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FACULTY OF HEALTH SCIENCES

THE PHARMACEUTICAL DEVELOPMENT OF A FIXED COMBINATION ANTI-TUBERCULOSIS DOSAGE FORM.

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Science in medicine (Pharmaceutical affairs)

Johannesburg, 1998

DECLARATION

I, Salima Ebrahim declare that this research report is my own work. It is being submitted for the degree of Master of Science in medicine (Pharmaceutical affairs) in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

.....

.....26 th.....day of.....MAY.....,1998.

Dedicated to my mum and dad, Ayesha and Abdool Hak Ebrahim

ABSTRACT

Despite the availability of highly effective treatment regimens for tuberculosis, cure rates remain low for tuberculosis mainly due to patient non-compliance which results in the occurrence of multidrug resistance tuberculosis. To avoid the problem of further creation and propagation of multidrug-resistant tuberculosis, patients should be given fixed-dose combinations of anti-tubercular drugs whenever self-administration of drugs is permitted.

During this study, an anti-tuberculosis extemporaneous powder for suspension was optimized in order to formulate a fixed combination of rifampicin, isoniazid, pyrazinamide and ethambutol hydrochloride as a powder to be reconstituted with water by the patient prior to administration. Once an effective manufacturing method was established, different suspending agents were evaluated by their influence on powder flow properties and sedimentation volume on the powder blends. Sodium starch glycollate was chosen as the suspending agent of choice because as the concentration of sodium starch glycollate was increased, the powder flow properties of the powder blends improved. The sedimentation volume also increased with the increasing concentration of sodium starch glycollate in the powder blends. A suitable flavour and colour was also determined to increase the acceptability of the preparation to the patient. Liquorice flavour was the most acceptable in terms of colour and flavour. An evaluation of the dissolution characteristics of the extemporaneous powder for suspension was also conducted in comparison to the dissolution profiles from commercially available tablet dosage forms. The dissolution rates from the powder for suspension for rifampicin, isoniazid and pyrazinamide was faster than from the

commercially available tablet dosage form, while the dissolution rate of ethambutol HCl from the powder closely resembles the dissolution profile from the Rolab-Ethambutol HCl® tablet dosage form.

Therefore, a fixed combination powder for suspension was achieved and with its ease of administration would increase the compliance amongst tuberculosis patients, and increase therapeutic outcomes.

ACKNOWLEDGMENTS

Dr M. P. Danckwerts	- for his supervision and invaluable guidance for this project.
Professor Indres Moodley	- for his support and motivation for the duration of my research.
Miss R. Vather and Mr N. Shingwenyana	- for their valuable advice and technical assistance.
University research committee	- for the financial assistance which greatly contributed to this study.
Mr. Nazeem Ebrahim	- for his encouragement and motivation throughout my studies.

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1.0 INTRODUCTION

1.1 BACKGROUND TO PROJECT

Mycobacterium tuberculosis kills 3 million people each year, and in 1993 the World Health Organization declared tuberculosis a global emergency (Department of Health, 1996a). South Africa has the highest incidence of tuberculosis in the world with an annual number of new tuberculosis cases at 377 per 100 000 members of the population, while in other problem areas of the world, the average is only 200 per 100 000 (Singer, 1997). This tuberculosis epidemic is being fuelled by the HIV epidemic, increased migration, poverty and an expanding population.

Despite the availability of highly effective treatment regimens for tuberculosis, cure rates remain unacceptably low, since patients do not take the prescribed medications with sufficient regularity and duration to achieve a cure. In particular, regular intake of drugs in the initial 2-month phase is often not achieved. While short-course (less than the conventional 18 to 24 months) chemotherapy of 6 months has led to a reduction in dropout rates from treatment programmes, this alone has not consistently overcome patient non-adherence and drop-out (World Health Organization, 1993a). The main problem in the delivery of the initial phase of treatment, which occurs in rural areas where the patients home may be far from the nearest health centre, is decreasing supervision of the disease and drug administration. In areas with a well-developed primary health care system, it may be

feasible for a community health worker to supervise drug administration each day during the initial phase.

Another element of patient non-adherence, which is potentially more serious than simply dropping out from the treatment programme, is partial adherence to a prescribed regimen. When some drugs are selectively discontinued and others are continued, there is an increased risk of developing acquired drug resistance (Kochi et al.,1993). Resistance of *M.tuberculosis* to antimycobacterial agents is a worldwide problem in both immunocompetent and HIV-infected populations (Edlin et al.,1992; Fischl et al.,1992).

To avoid the problem of further creation and propagation of multidrug-resistant tuberculosis due to noncompliance, patients should be given fixed-dose combinations adjusted for body weight whenever self-administration of anti-tubercular drugs is permitted (World Health Organization,1993b).The recommended protocol for chemotherapy is to give at least two drugs to which the patients bacilli are sensitive. Surveys of cultures or bacilli from previously untreated patients in Britain show that about 4% are initially resistant to one of the commonly used anti-tuberculosis drugs (Rainsbury,1981). In South Africa 1% of new patients and 4% of retreatment patients are multidrug resistant (Department of Health,1996b).

Multi-drug therapy will almost always prevent the emergence of drug resistant mutants. The initial intensive phase is therefore of enormous importance. The drugs used most commonly

are rifampicin, isoniazid, streptomycin, pyrazinamide and ethambutol hydrochloride. The key concept involved in short course treatment is that two phases are involved, one of early killing and another of sterilization. In clinical descriptions these are referred to as the initial phase and the continuation phase (Ross et al.,1983). In South Africa the Department of Health (1996c) stipulated the following treatment regimen for new adult cases diagnosed with tuberculosis:

TABLE 1.1 Treatment regimen for new adult cases diagnosed with *tuberculosis*

Intensive Phase 2 months	**Over 50 kg**
Rifampicin/Isoniazid/pyrazinamide Combination tablet 120/80/250 mg (Pyrifin®)	5 tablets
Ethambutol 400 mg (Rolab-Ethambutol Hcl 400®)	3 tablets
Daily Number Of Tablets	**8**
Continuation Phase 4 months	**Over 50 kg**
Rifampicin/ Isoniazid Combination tablet 150/100 mg 300/150 mg	2 tablets
Daily Number Of Tablets	**2**

Treatment regimens are divided into the initial or intensive phase and the continuation phase. During the intensive phase consisting usually of four drugs given daily, the bactericidal effect of the treatment leads to rapid bacteriological sputum conversion and a reduction in clinical symptoms. During the continuation phase, consisting usually of fewer

drugs given either daily or intermittently, the sterilizing effect of treatment eliminates remaining bacilli and prevents subsequent relapse.(World Health Organization,1993c). In order to comply with the above recommended dosage regime and to improve patient compliance, Rifater^R, a combination product comprising three major anti-tuberculosis drugs in one tablet (i.e. rifampicin, isoniazid and pyrazinamide) was introduced to the South African market. Rifater^R, has been shown to:

(i) be as effective as standard therapy.

(ii) have an acceptable low level of adverse effects.

(iii) offer convenience of dosing, administration and supervision.(Glatthaar et al.,1991).

Acocella et al. (1988) have demonstrated that no major changes in blood kinetics of isoniazid, rifampicin, and pyrazinamide occur when the three anti-tuberculosis drugs are administered orally in a fixed triple combination over a period of time corresponding to that of the initial intensive phase of short-course anti-tuberculosis chemotherapy. Furthermore, a study carried out by Ellard et al. (1986) has indicated that excellent bioavailability can be achieved when isoniazid, rifampicin and pyrazinamide are administered as a fixed combined product. Hence, there is good evidence that a fixed dose combination regimen will prove to overcome the problem of multidrug-resistant tuberculosis. Furthermore, the strain placed on patients who have to take drugs for long periods would be reduced and will assist us to achieve a higher cure rate in South Africa.

1.2 STATEMENT OF PROBLEM

The disadvantage with current anti-tuberculosis treatments is that with separate formulations of isoniazid, pyrazinamide and rifampicin, a large number and sheer bulk of tablets and capsules have to be ingested at a time. It can be as many as 8 large tablets with diameters up to 15 mm that have to be swallowed at a time. For some patients this causes difficulties in swallowing and is a major contributing factor to patient non-compliance.

Fixed combinations, are dosage forms designed to effect a planned interaction between the components or to provide convenience to the patient. Since each component is required for rational therapy, fixed combinations ensure compliance. Where the components interact, by design or otherwise, fixed combinations define the intensity and the duration of the net effect. For convenience dosage forms, the primary biopharmaceutic concern is to ensure that the bioavailability of each component in the fixed combination is equivalent to that following the concomitant administration of the single entities at the same dose by the same route (Kwan et al.,1986a). In addition, the principal objective of dosage form design is to achieve a predictable therapeutic response to a drug included in a formulation which is capable of large scale manufacture with reproducible product quality.

1.3 DELINEATION OF PROBLEM

At present, there exists no fixed combination dosage form of rifampicin, isoniazid, pyrazinamide and ethambutol to be administered as a single unit. Furthermore, in therapeutic concentrations combining rifampicin, isoniazid, pyrazinamide and ethambutol as a single unit would produce a tablet or capsule that would be much too large for swallowing. Therefore, for this study, a powder for suspension to be reconstituted with water prior to administration will be designed and evaluated. The powder blend will be conveniently packaged into single dose sachets. With this convenient packaging, all the patient needs to do is pour the powder into half a glass of water, stir for a few seconds and then drink the suspension. Suspensions have the following advantages over the current conventional dosage forms of tablets and capsules:

(i) Suspensions contain finely divided drugs suspended in a suitable vehicle, and are a useful means of administering large amounts of drugs which would be inconvenient if taken in tablet or capsule form.

(ii) They are useful for patients who have difficulty in swallowing tablets and capsules.

(iii) Dissolution of a drug is required prior to absorption following administration of drug, and in a suspension, the fine particles with a large surface area are presented to dissolving fluids which facilitates drug dissolution and thereby the onset of drug action. (Billany, 1988a).

(iv) Powder mixtures are also the simplest and most economical preparations to produce.

(iv) They present few stability problems as no heat or solvents are involved in their production.(Lund,1994a)

The basic concern in the development of a suspension dosage form is that suspensions settle, and it is necessary to redistribute them before using them as products. Since rifampicin, is slightly soluble in water (Lund,1994b), a suitable suspending agent will have to be added to the formulation. The suspending agent must be one which will suspend and thicken immediately in cold aqueous vehicles. A desirable powder for suspension should also have the desired flow properties for filling into sachets, be easily redispersed by shaking, and should suspend the active drugs long enough to withdraw an accurate dose (Patel et al.,1986). The use of suspension additives i.e. colour and flavour must also be considered, since these may materially affect the characteristics of the suspension system.

1.4 APPROACH TO PROBLEM

This project will be approached from a pharmaceutical formulation perspective and will take into account all factors which may optimize the formulation of the multi-combination anti-tubercular powder for suspension. For the study, factorial experimental design (Box et al.,1978) will be used as a basis of optimizing the experiments. This design enables one to test the existence of a statistical interaction between variables and allows for multitudes of comparisons to be made.

The following factors would require consideration during formulation of the suspension dosage form:

1.Particle size control

It is necessary to ensure that the drugs to be suspended are suitably reduced in size prior to formulation as the rate of sedimentation of a suspended particle can be retarded by a reduction in its size. Therefore, the use of different particle sizes of rifampicin will be evaluated.

2.The use of wetting agents

Since redispersability is one of the major considerations in assessing the acceptability of a suspension, and since the sediment formed should be easily dispersed by shaking to yield a homogeneous system, a suitable wetting agent would be required in the formulation. With the inclusion of a wetting agent, insoluble solids may be easily wetted by water and will be dispersed readily throughout the aqueous phase with minimum agitation. Sodium lauryl sulphate will be used for the formulations to suitably wet the active drugs and measurement of the sedimentation volume will be carried out. The nature of the sediment should be such that the particles which do settle, must not form a hard mass but should be easily redispersed into a uniform mixture by moderate agitation.

3.Powder flow

Of primary importance to the formulator, when handling drug powder for filling into containers and sachets, is an assessment of flow properties. These can be evaluated simply by the angle of repose (θ). Angle of repose measurements are extremely useful derived parameters for assessing the impact of changes in drug bulk, as new ingredients change in particle size and shape. (Wells, 1988a).

4. Formulation additives

In many instances, the use of only a sweetening agent in a formulation may not be sufficient to mask an unpleasant taste of a particular drug. Therefore, the inclusion of flavouring agents in formulations, not only increases patient compliance by masking unpleasant tastes, they have the additional advantage of enabling easy identification of products (Billany,1988b). Although, saccharin sodium will be used as the sweetening agent in the formulation, different flavouring and colouring agents will have to be assessed to mask the the bitter taste of pyrazinamide in the formulation.

2.0 EXPERIMENTAL

In experiment 3.1 of the research report, the suitability of different suspending agents for the extemporaneous powder for suspension will be evaluated. Once a suitable suspending agent is chosen in the preliminary part of the experiment, the extemporaneous powder for suspension will be optimized by evaluating different concentrations of suspending agent as well as the influence of different particle sizes on the properties of the powder formula. In experiment 3.2, a suitable flavour and colour for the suspension will be chosen while in the final experiment 3.3, the dissolution profiles of the active materials will be evaluated in both the extemporaneous suspension, as well as tablet dosage forms for comparison.

2.1 Materials

Rifampicin BP, pyrazinamide BP, and ethambutol HCl BP were obtained from Themis Chemicals Limited. Isoniazid BP was obtained from Marsing & Co. LTD. The SCMC, povidone, sodium starch glycollate, colloidal silicone dioxide and saccharin sodium were obtained from Lanpro Chemicals (Pty. Ltd.). Sodium lauryl sulphate was obtained from Saarchem (Pty. Ltd.). The liquorice LR 17896, cherry LR 11377 and raspberry LR 6285 powder flavours were obtained from Haarmann & Reimer. Pyrifin^{R} , a fixed combination tablet dosage form consisting of rifampicin, isoniazid and pyrazinamide manufactured by Noristan Generica was obtained for the comparative study. Rolab-Ethambutol HCl 400^{R}, a single tablet dosage manufactured by Rolab (Pty. Ltd.) was also obtained for the comparative study.

For the HPLC detections, HPLC grade water was obtained from a MilliQ system (Millipore, S.A.). Other reagents used were : Acetonitrile , methanol and ammonium perchlorate, obtained from BDH (S.A.). 0.45 μ Millex syringe filters were obtained from Microsept S.A. for the filtration of samples prior to analysis.

3.0 METHODS

3.1 OPTIMIZATION OF AN EXTEMPORANEOUS SUSPENDING AGENT FOR SUSPENSION OF RIFAMPICIN IN THE COMBINATION POWDER FOR SUSPENSION.

3.1.1 *Introduction*

Extemporaneous suspensions require a suitable suspending agent to produce a product that is not only elegant and stable but also capable of delivering the drug to the systemic circulation at an appropriate rate. The suspending agent for an extemporaneous suspension should allow for the following properties of a well formulated suspension to be easily met:
(1) The suspension must remain sufficiently homogeneous for at least the period between stirring the contents and drinking the required dose.
(2) The sediment produced must be easily resuspended by the use of moderate agitation.
(3) The viscosity must allow for easy pourability of the dose.

Many polymers have been used to produce extemporaneous suspensions. Some of the more popular ones include, sodium starch glycollate, sodium carboxy methyl cellulose (SCMC) and povidone. In studies carried out by Farley and Lund (1976), and Trevean (1981) , it was found that amongst other suspending agents, sodium starch glycollate and sodium carboxy methyl cellulose were suitable as extemporaneous suspending agents. Aqueous

solutions of povidone, a synthetic suspending agent are stable and can be stored for long periods in suitable containers (Lund,1994c).The quality of synthetic and semi-synthetic agents tend to be less variable than that of suspending agents derived from natural sources. It was therefore decided to investigate the use of these agents in the anti-tuberculosis formula in order to optimise the properties of the combination powder for suspension.

3.1.1.1 *Aim*

To optimise the suspension of rifampicin by determining the effect of the type of suspending agent, concentration of suspending agent, and particle size of rifampicin.

3.1.2 **Rifampicin particle size separation**

For this study, the rifampicin was separated into three different size fractions by passing the powder through a nest of sieves (Endecotts Limited, London), with aperture sizes of 180 μm, 125 μm and 63 μm. The sieves were arranged in descending order of size in an Endecotts Octagon 200 test sieve shaker. The powder was shaken for 30 minutes at an amplitude of 7.

3.1.3 Study design

The study followed a sequential 3^1 preliminary study followed by a 3^2 factorial experimental design. In the preliminary phase, the rifampicin was blended with three different suspending agents to allow for the assessment of powder flow and sedimentation behaviour of the different blends.The normalised factor levels of the independent variables (type of suspending agent) are presented in table 3.1. Powder flow and sediment volume were used as the dependent variables in order to evaluate the formulations.

TABLE 3.1 Normalised factor levels of the independent variables for the 3^1 factorial design.

Variable	Factor Level			Units
	-1	0	1	
Type of suspending agent	S.C.M.C.	Povidone	Explotab	%w/w

Once the most suitable suspending agent was found in the preliminary experiment, a 3^2 factorial design was conducted to determine the effect of concentration and particle size of rifampicin on the flow and sediment volume. The amount of suspending agent added to the formulation (c), and the particle size range (p) of the rifampicin were used as independent variables. The normalised factor levels of the independent variables are presented in table 3.2 below.

TABLE 3.2 Normalised factor levels of the independent variables for the 3^2 factorial design.

Variable	Factor Level			Units
	-1	0	1	
Concentration of suspending agent *(c)*	5	15	25	%w/w
Particle size of rifampicin *(p)*	32	94	156	μm

3.1.4 Formulations

For the preliminary study on the suitability of the suspending agents, all the powders in Table 3.3 were passed through a 710 μm sieve prior to blending in an Erweka AR400 rotatory cube mixer for 4 minutes. The dose of the actives per sachet are as stipulated by the Department of Health.The powders were blended with either povidone, sodium starch glycollate or SCMC as the suspending agent. Thereafter, each of the powder blends were tested for flow as well as sediment volume.

TABLE 3.3 Powders used for the preliminary formulation

Materials	Quantity/dose
Rifampicin	600 mg
Isoniazid	300 mg
Pyrazinamide	1500 mg
Ethambutol hydrochloride	1000 mg
Sodium lauryl sulphate	25 mg
Colloidal silicone dioxide	50 mg
Suspending agent	183 mg
Saccharin sodium	120 mg
Total Mass	3778mg

Once it was determined from the preliminary study that sodium starch glycollate was the most suitable suspending agent, each of the different size fractions (32 μm, 94 μm and 156 μm) of the rifampicin was blended with the different concentrations of sodium starch glycollate (5%w/w, 15%w/w, or 25%w/w), together with the other materials as in Table 3.4. The 10%w/w increments of sodium starch glycollate were chosen in order to enhance the response surface plots of the estimated effects of the concentration of sodium starch glycollate on the angle of repose and sediment volume of the powder blends. Colloidal silicone dioxide was included in the formulation to improve the flow properties of the dry powders (Wade et al.,1994). All the powders except rifampicin was passed through a 710 μm sieve prior to blending in an Erweka AR400 rotatory cube mixer for 4 minutes. Thereafter, each of the powder blends were tested for flow as well as sedimentation.

TABLE 3.4 Powders used for formulations in the second part of experiment 3.1

Materials	Quantity/dose
Rifampicin (mean particle size of 32 μm, 94 μm, 156 μm)	600 mg
Isoniazid	300 mg
Pyrazinamide	1500 mg
Ethambutol hydrochloride	1000 mg
Sodium lauryl sulphate	25 mg
Colloidal silicone dioxide	50 mg
Saccharin sodium	120 mg
sodium starch glycollate	$5\%^{w}/_{w}$, $15\%^{w}/_{w}$, $25\%^{w}/_{w}$

3.1.5 Measurement of powder flow

Powder flow of the powder blends can best be evaluated from their angle of repose measurements. This was achieved by using a powder funnel with a diameter of 10 mm which was fixed to a retort stand, such that the bottom of the orifice was 10 cm from the bench surface (covered with paper). The outlet was closed and the funnel carefully filled with 3.778 g of each powder formula to be tested. The contents were then allowed to pour out onto a piece of graph paper. The radius and height of the cone were measured and the angle of repose was then calculated from the following equation:

θ = arc tan h/r ---------- equation 3.1

where, arc tan = $\tan^{-1}$

θ = angle of repose

h= height of powder heap measured in millimeters (mm).

r = radius of powder heap measured in millimeters (mm).

3.1.6 Measurement of sediment

A powder sample equivalent to one unit dose (3.778g) was weighed out for each formulation in the study and dispersed in 100 mls of tap water by stirring for 30 seconds. Each mixed suspension was then placed into a 100 ml cylinder and the sediment was measured after one hour. One hour was sufficient time to allow for differentiation of sediment volumes amongst the formulations tested.

3.1.7 Statistical analysis

STATGRAPHICS *v* 5 (Statistical Graphics Corporation, U.S.A.) was used to analyse the data presented in table 2.6. The independent variables were concentration of suspending agent (*c*) and the average particle size (*p*). The dependent variables were flow (angle of repose) and sediment volume. Response-surface plots were constructed for these variables, in order to determine the optimal combination of the variables. Main effects and significant interactions were also calculated. A simple regression model for the concentration of

suspending agent and particle size that maximizes sediment and flow was developed from the results as follows:

$$Y(c,p) = a_0 + a_1c + a_2p + a_3pc + a_4c^2 + a_5p^2 \qquad \text{---- equation 3.2}$$

where; $a_0, \ldots\ldots, a_5$ are regression coefficients of the system.

c denotes the concentration of sodium starch glycollate (%w/w).

p denotes the particle size of rifampicin (μm).

Each term in the final regression equation for sediment was only included if the t-test p value was less than 0.05. The regression coefficients for effects that were considered insignificant were eliminated and the model was re-estimated. All statistical analysis was performed using STATGRAPHICS version 5 (Statistical Graphics Corporation, U.S.A.).

3.1.8 RESULTS AND DISCUSSION

3.1.8.1 Preliminary determination of suitability of type of suspending agent.

Table 3.5 shows the results from the preliminary investigation into the suitability of the extemporaneous suspending agents used.

TABLE 3.5 Angle of repose and sedimentation volumes of the preliminary powder blends.

Type	Flow ± S.D (Angle of repose)(n=3)	Sedimentation volume(cm^3) ± S.D. (n=3)
Sodium carboxy methyl cellulose	35.61 ± 2.36	8.70 ± 1.15
Sodium starch glycollate	31.62 ± 2.61	25.67 ± 1.15
Povidone	25.43 ± 0.85	9.67 ± 3.06

It is evident from table 3.5 that the greatest volume of sediment was achieved with the sodium starch glycollate. This was followed by povidone and SCMC with values of 9.67 cm^3 and 8.70 cm^3 respectively.

After analysis of powder flow, povidone exhibited the best flow with the lowest angle of repose followed by sodium starch glycollate and sodium carboxy methyl cellulose. Using the LSD analysis of variance test, it was shown that there was a significant difference between the groups for sediment volume as well as flow values at the 95% confidence level.

An important physical property of a well formulated suspension, is the degree of suspendability, i.e. the greater the sediment produced, the easier the redispersion with minimum agitation, provided such a suspension is uniformly and loosely packed. (Farley et al.,1976). Therefore, based on the results from the preliminary study, it was found that although povidone exhibited better flow, sodium starch glycollate would be the suspending agent of choice because of the greater sediment volume achieved. Thus, the use of sodium starch glycollate was investigated further to determine the ideal concentration to use.

3.1.8.2 **Determination of the effect of concentration of suspending agent and particle size of rifampicin on powder flow and sediment volume.**

Table 3.6 lists the angle of repose and sediment values of the different powder blends which were evaluated during the second part of experiment 3.1. The powder blends were formulated according to the factor levels in table 3.2.

Table 3.7 lists all the relevant statistical parameters calculated from the effect of the two independent variables on the angle of repose and sediment volume of the powder formulations. The statistical analysis was carried out on all the experimental runs (total 19).

TABLE 3.6

Angle of repose and sediment of powder blends. (*c;* concentration of suspending agent, *p*; particle size of rifampicin).

RUN	*C* (%w/w)	*P* (μm)	FLOW (angle of repose)	SEDIMENT (cm^3)
1_a	0	0	26.14	41.00
1_b	0	0	26.99	53.00
1_c	0	0	26.14	53.00
2_a	1	1	25.01	63.00
2_b	1	1	23.19	65.00
3_a	-1	1	30.43	23.00
3_b	-1	1	30.15	22.00
4_a	-1	-1	35.12	23.00
4_b	-1	-1	35.64	24.00
5_a	-1	0	33.67	25.00
5_b	-1	0	33.86	23.00
6_a	0	-1	28.86	52.00
6_b	0	-1	29.81	54.00
7_a	1	0	25.14	60.00
7_b	1	0	25.82	62.00
8_a	0	1	25.99	50.00
8_b	0	1	26.56	51.00
9_a	1	-1	26.57	67.00
9_b	1	-1	27.34	70.00

TABLE 3.7 Relevant statistical parameters for sediment and flow studies

Source	Estimated effects ±Standard error (13 *d.f.*)	*p* Value	Regression coefficients	Regression coefficients re-estimated
Sediment				
Average	49.196 ± 1.508			
Constant			8.7589	4.8795
c	41.083 ± 1.868	0.0000	4.2301	4.0381
p	-2.750 ± 1.868	0.1647	-0.1203	
cp	-1.625 ± 2.288	0.4975	1.3105×10^{-3}	
cc	-13.685± 3.091	0.0007	-0.0684	-0.0661
pp	4.815 ± 3.091	0.1433	6.2633×10^{-4}	
Angle of repose				
Average	27.057± 0.369			
Constant			42.1608	40.4785
c	-7.633 ± 0.457	0.0000	-1.0933	-1.0163
p	-3.668 ± 0.457	0.0000	-0.0564	-0.0296
c p	1.118 ± 0.559	0.0670	9.0121×10^{-4}	
cc	4.179 ± 0.756	0.0001	0.0209	0.0212
pp	0.544 ± 0.756	0.4918	7.0777×10^{-5}	

3.1.8.3 Powder Flow

Figure 3.1 is the response surface plot of the estimated effects of the concentration of sodium starch glycollate and particle size of rifampicin on the angle of repose of the powder blends. As the concentration of sodium starch glycollate in the powder blend is increased, the angle of repose decreases, resulting in better flow. This is evidenced by the high significance of the concentration c as well as statistical cc interaction variables with p values of <0.05 in table 3.7. Although there exists a statistical significance of particle size p on the angle of repose of the powder blends, ($p<0.05$), there is a very slight decrease (3.66833 $\pm$ 0.457) in the angle of repose as the particle size of rifampicin is increased. This small decrease may be attributed to the fact that as particle size of powders is increased, flow is increased because of minimal adhesion of powders resulting in lower angle of repose values. Equation 3.3 was developed from the re-estimated regression coefficients in order to calculate the concentration of suspending agent required to produce the optimal powder flow.

$$Y\ (angle\ of\ repose) = 40.4785 - 1.0163c - 0.0296p + 0.0212c^2 \quad \text{---- equation 3.3}$$

Using equation 3.3, excellent flow i.e. angle of repose of 20^{o} would be achieved with a concentration of 15%w/w of sodium starch glycollate and particle size of rifampicin of 13.04μ.

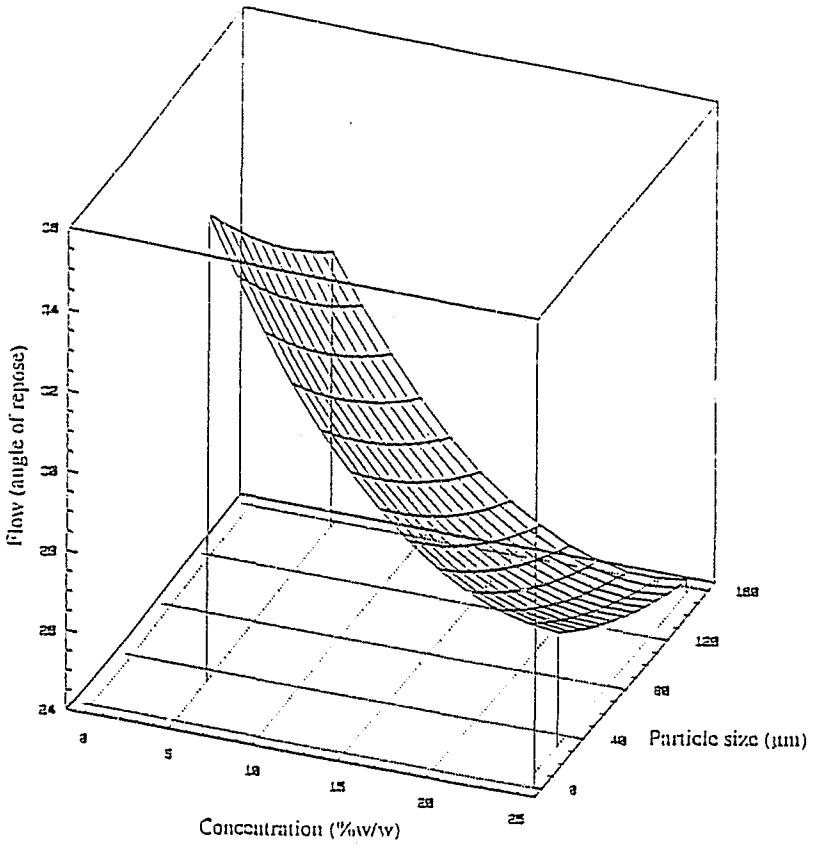


Figure 3.1 Response surface plot of the estimated effects of c and p on the flow of the powder blends.

3.1.8.4 Sediment

Figure 3.2 is the response surface plot of the concentration of sodium starch glycollate and particle size of rifampicin on the sedimentation volume of the powder blends. As the concentration of sodium starch glycollate increases, the sedimentation volume increases.

This is a significant change because as the concentration of sodium starch glycollate is increased by 10%, there is a 41.083 $\pm$ 1.868 cm^3 fold increase in the sediment volume (table 3.7).

This statistical significance is evident by the significant *c* and *cc* values with p values of <0.05. However, the change in particle size of rifampicin again has a very minimal effect on the sedimentation volume and is not statistically significant ($p>0.05$). An increase in particle size therefore results in a minimal decrease (2.750 $\pm$ 1.868) in sediment volume. Equation 3.4 was developed from the re-estimated regression coefficients in order to calculate the concentration of suspending agent required to produce the optimal sediment volume.

$$Y\ (\textit{sediment volume}) = 4.8795 + 4.0381c - 0.0661c^2 \qquad \text{---- equation 3.4}$$

Using equation 3.4, values of *c* that optimized the sediment volume were calculated. A maximum sediment volume of 44 cm^3 can be achieved using 49%w/w sodium starch glycollate. From the above results, it can be noted that concentration of sodium starch glycollate plays a significant role in improving flow of the powder blends. It also increases the sediment volume as the concentration is increased in the powder blends. However, since suspendability is a major criterion in suspension formulation, the concentration of sodium starch glycollate, 49%w/w that would maximize the sediment would be used in all future powder blends. Since, a change in the particle size of rifampicin contributed minimally to the flow and was not significant on the sediment volume, it was concluded that the particle

size will not have to be adjusted prior to formulation. Hence, the rifampicin will be used in the supplied form for all future formulations without any further manipulation on the particle size.

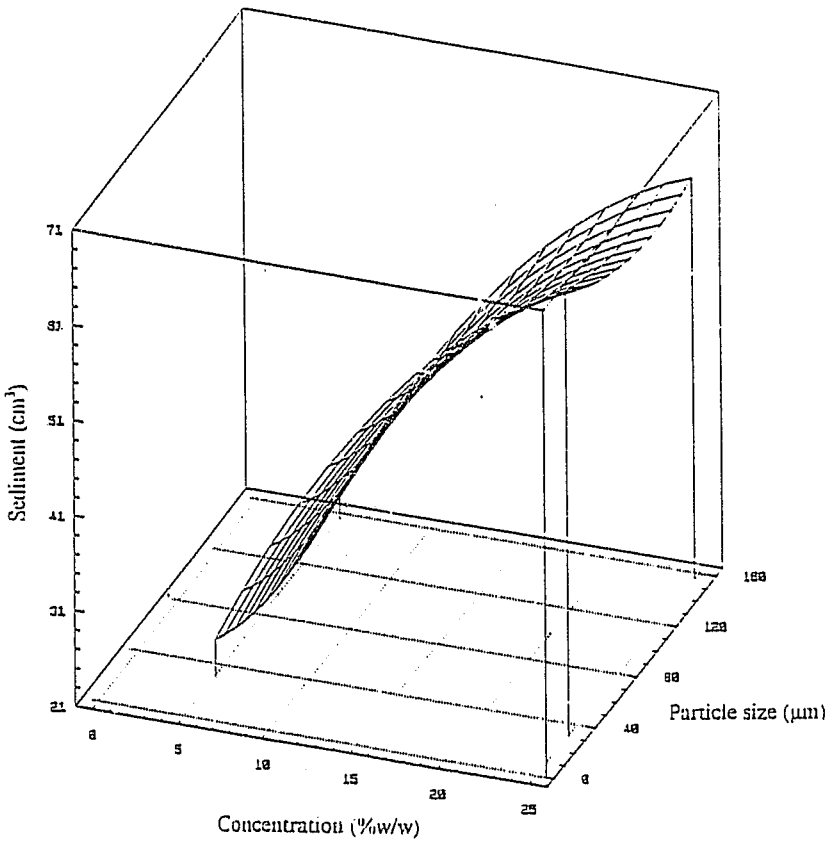


Figure 3.2 Response surface plots of the estimated effects of c and p on the sediment of the powder blends.

3.2 DETERMINATION OF A SUITABLE FLAVOUR AND COLOUR.

3.2.1 Introduction

Flavouring agents may be added to oral preparations in order to mask an unpleasant taste and increase the acceptability of the preparation to the patient (Billany,1988c). The inclusion of flavours has the additional advantage of enabling identification of liquid products to be achieved. Some generalizations concerning the selection of flavours to mask specific types of taste have been suggested by Wesley, 1957 and Janovsky, 1960. They recommend the flavours as listed in table 3.8 mask various taste sensations. Therefore, it was decided to assess at least three flavours i.e. liquorice, raspberry and cherry powder flavours to be included in the formulation in order to mask the slight bitter to sour taste of the powder when reconstituted with water.

TABLE 3.8 flavours recommended for specific taste sensations

Taste Sensation	Recommended Flavour
Salt	Butterscotch, maple, apricot, peach, vanilla, wintergreen mint
Bitter	Wild cherry, walnut, chocolate, mint combinations, anise, passion fruit, mint spice
Sweet	Fruit and berry, vanilla
Sour	Liquorice, raspberry, citrus flavours, root beer

3.2.1.1 *Aim*

To determine a suitable flavour and colour to improve the palatability of the extemporaneous suspension.

3.2.3 **Study design**

The type of flavour is usually chosen according to the taste to be disguised or masked, but the selection of a flavour for a particular medicine is difficult because of the highly subjective nature of taste preferences (Little Inc.,1958). Therefore, a panel of 25 volunteers were used in a single blind crossover study to evaluate the three different flavours i.e. liquorice, cherry and rasberry flavours. A questionaire was drawn up to assess the acceptability of the different flavours as well as the colour of the preparations. The volunteers rated the formulations according to the following ordinal scale of measurement;

3 = highest acceptable flavour/colour

2 = acceptable flavour/colour

1 = unacceptable flavour/colour.

3.2.4 Formulations

For this study, the formulations consisted of all the powders listed in table 3.9. All the powders were passed through a 710 μm sieve prior to blending together in an Erweka AR400 rotatory cube mixer for 4 minutes. Thereafter, each of the powder blends were dispersed into 100 mls of water and evaluated by the volunteers.

TABLE 3.9 Powders used for formulations in experiment 3.2

Materials	Quantity/dose
Rifampicin	600 mg
Isoniazid	300 mg
Pyrazinamide	1500 mg
Ethambutol hydrochloride	1000 mg
Sodium lauryl sulphate	25 mg
Colloidal silicone dioxide	50 mg
Saccharin sodium	120 mg
Powder flavours	250 mg
sodium starch glycollate	3750 mg

3.2.5 Results and discussion

From figure 3.3 it is evident that Formula A with a liquorice flavour and liquorice-brown colour was the most acceptable for flavour (80%), as well as colour (72%), respectively by the volunteers.

Figure 3.3 depicts that the raspberry flavour for Formula B is acceptable as a flavourant by 76% of the volunteers, while the red colour was least acceptable (72%) for the formulation.

It is evident from figure 3.3 that Formula C with a cherry flavour and orange/red colour was the least acceptable for flavour by 92% of the volunteers. The colour of this preparation was also the least acceptable by 80% of the volunteers.

Therefore, Formula A was the most acceptable in terms of colour and flavour, resembling most pharmaceutical liquid formulations available on the market. Formula B followed with its rasberry flavour and red colour, whilst the most unpopular cherry flavour and colour followed.

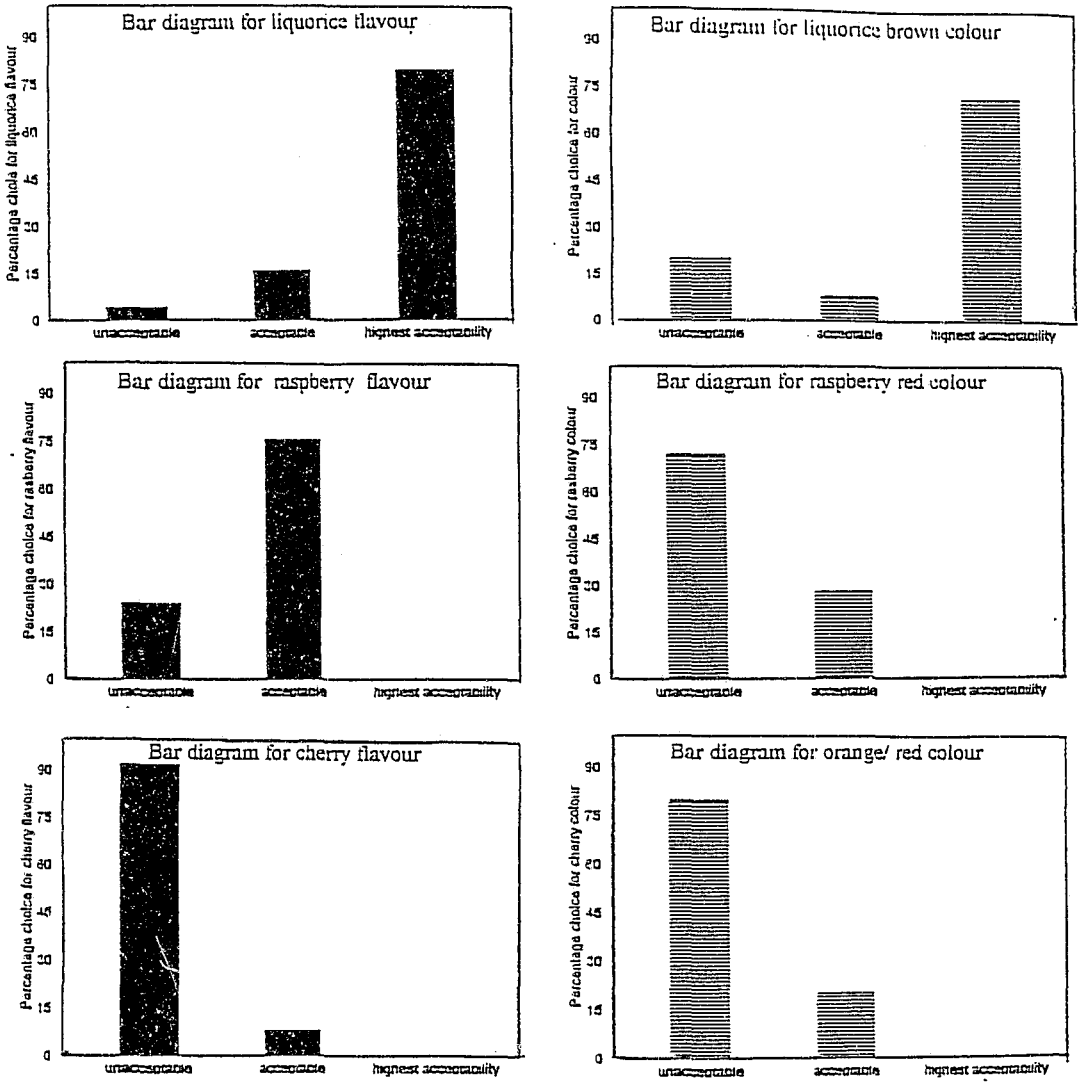


Figure 3.3 Bar graphs depicting % choice for the flavours and colours in formulas A, B and C.

3.3 EVALUATION OF THE DISSOLUTION CHARACTERISTICS OF THE EXTEMPORANEOUS POWDER FOR SUSPENSION.

3.3.1 Introduction

There are many different methods for the in vitro and in vivo testing of dosage forms (McGinty et al.,1981; Skelley et al.,1991) . The purpose of an vitro study is to provide a fast and inexpensive method that correlates with the performance of a dosage form in human subjects (Banker et al.,1986). Dissolution tests of powdered drugs are also currently used for drug characterization (De Almeida et al.,1997). Although they are mostly utilized as quality control methods (to ensure end product quality or batch to batch consistency), they may also be correlated to in vivo activity. It is well recognized that the rate of dissolution often controls the drug bioavailability, in particular for poorly soluble drugs where dissolution is the rate limiting step. Therefore, dissolution kinetics has an important effect on the extent and rate of drug absorption and requires further investigation for the powder dosage form.

3.3.1.1 *Aim*

To compare the dissolution profiles of rifampicin, isoniazid, pyrazinamide, and ethambutol HCl from the powder for suspension to dissolution profiles of these drugs from commercially available tablet dosage forms.

3.3.2 Dissolution studies

To achieve dissolution profiles for all the dosage forms under study, the BP 1993 paddle method was used. The release rates of three tablets and three powders for suspension were monitored using a three beaker model dissolution apparatus (Scientific S.A. figure 3.4). The dissolution medium used was 900 ml of deionized water, equilibrated at 37.5°C. Due to degradation of rifampicin in extreme acidic and basic conditions, deionized water was chosen as the dissolution medium of choice. Aqueous solutions of rifampicin show maximum stability at 37°C at ± pH 5 (Lund, 1994d). At appropriate time intervals 2.0 ml samples were withdrawn and filtered through a 0.45μ Millex syringe filter for analysis. To analyse the amount of rifampicin, isoniazid and pyrazinamide released from the tablets as well as powder for reconstitution, the HPLC method developed by Ebrahim Salma (1998) was used. Very briefly, the method consisted of a Beckman Gold HPLC system consisting of a 126 programmable pump, a Rheodyne injector connected to a diode array detector. The analytical wavelength was set at 250 nm, with acetonitrile and phosphate buffer with a pH of 3.45 as the mobile phase for the gradient run. The mobile phase was perfused through a Licrosphere C 8 column at a rate of 1 ml/min. The release of ethambutol HCl was analysed electrochemically with an applied potential of 1 volt for all samples using a Hewlett Packard 1050 HPLC system with a Rheodyne (20μl) loop (Ebrahim ,1998). For the release of ethambutol HCl, 2 mls methanol was added to 1ml of the sample withdrawn and mixed. The release profiles of a minimum of 3 powders for suspension and 3

comparative tablets were assessed.

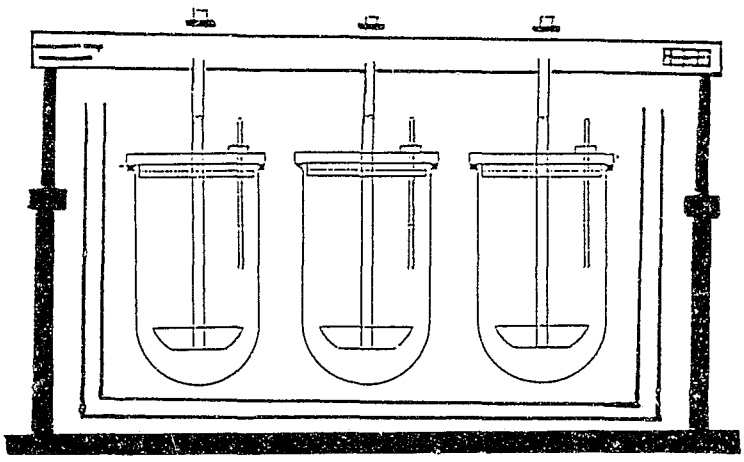


Figure 3.4 Three beaker model dissolution apparatus,Scientific S.A.

3.3.3 Formulations

The final formulation to be tested is listed in table 3.10. The suspensions were made according to the method discussed in section 3.2.4 above. Pyrifin^{R} tablets consisting of rifampicin, isoniazid and pyrazinamide as well as Rolab-Ethambutol HCl 400^{R} were used for the comparative study, (Refer to Table 1.1).

TABLE 3.10 Powders used for formulations in experiment 3.3

Materials	Quantity/dose
Rifampicin	600 mg
Isoniazid	300 mg
Pyrazinamide	1500 mg
Ethambutol hydrochloride	1000 mg
Sodium lauryl sulphate	25 mg
Colloidal silicone dioxide	50 mg
Saccharin sodium	120 mg
Liquorice flavour	250 mg
sodium starch glycollate	3750 mg

3.3.4 Results and discussion

Once analysis of the samples were completed, the dissolution profiles of the active drugs were plotted from the data shown in appendix 1. Figures 3.5 to 3.8 depict the dissolution profiles of the active drugs from the extemporaneous powder for suspension in comparison to the dissolution profiles from the commercially available tablet dosage forms.

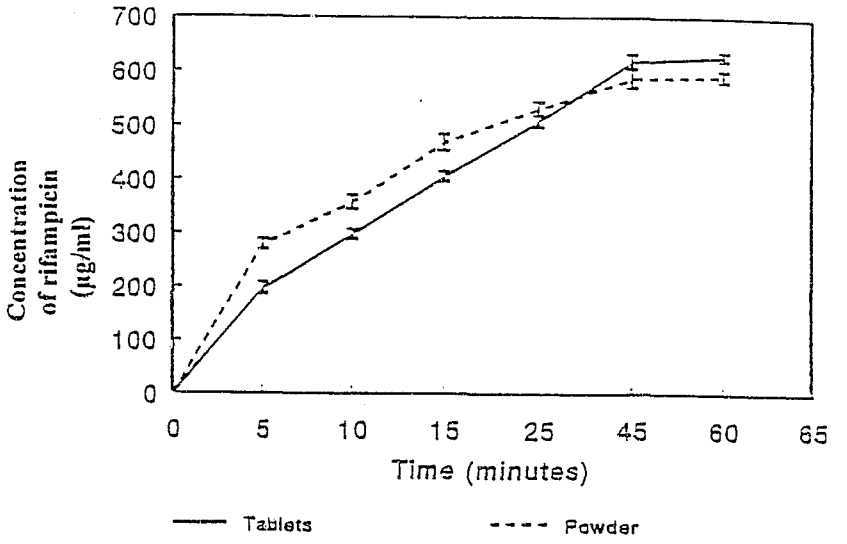


Figure 3.5 Dissolution profiles of rifampicin from the powder for suspension and Pyrifin^{R} tablet dosage form.

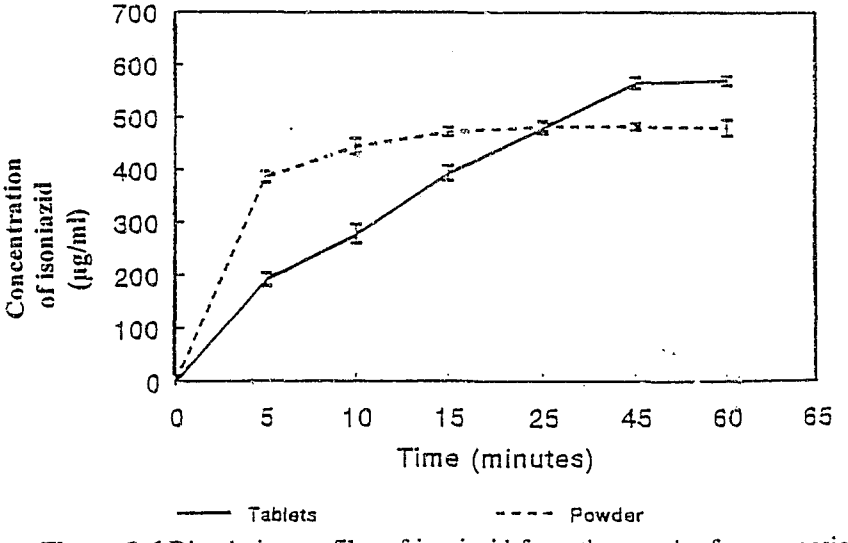


Figure 3.6 Dissolution profiles of isoniazid from the powder for suspension and Pyrifin^{R} tablet dosage form.

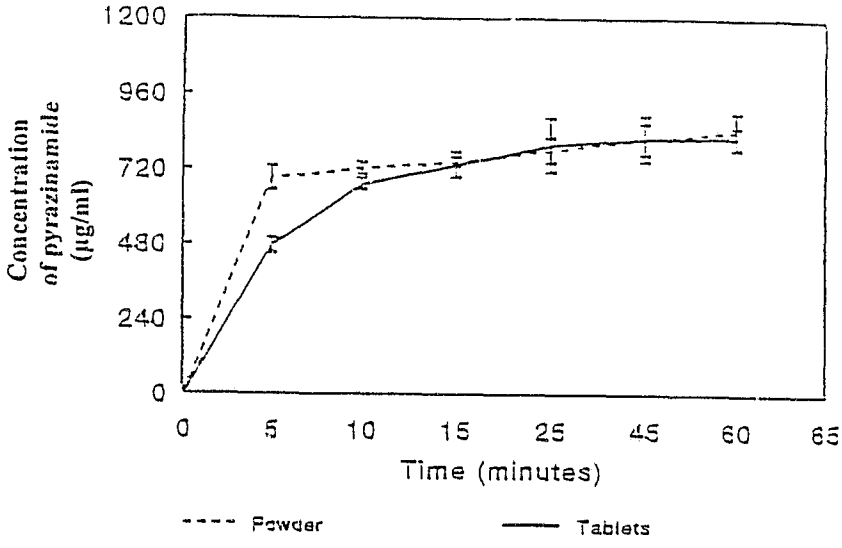


Figure 3.7 Dissolution profiles of pyrazinamide from the powder for suspension and Pyrifin^R tablet dosage form.

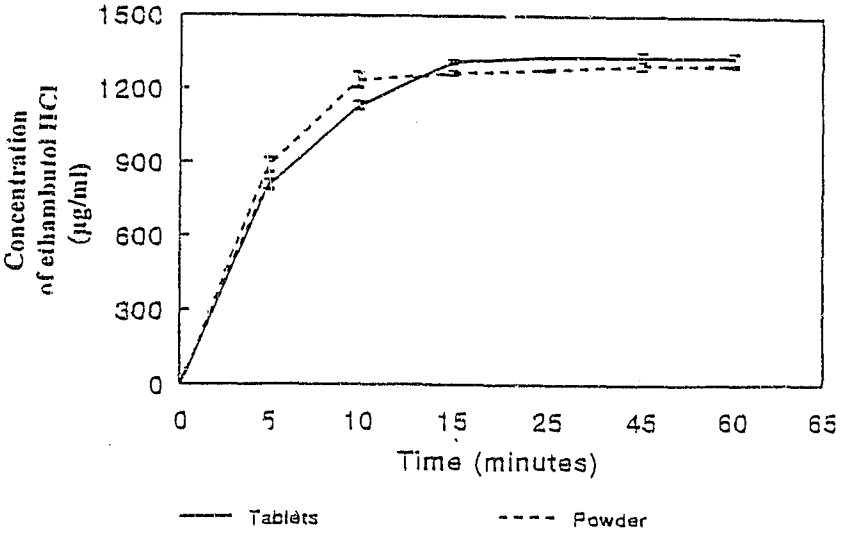


Figure 3.8 Dissolution profiles of ethambutol hydrochloride from the powder for suspension and Rolab-Ethambutol HCl^R tablet dosage form.

From figure 3.5, it can be seen that rifampicin reached higher concentrations within the dissolution medium within a shorter time period from the powder formulation when compared to dissolution from the tablet which was slower. The dissolution profile for the suspension was not significantly decreased over 30 minutes when compared to the slightly higher concentration of Rifampicin released from the tablet dosage form at 30 minutes. The dissolution of isoniazid from the powder formulation was more rapid (figure 3.6) than from the tablet dosage form, therefore resulting in a higher concentration of isoniazid being reached within five minutes from the powder formulation. Figures 3.7 and 3.8 also depict higher concentrations of pyrazinamide and ethambutol HCl within the first 5 minutes from the extemporaneous powder being dissoluted when compared to the tablet dosage form. However, with increasing dissolution time, dissolution from both the powder and tablet dosage forms resulted in similar concentrations being dissoluted within the dissolution medium.

TABLE 3.11 The rate of dissolution of anti-tubercular drugs from powder and tablet dosage forms.

Active drugs	Powder μg/ml/min.	Commercial tablet dosage forms μg/ml
Rifampicin	1.75	1.59
Isoniazid	1.89	1.58
Pyrazinamide	2.14	1.98
Ethambutol HCl	2.25	2.21

From the above results (table 3.11), it can be seen that the active drugs in the extemporaneous powder for reconstitution developed during this research have a faster dissolution rate than the drugs from the commercial tablets. This difference is significant at the 95% confidence limit for the release of rifampicin, isoniazid and pyrazinamide, while the dissolution rate of ethambutol HCl from the powder closely resembles the dissolution profile from the Rolab-Ethambutol HCl^{R} tablet dosage form. Therefore, it seems that the sodium starch glycollate has no noticeable retarding effect on the dissolution of the drugs from the extemporaneous suspension as compared to the commercial tablets. Drug absorption from such preparations should therefore be faster or at least the same as from the corresponding commercial tablets.

4.0 CONCLUSION AND FUTURE OBJECTIVES

During this research project, an anti-tuberculosis powder for suspension was optimized in order to formulate a fixed combination of rifampicin, isoniazid, pyrazinamide and ethambutol hydrochloride as a powder to be reconstituted with water by the patient prior to administration.

Not only is the powder for suspension a convenient and acceptable form in which to dispense drugs, it is simple and economical to manufacture. The only major equipment required is a tumbler mixer and suitable sieve for particle sizing. Therefore, this formulation will be cost effective to meet the needs of tuberculosis sufferers throughout South Africa. Administration of this fixed dose combination preparation will assist in overcoming the problem of multidrug-resistant tuberculosis because it will achieve greater patient compliance because of the ease of administration. This is because it is pleasant tasting and easy to swallow. All the patient needs to do is place it in half a glass of water, stir it for a few seconds and then drink it. A once daily dosage of the suspension containing the dosages of anti-tubercular drugs as stipulated by the World Health Organization would increase therapeutic outcomes of tuberculosis patients the first time around by ensuring that they complete their treatment.

For the final packaging, a sachet, would be a suitable airtight, sealed container which would be convenient to use by the patient and would not be too bulky for storage.

Although there is no evidence to suggest any chemical stability problems or any interactions, it is recommended that a suitable shelf life stability study as well as a controlled clinical trial should be carried out on the powder for suspension. There also exists the need to determine the effect of administration of the powder for suspension concomitantly with the directly observed therapy strategy implemented by the Department of Health.

APPENDIX 1

TIME (minutes)	Average concentration of rifampicin from tablet dosage form (μg/ml)	Average concentration of rifampicin from powder dosage form (μg/ml)
5	197.59	279.63
10	298.22	357.84
15	405.99	469.13
25	507.79	531.12
45	620.67	589.34
60	628.67	591.96

TIME (minutes)	Average concentration of isoniazid from tablet dosage form (μg/ml)	Average concentration of isoniazid from powder dosage form (μg/ml)
5	191.44	386.21
10	278.46	445.11
15	393.33	472.76
25	481.17	482.4
45	565.79	482.25
60	570.78	480

TIME (minutes)	Average concentration of pyrazinamide from tablet dosage form (μg/ml)	Average concentration of pyrazinamide from powder dosage form (μg/ml)
5	474.83	691.36
10	674.07	724.57
15	735.61	744.58
25	799.06	782.24
45	819.37	817.87
60	818.85	842.26

TIME (minutes)	Average concentration of ethambutol HCl from tablet dosage form (μg/ml)	Average concentration of ethambutol HCl from powder dosage form (μg/ml)
5	808.22	888.35
10	1133.37	1237.03
15	1312.84	1266.37
25	1332.5	1280.37
45	1333.25	1300.11
60	1330	1301.05

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Author Ebrahim S
Name of thesis The Parmaceutical Development Of A Fixed Combination Anti-Tuberculosis Dosage Form Ebrahim S 1998

PUBLISHER:

University of the Witwatersrand, Johannesburg
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