and permeation profiles. My key finding is, ex vivo release profiles of the optimized formulation revealed first order release and later zero order.

Conclusions: Therefore, it is evident that rice paper IDF could efficiently deliver natural drugs into the blood.

Biography

Mukasa Eliphaz has worked at Medipharm Industries EA Ltd. Uganda factory for 15 years. He is specialized in cGMP and ORS Manufacture. He has studied at Mulago Hospital School of Dispensing for a Diploma Pharm in 1988. He taught at Mulago Paramedical School for 2 years. He has attended a Clinical Instructor's course at Mbale Health Manpower Development Center in 1999 and worked as an Assistant Drugs Inspector at Uganda National Drug Authority for 7 years. He has also attended NIPER Chandigarh India for Assessment of quality of Pharmaceuticals. He did his BPharm in 2012 at NMMU Port Elizabeth South Africa. He has worked at Johannesburg General Hospital Charlotte Maxeke for his Pharmacist Internship in 2013. Presently he is an MPharm student at the University of the Witwatersrand, SA 2013 to 2015.

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Notes: New Email address: eliphaz.mukasa@yahoo.com
Cell Nos. + 27 78 1080 535 + 256 772462479 + 256 772 984 101

APPENDIX L BATCH MANUFACTURING RECORDS

BATCH MANUFACTURING RECORD – 16 A

Product:									
Product Number:									
Approval Date:									
Batch Number:									
Manufacturing Date:									
Theoretical yield				**Actual yield**			**%age yield**		
Batch Number	Ingredients	Quantity	Expiry Date	Added by	Signature	Date	Checked by	Signature	Date
	Curcumin	15 g (5%)							
	Rice starch	30 g (15%)							
	Glycerine	45 g (15%)							
	Honey	15 g (5%)							
	Mannitol	6 g (2%)							
	Peppermint oil	2 drops (0.013%)							
	Stevia soln. 10%	12 ml (4%)							
	Cocoa flavour	6 g (2%)							
	Distilled water	300 ml (100%)							

Method:

1. Accurately weigh 45 g of Rice starch in a 600ml glass beaker
2. Add 15 ml of glycerine to the beaker
3. Add 100ml of distilled water to the rice in the beaker and homogenise using a Silverson emulsifier for 1 hour to make a smooth suspension.
4. Fill the water bath with 1/3 full of distilled water to cover the elements and keep it boiling at 100^0C.This may be done earlier to save time.
5. Place the second rice suspension beaker into the boiling water bath and keep steaming it between 85 – 90 ^{0}C for 2 hrs.
6. Accurately measure 15 g of Mannitol and put it in the beaker
7. Accurately measure 6 g of Cocoa and dissolved it in 10 ml of hot water and add it to the beaker
8. Add 15g of Honey to the beaker and stir with a glass rod to make a smooth paste
9. Add this paste to the Rice paste while is still very hot in 600 ml glass jar and stir with a stainless steel spatula to blend the Cocoa.
10. Add 100ml of distilled water to the rice in the beaker and homogenise using a silverson emulsifier at 500 rpm for 1 hour to make a smooth suspension.
11. Using a micropipette, add 2 drops of the peppermint oil t the cooled suspension and continue to stir gently at 100 rpm for 15 minutes to blend.
12. Keep aside to cool to room temperature about 25^0C
13. Accurately weigh the 15 g of Curcumin, add to the cooled emulsion, and stir gently using a motorised stirrer at 100 rpm.
14. Keep the suspension for 1 hour to settle in a tightly covered glass bottle to eliminate air bubbles.
15. Keep tightly covered ready for casting onto the 3.5 cm plastic Petri dishes. .
16. Measure off exactly one table spoonful (10 ml) of the suspension and triturate on a white tile to make sure you have a very smooth thick suspension and cast onto the 3.5 cm diameter plastic Petri dish.
17. Leave undisturbed for three (3) days (72 hrs).
18. Repeat in the same way to cast the films for the rest of the suspension.
19. If not all the rice suspension will be casted, keep the remaining rice suspension without the Curcumin in the fridge at 20^0C.
20. Once the film is formed, carefully remove it from the Petri dish. Trim the film to the required shape and size then wrap it immediately in aluminium foil to keep moist.
21. Keep in a tightly closed glass jar.
22. Label the container and store at room temperature 25^0C- 30^0C and Relative Humidity (HR) 60% ± 5% until further analysis or use.
23. Use the remaining suspension to cast more of Curcumin rice paper films as required in the same way

Storage and Manufacturing Conditions: Sterile, Temperature 25^0C – 30^0C and Relative Humidity (RH 60% ± 5%

Label: **Schedule ---**

Name of product: Curcumin rice paper film (Curcumin Rice Film®)
Usage: For Intraoral use only as directed
Store at 25^0C – 30^0C and Relative Humidity (HR) 60% ± 5% wrapped in Aluminium foil in a tightly closed glass jar.
Batch Number:
Manufacturing Date:
The Expiry: (Date of the rice starch must be retained on the label of the final product).



Manufactured by Wits Pharmacy

Containers:
Aluminium foil 1 roll
Tightly closed glass jar

Presentation:
1 x 28s x 20 mg (1cm x 2cm)

Utensils and Utilities:
Petri dishes 3 spatula, Bunsen Burner, Paper tissue, Glass beakers,

Equipment:
2 Mixing bowls, Water Bath, 1 Thermometer, I Stop watch, A pair of seizers, 1Disecting set, Bunsen burner, 1 Coking pot, 1 Viscometer.
We need Hot film rollers in the Industry for large scale production

Bulk Compounding Pharmacist	Signature	Date

APPENDIX L BATCH MANUFACTURING RECORDS

BATCH MANUFACTURING RECORD – 16 B

Product:									
Product Number:									
Approval Date:									
Batch Number:									
Manufacturing Date:									
Theoretical yield				**Actual yield**			**%age yield**		
Batch Number	Ingredients	Quantity	Expiry Date	Added by	Signature	Date	Checked by	Signature	Date
	Resveratrol	15 g (5%)							
	Rice starch	30 g (15%)							
	Glycerine	45 g (15%)							
	Honey	15 g (5%)							
	Mannitol	6 g (2%)							
	Peppermint oil	2 drops (0.013%)							
	Stevia soln. 10%	12 ml (4%)							
	Cocoa flavour	6 g (2%)							
	Distilled water	300 ml (100%)							

Method:

1. Accurately weigh 45 g of Rice starch in a 600ml glass beaker
2. Add 15 ml of glycerine to the beaker
3. Add 100ml of distilled water to the rice in the beaker and homogenise using a silverson emulsifier for 1 hour to make a smooth suspension.
4. Fill the water bath with 1/3 full of distilled water to cover the elements and keep it boiling at 100^0C.This may be done earlier to save time.
5. Place the second rice suspension beaker into the boiling water bath and keep steaming it between 85 – 90 ^{0}C for 2 hrs.
6. Accurately measure 15 g of Mannitol and put it in the beaker
7. Accurately measure 6 g of Coca and dissolved it in 10 ml of hot water and add it to the beaker
8. Add 15g of Honey to the beaker and stir with a glass rod to make a smooth paste
9. Add this paste to the Rice paste while is still very hot in 600 ml glass jar and stir with a stainless steel spatula to blend the Cocoa.
10. Add 100ml of distilled water to the rice in the beaker and homogenise using a Silverson emulsifier at 500 rpm for 1 hour to make a smooth suspension.
11. Using a micropipette, add 2 drops of the peppermint oil t the cooled suspension and continue to stir gently at 100 rpm for 15 minutes to blend.
12. Keep aside to cool to room temperature about 25^0C
13. Accurately weigh the 15 g of Resveratrol, add to the cooled emulsion, and stir gently using a motorised stirrer at 100 rpm.
14. Keep the suspension for 1 hour to settle in a tightly covered glass bottle to eliminate air bubbles.
15. Keep tightly covered ready for casting onto the 3.5 cm plastic Petri dishes.

    .
16. Measure off exactly one tablespoonful (10 ml) of the suspension and triturate on a white tile to make sure you have a very smooth thick suspension and cast onto the 3.5 cm diameter plastic

Petri dish.

17. Leave undisturbed for three (3) days (72 hrs).
18. Repeat in the same way to cast the films for the rest of the suspension.
19. If not all the rice suspension will be casted, keep the remaining rice suspension without the Curcumin in the fridge at 20^0C.
20. Once the film is formed, carefully remove it from the Petri dish. Trim the film to the required shape and size then wrap it immediately in aluminium foil to keep moist.
21. Keep in a tightly closed glass jar.
22. Label the container and store at room temperature 25^0C- 30^0C and Relative Humidity (HR) 60% ± 5% until further analysis or use.
23. Use the remaining suspension to cast more films of Resveratrol rice paper as required.

Storage and Manufacturing Conditions: **Sterile,** Temperature 25^0C – 30^0C and Relative Humidity (RH)

60% ± 5%

Label: **Schedule ---**

Name of product: Resveratrol rice paper film (Retro Rice Film®)

Usage: For Intraoral use only as directed

Store at 25^0C – 30^0C and Relative Humidity (HR) 60% ± 5% wrapped in Aluminium foil in a tightly closed glass jar.

Batch Number:

Manufacturing Date:

The Expiry: (Date of the rice starch must be retained on the label of the final product).



Manufactured by Wits Pharmacy

Containers:

Aluminium foil 1 roll

Tightly closed glass jar

Presentation:

1 x 28s x 20 mg (1cm x 2cm)

Utensils and Utilities:

Petri dishes 3 spatula, Bunsen Burner, Paper tissue, Glass beakers,

Equipment:

2 Mixing bowls, Water Bath, 1 Thermometer, I Stop watch, A pair of seizers, 1Disecting set, Bunsen burner, 1 Coking pot, 1 Viscometer.

We need Hot film rollers in the Industry for large scale production

Bulk Compounding Pharmacist	Signature	Date

APPENDIX M
PROFESSIONAL RESEARCH POSTER