Formulation of a topical haemostatic drug delivery system for minor wounds and abrasions

Aysha Hafeji

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Masters of Science in Medicine, Pharmacotherapy

Johannesburg, 2019

Supervisor

Ass. Prof M.P. Danckwerts

University of the Witwatersrand, Department of Pharmacy and Pharmacology, Johannesburg,

South Africa

Declaration

Faculty of Health Sciences, Postgraduate Office

Phillip V Tobias Building, 2nd Floor

Cnr York & Princess of Wales Terrace, Parktown 2193

Tel: (011) 717 2745 | Fax: (011) 717 2119

Email: Mathoto.senamela@wits.ac.za

I, Mrs. Aysha Hafeji (Student number: 1562286), am a student registered for the degree of Master of Science in Medicine Pharmacotherapy in the academic year 2016.

I hereby declare the following:

I am aware that plagiarism (the use of someone else's work without their permission And/or without acknowledging the original source) is wrong.

I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.

I have followed the required conventions in referencing the thoughts and ideas of others. I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.

I have included as an appendix a report from "Turnitin" software indicating the level of plagiarism in my research document.

Signature: Date: 22nd June 2019

Dedication

This research report is dedicated to my three greatest gifts from the Almighty: Amira, Abdullah and Amanullah, my beloved husband, Asif Hafeji. You are my life, my love, my happiness. Your love, prayers, support and sacrifice has made all this come together and I will forever remain grateful. My dearest parents, Yunus and Mariyam Patel, to my siblings Asma Bhamji and Yamin Patel for your unconditional love, motivations and prayers.

Acknowledgments

All praise and gratitude is due, first and foremost, to **the Almighty** for His infinite mercy, blessings, and guidance throughout this journey.

It brings me great pleasure to acknowledge the countless number of individuals whose invaluable contribution assisted in the completion of this research project.

I would like to express my sincere gratitude to my supervisor**, Prof Paul Dancwerts** for the kind assistance and guidance in any enquiry. I am immensely grateful to him for infusing his wealth of knowledge and wisdom into this project without which this project would not have been completed.

I would like to extend my heartfelt appreciation to my wonderful parents **Yunus** and **Mariyam Patel** it is through your encouragement, support, and prayers that I am here today. Your unconditional love and belief in me have been an infinite source of inspiration always. Thank you for teaching me that with hard work and honesty and believing in almighty, anything is possible.

To my husband and friend **Asif Hafeji**, to my amazing kids **Amira, Abdullah** and **Amanullah.** Thank you for your all small and big help, understanding, motivation and sacrifices. It means a lot to me.

I would like to thank **Prof. Sandy van Vuuren** for her help in sterility test of my formulation.

To Ms. **Mariambibi Patel** for your input in dissertation writing. I am immensely grateful.

I would also like to express my gratitude to the **students and staff** of the Department of Pharmacy and Pharmacology, University of the Witwatersrand their contributions, great and small, have aided me in numerous ways. I thank you.

I also thank The **University of the Witwatersrand** for its support during my studies and for giving me the opportunity to purse my education at this institution in this wonderful country.

Table of contents

List of Figures

List of Tables

List of Appendix

List of abbreviations

ACT: anticoagulant therapy

ACS: American College of Surgeons

OAC: oral anticoagulant

TA: tannic acid

CS: CS

CX: Celox©

CoTCCC: Committee on tactical combat casualty care p(TA):

poly Tannic Acid

Tris: Trishydroxymethylaminomethane

TSB: Trypticase soy broth

Thio: Fluid Thioglycolate medium

Abstract

Haemostatic agents accelerate blood clotting and help in wound healing processes for minor wounds or abrasions. Haemostatic gauzes and bandages have been formulated to achieve quick clotting and haemostatic control in emergency combat situations. Current available haemostatic gauzes and bandages in South Africa have to be imported and are thus expensive. The purpose of this study was to prepare and evaluate a haemostatic gauze impregnated with chitosan and tannic acid as two effective and cheap haemostatic agents. The haemostatic gauze was easy to prepare and produce by means of a simple solvent casting method followed by lyophylisation.

Nine different concentration combinations of tannic acid and chitosan were impregnated onto a 50mm x 50mm 8 ply gauze strip and were prepared and evaluated for various physicochemical properties, mechanical properties, dissolution into simulated body fluid, and finally tested for *in vitro* blood clotting ability. The different formulations were also tested to see if they maintained their sterility after 30 days of storage. The final in *vitro* blood clotting ability of the tannic acid and chitosan haemostatic gauze formulations were compared to Quikclot© a commercially imported haemostatic gauze.

During the physicochemical analyses of the haemostatic gauze formulations, the concentration of tannic acid and chitosan in the haemostatic gauze formulations significantly affected some properties. It was found that chitosan % $^{w}/_{v}$ significantly affected the viscosity, thickness, swellability and clotting ability of the haemostatic gauze formulations. However, the tannic acid % $^{w}/_{v}$ had a significant effect on the swellability, dissolution and in addition to clotting ability of the haemostatic gauze formulation.

As the tannic acid % $^{w}/_{v}$ increased from 1% $^{w}/_{v}$ to 5% $^{w}/_{v}$ and the chitosan % $^{w}/_{v}$ increased from 0.5% $^{w}/_{v}$ to 1.5 % $^{w}/_{v}$, viscosity increased and the weight of the haemostatic gauze strips varied from 0.3884 ± 0.02 to 0.6151 ± 0.07 g. The thickness variation of the haemostatic gauze strips were found to be in between 1.023 ± 0.12 and 2.615 ± 0.29 mm. Drug content uniformity of the haemostatic gauze strips was found to be uniform throughout the various formulations (all above 94%). Swellability of the haemostatic gauze strips in simulated body fluid (SBF) varied between 654.43 ± 45.22 % and 1203.71 ± 100.2%. The tensile strength for all haemostatic gauze strips were found to be > 0.57 kg/cm^2 where some of gauze strips did not break. It was also found that there was an initial quick release of tannic acid from the haemostatic gauze strips within 30 seconds and after that there was a constant release of tannic acid. Dissolution of tannic acid at 300 second has been found between 32.62 and 10.56 % µg/ml of total amount. The *in vitro* blood clotting ability was found to be better in our formulated haemostatic gauze than that of Quikclot© commercial gauze. Best clotting extent was found from 0.5C1TA, 1C1TA and 1C2TA formulations at 180 sec. excellent clotting rate has been found for 1C2TA formulation.

However, most of the haemostatic gauze strip formulations did not found to be sterile after 30 days of storage even though tannic acid has been reported to have antimicrobial properties. In future, an effective preservative will have to be added to the formulation, or they would have to be prepared aseptically.

In conclusion, the tannic acid and chitosan haemostatic gauze developed in this study presents an inexpensive and effective alternative to importing haemostatic gauzes and bandages.

Chapter 1 Introduction

1.1 Rational and motivation

A haemostatic agent could be a life-saving step to control accidental bleeding in rural areas or in a military setting where a hospital is not nearby. If used immediately, in a pre-hospital setting, patients will have sufficient time to be stabilize prior to treatment (Zhang *et al*., 2015). Minor bleeding from wounds or abrasion also present a day-today problem for patients on (ACT) anticoagulant therapy. 20 minutes or longer bleeding can be seen for a simple surgical wound from a patient on ACT treated. Furthermore, it has been found that long-term ACT of oral anticoagulants is associated with lessened quality of life, including complications with bleeding and the fright of such injuries (Hasan *et al*., 2015).

The quickest and easiest method to stop topical bleeding is the application of direct pressure to the bleeding site (McGee and Lynch, 2016). When the wound is not open to tourniquet use and a pressure dressing is ineffective, topical haemostatic agents can be used to help stabilize blood flow. According to the Tactical Combat Casualty Care Committee (TCCC), many topical haemostatic agent dressings are used to treat extensive bleeding in military casualties (Ong *et al*., 2016). Although most haemostatic agent dressings are used in the military, they may also be useful in non-military settings, i.e. motor vehicle accidents and other domestic accidents. Consequently, many currently approved haemostatic products could be useful in civilian settings. This comprises in all civilian settings, as important part of a program for controlling severe bleeding (Bennett, 2017).

Coagulopathy, infection, hypothermia and organ failure are possible complications developing from acute blood loss (Carraway *et al*., 2008). Therefore, quick intervention in hemorrhagic control will change in better patient outcomes and decreased demand for blood products (Gruen *et al*., 2012). Thus, topical haemostatic agents can play a major role in the emergency control of trauma like hemorrhage and could reduce the associated morbidity and mortality in life-threatening situations (Khoshmohabat *et al*., 2016).

Ideally, a topical haemostatic dressing should be easy to store, lightweight, and able to be rapidly applied to a bleeding wound or abrasion. It should be comfortable for the patient, cause minimal local tissue destruction, be easily removable from the wound, not contain any particles which can disperse systemically and allow the haemostatic agent to reach areas of injury or wound which are difficult to operate with direct pressure (e.g. deep groin wounds). The haemostatic dressing must not be removed by rapid bleeding (Mani *et al*., 2015; Boateng *et al*., 2015). Additionally, the dressing should be cost effective, easily available, decrease pain (if possible) and prevent contamination and microbial growth (Weir *et al*., 2015). The cessation of bleeding, as well as associated hypothermia and acidosis, is crucial for the treatment of hemorrhagic coagulopathy (Lier *et al*., 2008). The ideal haemostatic agent should also be safe, effective in an abundance of coagulopathies, and act site-specific destitute of pathologic thrombotic and immunogenic side-effects (Hangge *et al*., 2017).

There are currently a number of formulations available, including

Quikclot© clotting sponge which contains kaolin,

Advanced clotting sponge® which contains zeolite.

Celox© haemostatic agent which contains Chitosan,

BloodSTOP® patches which are made up of the natural fiber cellulose,

WoundSeal© powder which contains hydrophilic polymer and potassium ferrate,

Quick stop© flex fabric bandage which is made from micro dispersed oxidized cellulose, and

Medi-First® blood clotting spray which contains benzethonium chloride and lidocaine.

1.2 Statement of problem

Currently, there are no commercial topical haemostatic gauzes or bandages available on the South African market. Furthermore, imported haemostatic topical applications such as Quikclot© dressing are extremely expensive. An imported single Quikclot© 10 x 10cm gauze strip retails approximately R249-95 (11th December 2018).

For any new medical product to be commercially viable, it needs to be relatively inexpensive to produce and market. One such a haemostatic agent is tannic acid. Tannic acid has been shown to be an effective topical haemostatic agent (Xu *et al*., 2015). Tannic acid (extracted from gallnuts from *Rhus semialata)* is readily available, inexpensive (about R 1423-00 for 500g, Sigma Aldrich, South Africa), non-allergenic, has no systemic effects side effects, and is easy to use as a stable powder. It is also reported that tannic acid reduces postoperative pain and improves wound healing (Zboralske *et al*., 1966; Abd El-Mabood *et al*., 2014; Soleiman *et al*., 2018). It is a water soluble compound which acts as an antioxidant, antimicrobial and has wound healing

properties by forming complexes with metal ions and macromolecules (Antonia *et al*., 2015; Nakamura *et al*., 2018).

Chitosan is another inexpensive haemostatic agent that is cheap (R 1423-00 for 500g Sigma Aldrich, South Africa) and readily available. It is useful biological material that is popular for its haemostatic nature (Dash *et al*., 2011). Lan *et al* (2015) have shown that an excellent blood clotting index (BCI) was achieved *in-vitro* using a chitosan-gelatin sponge in a 50-50 ($^{w}/_{w}$) ratio.

Therefore, the purpose of this research is to prepare a gauze strip that is impregnated with tannic acid in a chitosan gel. The process of impregnating a standard gauze strip or bandage with chitosan gel and tannic acid is simple and cost-effective. The soluble tannic acid can be incorporated into a chitosan gel and simply poured onto a strip of standard gauze which can then be lyophilized so that it effectively adsorbs blood plasma and enhances clot formation.

1.3 Aim of study

The aim of this research study is to develop and characterize a simple haemostatic gauze strip containing chitosan as a plasma absorbing polymer and tannic acid as a topical haemostatic agent, for minor wounds and abrasions.

1.4 Objectives of study

1.4.1 To determine the ideal composition of tannic acid and chitosan to impregnate the gauze.

1.4.2 To develop a suitable simple solvent casting method to produce the tannic acid and chitosan impregnated gauze.

1.4.3 To test the physicochemical properties of the haemostatic gauze strip.

1.4.4 To test the release of the tannic acid from the impregnated gauze in simulated body fluid (plasma).

1.4.5 To test the haemostatic properties of the impregnated gauze strip by means of platelet aggregation in human blood.

1.4.6 To compare the haemostatic properties of the novel gauze prepared in this study with that of the imported Quikclot©.

1.4.7 To test the sterility of the final impregnated haemostatic gauze drug delivery system.

1.5 Overview of research report

Chapter one contains a brief introduction of the use of haemostatic agents in wound management. The need for a commercially available haemostatic topical drug delivery system that is cost-effective is also discussed. The rationale behind the selection and combination of chitosan and tannic acid is given. This chapter concludes with the aim and objectives of this study.

Chapter two describes the phases of haemostasis in the human body. It also details the current use of topical haemostatic drug delivery systems on the international market in addition to advantages offered by haemostatic formulations. It also explains the useful properties of chitosan and tannic acid.

Chapter three describes the methods and materials used to manufacture, design and optimization of haemostatic gauze. This includes the rheological study of chitosan and tannic acid gel, to determine the ideal composition of tannic acid and chitosan gel, solvent casting

method along with formulation of simulated body fluid used to determine the release of tannic acid from the gauze formulation. The methods used to characterize the physicochemical properties, UV assay method, and *in-vitro* drug release study are also described. Furthermore, it describes the *in-vitro* study of measuring blood clotting ability by spectrophotometry analysis of platelet aggregation and provides details of the gauze sterility test.

Chapter four presents and discusses results for the haemostatic properties and physiochemical properties of tannic acid and chitosan impregnated gauze. It also presents the sterility test results.

Chapter five is the study conclusion and future recommendations.

Chapter 2 Literature review

2.1 Haemostasis

Haemostasis is derived from Greek, defined as arrest of bleeding, *haem* meaning 'blood' and *stasis* meaning 'to stop' (Thornton and Douglas, 2010). Haemostasis is an initial biological response to injury. In basic first aid, the first priority is to control external bleeding from injury. It starts with the first responders before transportation to the hospital (if required) and continues with physicians in the emergency care unit. To manage traumatic injuries and optimize wound management, methods for rapid and effective control of bleeding are indispensable (Inaba *et al*., 2015; Freeman and Wilkinson, 2018). Patient himself can apply haemostatic agent to stop any bleeding abruptly which could decrease the need for further medical interventions (Ho and Hruza, 2007).

Haemostatics are formulations designed to stop blooding from the faster promotion of clotting (Breeze *et al*., 2017). Well-designed, efficacious topical haemostatic agents are also invaluable during minimally invasive surgical operation (Sundaram and Keenan, 2010). Haemostatic (also called antihemorrhagic) agents are substances that promote haemostasis and prevent bleeding. The ideal haemostatic agent should be effective, affordable, safe and easy to use within the body (Kamoh and Swantek, 2012).

A fundamental property of the blood is the ability to clot, which prevents blood loss. Precisely regulated interactions between circulating platelets, blood vessel walls and clotting proteins in the plasma are responsible for the ability to quickly stop bleeding. Development of a simple blood clot is followed by the orderly breakdown of the clot (fibrinolysis) as wound healing proceeds (Kibble and Halsey, 2015).

Haemostatic system gives a natural balance between anticoagulant and procoagulant forces. The procoagulant forces consist of adherence of platelets, aggregation and formation of a fibrin clot. The anticoagulant forces consist inhibition of coagulation and promotion of fibrinolytic processes (Konkle, 2018).

Haemostasis is a combination of different physiological processes. These processes maintain the fluidity of blood and are controlled by a fragile balance between anti-thrombogenic and thrombogenic mechanisms in the body (Palta *et al*., 2014). Haemostasis is divided into three major processes namely are primary, secondary haemostasis and fibrinolysis.

2.1.1 Primary haemostasis

Primary haemostasis involves the vasoconstriction and adhesion of platelets and activation at sites of endothelial injury. Collagen and thrombin activate platelets, resulting an increase in intracellular secretion of platelet granules, calcium and activation of various signaling pathways. Vasoconstriction of small vessels in the body reduces blood flow and increases the likelihood of vessel closure. At the site of injury, activation of platelet and clot formation lead to the release of the vasoconstrictors. Platelets release the vasoconstrictors serotonin and thromboxane A_2. Thrombin, a clotting factor, stimulates endothelial cells to secrete the highly potent vasoconstrictor called endothelin$_1$. Platelet plug formation occurs at the place of damage in arterioles, veins and capillaries. Formation of the platelet plug is referred to as primary haemostasis (Gale, 2011).

2.1.2 Secondary haemostasis

Secondary haemostasis is the process whereby fibrin is formed. Formation of a clot occurs when a fibrin mesh forms together with platelets and other trapped blood cells. Formation of the clot is known as secondary haemostasis**,** and is closely coordinated with primary haemostasis (Kibble and Halsey, 2015). Primary haemostasis is stabilized as a result of the cross-linking of fibrin during secondary haemostasis. The principle in primary as well as secondary haemostasis is the activation of the coagulation cascade (Hess *et al*., 2008). The coagulation cascade consists of a chain of sequenced enzymatic processes whose final result is the formation of a sturdy clot. There are two branches of the clotting cascade, the first is called the extrinsic pathway and second, the intrinsic pathway (Porteus and Mantanona, 2018). When a blood vessel is damaged collagen activates the intrinsic pathway, which is exposed. Similarly the extrinsic pathway is activated because of tissue damage and the result is releasing a of tissue factor. Both the intrinsic and extrinsic pathways merge into the common pathway, which starts with the transformation of Factor X (prothrombin) to Xa (thrombin). Thrombin mediates the conversion of fibrinogen to fibrin, which stabilizes the clot (Barrett *et al*., 2017). In both pathways inactive circulating precursor proteins (clotting factors) become activated by proteolytic cleavage. These chain reactions are not normally activated in blood circulation because clotting factors are present at low concentration in plasma. The intrinsic pathway is activated when blood comes in contact with a negatively charged surface (e.g., exposed sub endothelial collagen, aggregations of platelets). The extrinsic pathway is activated when blood contacts cells outside the vascular endothelium. Nonvascular cells express a ubiquitous membrane protein called tissue factor**,** which initiates the extrinsic pathway (Kibble and Halsey, 2015).

Secondary haemostasis is sub-divided into three overlapping stages

2.1.2.1 Initiation

Initiation occurs at the surface of injured cells. It starts with the exposure of tissue factor (TF) on sub endothelial cells. This causes an initial burst of thrombin to form through the extrinsic pathway. However, the amount of thrombin formed at the site of the injured cell is insufficient by itself to produce enough fibrin to stabilize the platelet plug.

2.1.2.2. Amplification

Amplification unlike the initiation phase, occurs at the surface of platelets. During this phase, thrombin produced in the initiation phase activates platelets and coagulation factors V, VIII, and XI found circulating in the blood.

2.1.2.3. Propagation

involves activated platelets recruiting other circulating platelets to the site of vessel injury (platelet aggregation) as well as the formation of two major clotting factor complexes, tenase and prothrombinase, which are crucial to fibrin production.

2.1.3 Fibrinolysis

This is the process of breaking down fibrin into its degradation products such as D-dimer (Gale, 2011; Davoren and Hsu, 2019). Fibrinolysis exists to limit the size of the clot.

2.2 Current haemostatics

Topical haemostatic agents are used when a wound is not suitable for tourniquet use and the use of a pressure dressing is inadequate to stop bleeding. Many haemostatic agents are used by the military to combat extensive bleeding in casualties (Ong *et al*., 2016).

Haemostatic agents can be classified into different groups depending upon their mechanism of action (Bennett and Littlejohn, 2014; Güven, 2017a, 2017b).

2.2.1 Factor concentrators

The mechanism of activity of these agents is the rapid absorption of the aqueous content of blood which activates clotting factors and platelets by increasing the concentration of its protein and cellular components, resulting in formation of clot, e.g. granules of zeolite- QuikClot®, gauze impregnated with kaolin - QuikClot Combat Gauze® (Güven, 2017b).

2.2.2 Mucoadhesive agents

Mucoadhesive agents work through a strong attachment to the tissues, and physically stop bleeding from wounds. Chitosan salts get rapidly cross-linked when they comes in contact with anionic charged erythrocytes, adhering firmly with the wound surface. This adhesion is basic mechanism of action and free of platelets or clotting factors, .e.g. chitosan/alginate impregnated gauze, freeze dried chitosan and chitosan granules (Zielińska *et al*., 2017).

2.2.3 Procoagulant supplementors

These agents act mainly through transporting procoagulant factors to the hemorrhagic wound.

e.g., dry fibrin sealant dressing (DFSD) consists of powdered fibrinogen, thrombin, factor XIII and calcium (Khoshmohabat *et al*., 2016).

2.2.4 Caustic and non-caustic agents

In dermatology, topical haemostatics are also classified by their chemical nature as either noncaustic agents or caustic agents.

2.2.4.1 Caustic agents

Caustic agents work by coagulating proteins leading to tissue necrosis and eschar formation, enhancing thrombus formation and haemostasis, e.g. aluminum chloride, ferric sulfate (Monsel's solution), silver nitrate, and zinc chloride (Howe et al., 2013).

2.2.4.2 Non-caustic agents

They **are** active in the amplification and propagation phases of haemostasis and create a physical mesh that facilitates platelet aggregation and coagulation. E.g. Porcine gelatins, microporous polysaccharide spheres, hydrophilic polymers with potassium salts (HPPS), oxidized regenerated cellulose and microfibrillar collagens (Glick *et al*., 2013).

2.3 Advantages offered by haemostatic gauze

Extended primary haemostasis could improve process of the wound healing and raise the chronification risk. (Rhee *et al*., 2008). Haemostatic dressings, for example Quikclot© used in emergency treatment, reveals a useful implement to stop bleeding rapidly (Gegel *et al*., 2013). The haemostatic formulation which is effective, inexpensive, easy to carry, and convenient to use may drastically decrease mortality of patients in an emergency or on the battlefield (Neuffer *et al*., 2004). Clinical benefits of haemostatic agents include prevention of thermal destruction on nerve or vascular structures in electro surgery, achieving quick and enough haemostatic control in soft tissues and to get results in patients with bleeding diathesis (Parker *et al*., 2010; Schreiber *et al*., 2011; Kleiner *et al*., 2014). Figure 1 illustrates the clotting process of haemostatic gauzes.

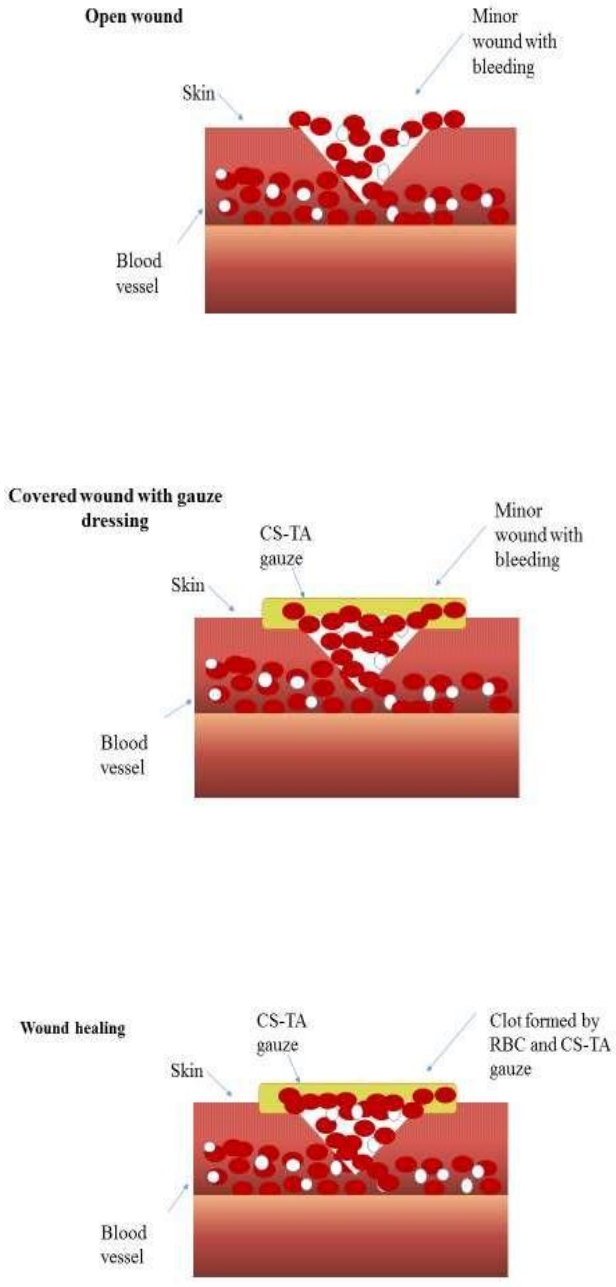


Figure 1: Simplified illustration of blood clotting by a tannic acid and chitosan (CS-TA)

2.4 Rationale for selecting the specific active pharmaceutical ingredient combination

2.4.1 Chitosan as a plasma absorbing polymer

In most biomedical applications, polymers play an important role (Shariatinia, 2018; Mohamed *et al*., 2018). Biopolymers contain polysaccharides such as cellulose and starch (the carbohydrate polymers produced by bacteria) plus animal and fungi protein based biopolymers such as gelatin, collagen, chitin, silk, chitosan, wool, inorganic polymer such as zeolite, have been found to have favorable applications in many diverse forms (Dai *et al*., 2010; Ninan *et al*., 2015).

Commercial neutral or acidic polysaccharides include alginic acid, cellulose, dextran, agar, pectin, agarose, carrageenan, heparin, and starch. While, chitosan is basic polysaccharide derived from shrimp shells or crab shells (Millner *et al*., 2009). Because of its crystalline and stable structure (Figure 2), chitosan is insoluble in aqueous solutions pH > 7. However, due to protonation of free amino groups chitosan is soluble in mild acidic solution pH < 5 (Kumari *et al*., 2015; El Knidri *et al*., 2018).

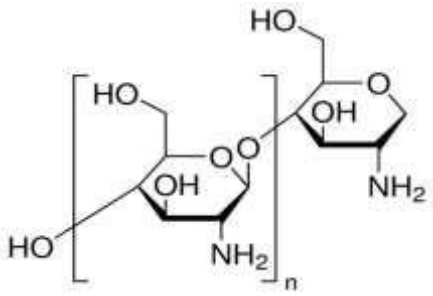


Figure 2 : Structure of chitosan (Muxika *et al*., 2017)

To achieve the biological properties and expand the possible uses of chitosan in the field of biomedical, many chemical modifications have been attempted, for instance carboxymethylation (Zhao *et al*., 2017), alkylation (Desbri *et al*., 1996) or quaternisation (Sajomsang *et al*., 2012).

Thus the main advantage of chitosan is it can easily be converted into different forms, which is important for the formulation of various biomedicals. (Ahmed and Ikram, 2016). Chitosan has a positive-charge which helps to coagulate the blood electrostatically from wounds by interacting with negatively charged erythrocytes of cell membranes. This then results in erythrocyte agglutination which seals wounds through tissue adhesion (Chan *et al*., 2016). This cationic nature provides chitosan the ability to help cell growth, accelerate the blood coagulation and increase the efficacy of chitosan dressings (Dash *et al*., 2011; Croisier and Jérôme, 2013; Archana *et al*., 2013).

In impaired coagulation rat model such as hypothermia and warfarin therapy the use of chitosan improved haemostasis (Koksal *et al*., 2011). Numerous case reports have reported effective haemostasis clinically. Successful haemostasis described by one report of combat gunshot causalities in Afghanistan by all patients in the field using chitosan based Celox© products. By using Celox© haemostasis was achieved in 18 of 21 wounds within a minute. The remaining haemostasis required additional application of Celox©, however in the end haemostasis was achieved completely (Pozza and Millner, 2011).

In the biomaterials field, chitosan is found to be versatile material in the design of a wide range of applications viz. wound dressings (Saral *et al*., 2019; Mohandas *et al*., 2018b), anticoagulants (Ouerghemmi *et al*., 2018), injectable hydrogels (Liu *et al*., 2016), ocular applications (Başaran *et al*., 2012; Makwana *et al*., 2016), scaffolds for tissue engineering (Oryan and Sahvieh, 2017), and antibiotic delivery (Sivakumar, 2016).

In a study by Kozen *et al*., (2008) using a porcine model of uncontrolled hemorrhage, Celox© haemostatic gauze provided hemorrhage control in patients and improved survival. They

concluded that Celox© haemostatic gauze is a possible substitute for the treatment of critical hemorrhage. From a wound healing perspective, chitosan increases the granulation of wounds, which accelerates the healing of deep and open wounds. Due to its haemostatic effect, it accelerates formation of fibroblast layer which also increases the rate of healing (Mi *et al*., 2002). Numerous studies report an innovative properties, functions and diverse applications of chitosan, which include antimicrobial, antifungal, anticoagulant, antinecrotic, analgesic, wound healing and antioxidant properties of chitosan (Okamoto *et al*., 2002; Rinaudo 2006; Reddy *et al*., 2015; Ahmed and Ikram, 2016; Bano *et al*., 2017; Kamble, 2018; Escárcega-Galaz *et al*., 2018; Heise *et al*., 2018; Bano *et al*., 2018; Kurniasih *et al*., 2018; Biranje *et al*., 2019). Chitosan also exhibits analgesic effect which is due to the adsorption of proton ions from the inflammatory site (Okamoto *et al*., 2002). Another advantage of chitosan is that it is a biodegradable polymer and thus eco-friendly (Muxika *et al*., 2017). Chitosan production is planned as an alternative procedure for the waste management of fishery industries and for end of the life cycle stage chitosan disposal generated to compost that can be valuable as a soil improver (Leceta *et al.* 2013).

2.4.2 Tannic acid as a haemostatic agent

Tannic acid is d-glucose gallic acid ester which contains many hydroxyl, phenolic groups and aromatic rings (Figure 3). Found mostly in fruits, seeds of leguminous plants, grains and a variety of drinks like tea, wine, apple juice and cocoa (Santos-Buelga *et al*., 2000).

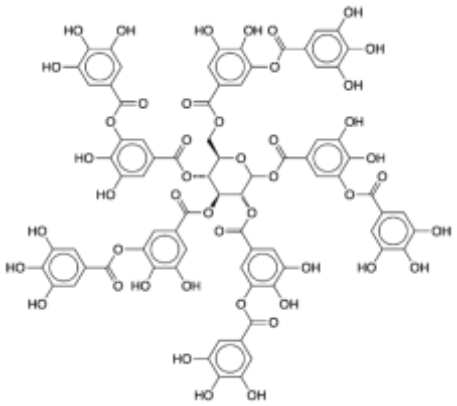


Figure 3: Structure of tannic acid (Bueno *et al*., 2012)

Tannic acid is used as an effective anti-hemorrhoid agent (Lim, 2012; Tomiki *et al*., 2015;

Huang *et al*., 2015) and also stops bleeding from mucous membranes via vasoconstriction (Kumar, 2016). Tannic acid also has antibacterial properties effective against *methicillinresistant Staphylococcus aureus* (MRSA) strains (Cipriano-Salazar *et al*., 2018), inhibits Hepatitis C virus (Liu *et al*., 2015) as well as antimitotic and anti-inflammatory properties (Booth *et al*., 2013; Jian Zhang *et al*., 2018).

A recent study by (Sahiner *et al*., 2016) has shown poly tannic acid particles with a 20–200 μm size distribution were to be non-toxic as well as more hemocompatible to blood. Tannic acid is an antioxidant compound which has polyphenolic group that have been used to mitigate various state of oxidative stress (Crispo *et al*., 2010; Gülçin *et al*., 2010; Turgut Coşan *et al*., 2015). Due to antioxidant and antimicrobial properties tannic acid has been reported in many studies to control oxidation of protein and lipid in meat, fruits and vegetables (Al-Hijazeen *et al*., 2016) as well as in formulation of a wound dressings (Sagbas *et al*., 2015; Sahiner *et al*., 2017).

Chung *et al*., (1998) reported that tannins have many physiological effects such as natural defense mechanisms against microbial infections, increase blood clotting, and decrease blood

pressure and serum lipid levels, as well as exhibit anti-carcinogenic properties. Thus, tannic acid has many health effects (Chu *et al*., 2016).

2.5 Chitosan and tannic acid crosslinking

The multiple phenolic groups in tannic acid can interact with biological macromolecules (Aelenei *et al*., 2009). Crosslinking occurs mainly through two different reactions either Michael-type adducts or Schiff base (Kumar *et al*., 2004). Figure 4 shows crosslinking of Chitosan and Tannic acid.

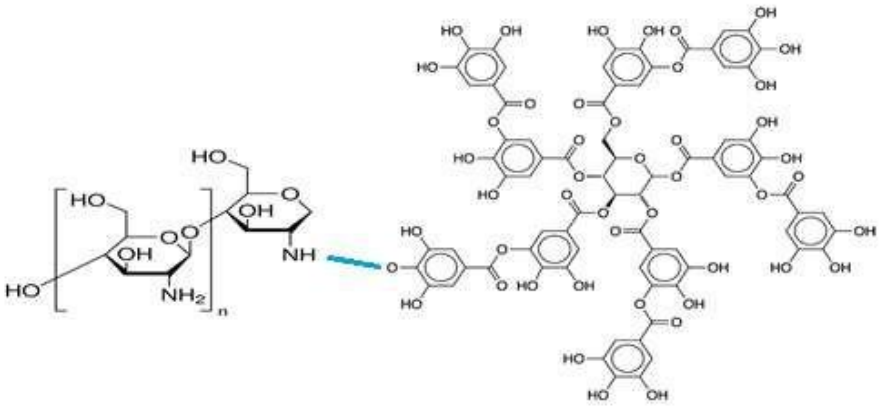


Figure 4 : Chemical crosslinking between chitosan and tannic acid

Plant derived phenolic compounds such as tannic acid can crosslink polymers like chitosan. It adds benefits like antioxidant and wound healing properties to the polymer (Azeredo *et al*., 2016). When compared to chitosan films, the addition of tannic acid changes the structure into an anhydrous and crystalline form (Rubentheren *et al*., 2015). Addition of tannic acid to the chitosan matrix leads to more rigid structure which increases the tensile strength of a film (Rivero *et al*., 2010; Sionkowska *et al*., 2014; Rubentheren *et al*., 2015). A wound dressing

which has an antimicrobial application has been prepare by crosslinking chitosan, pullulan and tannic acid (Xu *et al*., 2015).

Chapter 3 Formulation design and optimization of the tannic acid (TA) and chitosan (CS) haemostatic gauze

3.1 Introduction

This section describes the development and optimization of the haemostatic gauze by solvent casting and lyophylisation. When using solvent casting an active pharmaceutical ingredient dissolved in a polymer solution and then poured onto a gauze strip. In order to ensure rapid release an impregnated gauze is subsequently frozen and then lyophilized. In order to get a quick release of haemostatic drug at an injured site, to maintain the texture, color, and porosity of the film which contains the CS polymer, lyophylisation is required (Ruiz-Caro and Veiga-Ochoa, 2009). Lyophylisation has many applications, especially for micro- and nano-particle technology with the advantage of preventing particle aggregation. Lyophylisation is also considered a feasible strategy to improve the physicochemical stability of colloidal systems, to get sophisticated films, including CS-based delivery systems (Deville, 2006; Aranaz *et al*., 2014).

3.2 Materials

Tannic acid (TA) and medium molecular mass (448877g/mol) chitosan (CS) was purchased from Sigma-Aldrich, South Africa. Standard 8 ply 50mm×50mm gauze (Dis-Chem brand) swabs were purchased from a pharmacy. Other ACS reagents, sodium hydrogen carbonate, sodium chloride, potassium chloride, magnesium chloride hexahydrate, di-potassium hydrogen phosphate trihydrate, calcium chloride, hydrochloric acid, sodium sulfate, glacial acetic acid, tris-hydroxymethyl aminomethane, and glycerol were also purchased from Sigma-Aldrich, South Africa. Each material was used as unaltered.

3.3 Methods

3.3.1 Formulation of haemostatic gauze strip

3.3.1.1 Formulation of CS and TA gels

CS gels containing 0.5% $^{w}/_{v}$, 1.0% $^{w}/_{v}$, and 1.5%$^{w}/_{v}$ powder were prepared by means of slowly adding the CS powder to 100ml 0.1 M acetic acid stirred at 200 rpm with an overhead stirrer (Heldolph instruments D91126 Schwabach, Germany) in a water bath (G.F.L., Hannover;Vinnhorst, Germany) set at 50°C until dissolved. Each of the reagents were measured accurately on a digital weighing balance (Mettler instrument, AG CH-8606, GreifenseeZurich,Switzerland). In preliminary experiments, gels containing 2, 4 and 6 % $^{w}/_{v}$ CS were prepared but they were too viscous to effectively pour onto the gauze strips. Therefore 0.5, 1 and 1.5 % $^{w}/_{v}$ CS gels were prepared and 1 % $^{w}/_{v}$ TA, 2 % $^{w}/_{v}$ TA and 5 % $^{w}/_{v}$ TA was then added to each of the gels to produce the nine different formulations as shown in (Table 1). To increase the plasticity of the gels, 1 % $^{v}/_{v}$ glycerol was added to each of the formulations. Five ml each gel formulation was poured using syringe onto a 50 x 50 mm 8 ply gauze strip in glass petri dishes (6 cm diameter). The gel impregnated haemostatic gauze strips were then covered with the lids of the petri dishes and frozen in petri dishes overnight at a -75°C fridge (Sanyo VIP™ Series, Sanyo North America Corporation, Wood Dale, USA). After freezing, the gauze strips were then lyophilized in a freeze dryer (FreeZone® 2.5, Labconco®, and Kansas City, Missouri, USA) at -64'C temperature, 1.5 mtorr pressure over a period of 24 hours. Once lyophilized, the covers of the petri dishes were replaced and left sealed in a dark dry cupboard until testing. Two batches of three replicates (n=6) (Table 1) were used for each of the tests done on the different formulations.

Table 1 Different formulations of TA and CS gels

Formulation	% $^{w}/_{v}$ CS	% $^{w}/_{v}$ TA	% $^{v}/_{v}$ Glycerol	Formulation code
1	0.5	1.0	1.0	0.5C1TA
2	0.5	2.0	1.0	0.5C2TA
3	0.5	5-0	1.0	0.5C5TA
4	1.0	1.0	1.0	1.0C1TA
5	1.0	2.0	1.0	1.0C2TA
6	1.0	5.0	1.0	1.0C5TA
7	1.5	1.0	1.0	1.5C1TA
8	1.5	2.0	1.0	1.5C2TA
9	1.5	5.0	1.0	1.5C5TA

3.3.2 Assay method for TA

3.3.2.1 Instrumentations and settings

A PerkinElmer Lambda 25 (Perkin Elmer, USA) UV spectrophotometer was used to quantitate the TA during the content uniformity and the dissolution in simulated body fluid (SBF) studies. Stock and standard solutions of TA in SBF were prepared in triplicate. The absorbance of tannic acid was measured at 280nm. No other ingredients of the gel were absorbed in this range e.g. CS, glycerol. The absorbance mean and standard deviation (SD) and a standard curve was the constructed for the % $^{w}/_{v}$ TA versus absorbance using Microsoft Excel©.

3.3.2.2 Preparation of simulated body fluid (SBF)

SBF was used to measure TA release from the haemostatic gauze formulations and to check the TA content uniformity of the haemostatic gauze strips. SBF has an ionic concentration similar to that of human blood plasma and at pH 7.40 it was buffered with Tris buffer and 1M hydrochloric acid by maintaining temperature at 36.5°C. The procedure to prepare SBF was adapted from the method used by Oyane *et al*., (2003) and Kokubo and Takadama (2006). All the glassware used (100 ml beakers, 100 ml Measuring cylinder, 1 liter volumetric flasks, weighing boats) to make SBF was cleaned with 1M HCl and doubled distilled water and then dried in oven. This was to ensure no precipitation or turbidity in preparation of the SBF. One liter of SBF was prepared in doubled distilled water at 36.5°C (same as body temperature) where temperature was maintained using water bath (G.F.L.,Hannover, Germany) and by means of adding the reagents listed in Table 2 in the sequence in which they are listed. Once all ingredients were dissolved using electric stirrer (Heildolph instruments, D91126 Schwabach, Germany) at 100 rpm, the pH was adjusted (Eutech instruments, Part of thermo fisher Scientific, Singapore) to 7.4 using 1M HCl and Tris buffer. Lastly, total volume was adjusted with distilled water to make 1 liter.

3.3.2.3 Preparation of TA calibration curve

Stock solutions of TA were prepared in SBF as a solvent. A 1 % $^{w}/_{v}$ TA stock solution was formulated by means of dissolving 0.5 g of TA in to 50 ml SBF. This stock solution was then diluted (5ml in 50ml SBF) to obtain a stock solution of 0.1 % $^{w}/_{v}$ TA. The stock solution was then serially diluted to obtain standard solutions of 0.01 %, 0.005 %, 0.0025 %, 0.00125 %, 0.000625 %, 0.000325 % $^{w}/_{v}$ TA.

Table 2 : Reagents used to prepare 1 liter SBF pH 7.45 Temp 36.5'C

Order	Reagent	Amount
1.	Sodium chloride (NaCl)	7.996 g
2.	Sodium hydrogen carbon ($NaHCO_3$)	0.350 g
3.	Potassium chloride (KCl)	0.224 g
4.	Di-potassium hydrogen phosphate trihydrate($K_2HPO_4 \cdot 3H_2O$)	0.228 g
5.	Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$)	0.305 g
6.	1M-Hydrocloric acid(HCL)	40 mL
(About 90 % of HCl was added)		
7.	Calcium chloride ($CaCl_2$)	0.278 g
8.	Sodium sulfate (Na_2So_4)	0.071 g
9.	Tris-hydroxymethyl aminomethane ($(CH_2OH)_3CNH_2$)	

3.3.3 Physicochemical evaluation of haemostatic gauze

Prepared gauze strips were characterized for the following physicochemical parameters:

3.3.3.1 Rheological study of haemostatic gel formulations

Rheological study of a polymeric gel formulation is very important as it affects the casting method (mixing, pourability, and drying (Karki *et al*., 2016)). A Brookfield viscometer Model DV-II with a number 6 disc spindle was used to measure the viscosity of the resultant gels. Viscosity was measured at different speeds of 10, 20, 50 and 100 rpm for each gel formulations to determine if the gels exhibited Non Newtonian flow. Mean and SD of the viscosity for each

gel was calculated and plotted as a 3D plot to check the effect of TA and CS concentrations on viscosity of the gel formulations.

3.3.3.2 Weight uniformity

Gauze strips from each of the 3 x 2 (n = 6) batches were weighed individually on a digital balance (Mettler, Model AE 240, Switzerland) and the average weight and standard deviation (SD) of each haemostatic gauze strip formulation was calculated and plotted as a 3D graph to check the effect of TA and CS on weight uniformity (section 4.1.2) of haemostatic gauze strips.

3.3.3.3 Thickness

The thickness of each haemostatic gauze strip formulation was measured using a digital vernier caliper (RS Pro, South Africa). Measurements were taken at three different places of each gauze strip. These were done at 0.5 cm from the edge on each side, as well as the middle (2.5 cm) of the 5 x 5cm strip. Average thickness and SD were calculated for each strip. The average of the SD (of the three points of measurement along each strip) of the thickness of each haemostatic strip formulation was calculated (section4.1.3) and plotted on a 3D plot to see the effect of TA and CS on the SD.

3.3.3.4 Swellability

The percent swelling was calculated after 5 min because coagulation within a few minutes is one of the most potent features of an ideal haemostatic agent (Pusateri *et al*., 2003). The quicker the haemostatic gauze absorbs liquid, the sooner haemostasis can be achieved (Bharkatiya M. *et al*., 2010). A 1x1cm square of each of the haemostatic gauze strips were placed in 5ml SBF fluid in 100 ml beaker at 36.5^0C for 5 minutes. Temperature of SBF was maintained using water bath.

The weight was measured in weighing boat before and after swelling using digital balance. The percentage degree of swelling (DS) was calculated by using equation 1.

DS (%) = (Ws –Wd)/Wd ×100 ---------- equation 1

Ws equal to weight of swollen strip and Wd equal to weight of the dry strip. Mean and SD of degree of swelling was determined and plotted (section 4.1.4) as 3D graph to see effect of TA and CS on swellability of haemostatic gauze strip formulations.

3.3.3.5 Tensile strength

The crosslinking effect between polyphenol TA and CS results in a \higher tensile strength which results in a greater breaking force and higher degree of stiffness (Talón *et al*., 2017). The tensile strength of each gauze strip formulation was measured on a *TA.XTplus* Texture Analyzer (Stable Micro Systems, England). The haemostatic gauze strip was cut into 5×1 cm size strips and the tensile strength was measured using Exponent© software. Test mode was set at Tension.

Mean and SD of the tensile strength for each haemostatic strip was calculated and plotted as a 3D plot to see the effect of TA and CS on the tensile strength of the haemostatic gauze formulations (section4.1.5). The input parameters are listed in table 3 below.

Table 3 : *TA.XTplus* Texture Analyzer settings for tensile strength analysis of impregnated gauze

Mode of test	Tension
Stop plot at trigger	Return
Pretest speed	0.300 cm/sec
Strain rate	20 % /sec
Posttest speed	3.0 cm/sec
Target mode distance	10cm
Trigger type force	Auto – 1kg
Break mode level	0.01 kg

3.3.3.6 Content uniformity of TA

In order to determine the content uniformity of TA, each haemostatic gauze strip was placed in a 100 ml SBF at room temperature 20'C for 24 hours. Then, 1ml the solution was diluted to 10 ml with SBF and filtered with a 0.22µm filter to get a clear solution. The UV- absorption of TA was measured and the content of TA was calculated and plotted (section 4.1.7) on a 3D plot to see the effect of TA and CS on the content uniformity of the haemostatic gauze strip formulation.

3.3.3.7 Dissolution of TA

To determine the dissolution rate and release of TA from the haemostatic gauze strips, a Franz diffusion cell (A Franz diffusion cell, United Scientific Pty ltd, RSA) was used. Haemostatic gauze strips from each of the 3 x 2 (n = 6) batches were cut into 1 cm^2 squares and then clamped into the Franz diffusion cell (impregnated side facing down towards the SBF) containing 20ml SBF and maintained at a body temperature 36.5º C with continuous magnetic stirring. After predetermined time intervals (30, 60, 120, and 300 seconds) 2 ml SBF was removed by syringe and analyzed for TA content. 2 ml SBF (preheated to 36.5°C) was replaced to maintain the same total volume. The withdrawn solution was filtered through a 22 µm filter and the UV absorbance

of TA was measured at 280 nm. The values were corrected for a 10% $^{v}/_{v}$ dilution after each sample removal. The dissolution rate was calculated from the slope of the plot of % $^{w}/_{v}$ of TA released in SBF versus time.

3.3.4 Coagulation ability

3.3.4.1 Ethical consideration

The study was conducted after getting ethical approval from the Human Ethics committee WITS.

Ethics approval letter is attached in appendix 2.

3.3.4.2 Blood withdrawal

Thirty ml blood was taken from both the student and the supervisor each in the Day Clinic of the Charlotte Maxeke Hospital by a registered phlebotomist. Blood was taken in vacuum whole blood collection tubes, containing 6ml ACD-B (Acid Citrate dextrose-solution B) (Greiner-bio one GmbH Bad Haller Str-32, 4550-Austria). The blood samples were then stored in a -75°C refrigerator (Sanyo VIP™ Series, Sanyo North America Corporation, Wood Dale, IL, USA) until used.

3.3.4.3 Development of a method for in-*vitro* blood clotting ability

When whole blood is exposed to distilled water, due to the osmotic pressure differences between water and RBC, water is taken in to RBC and consequently RBC burst, and haemoglobin is released. So, blood that is clotted is adsorbed onto the haemostatic gauze and is thus not available for hemolysis of cells (Kang *et al*., 2011).

Haemostatic gauze strips from each of the 3 x 2 (n = 6) batches were cut into 1 cm^2 squares. The haemostatic gauze squares were then placed into clean 50ml glass beakers preheated to the temperature 37.5°C. Then, 0.25 ml whole human blood (at 37.5°C) was pippeted onto the surface of the various the gauze strips. The haemostatic gauze strips were then left to stand for 1, 2, and 3 minutes. After allowing the blood soaked haemostatic gauze strips to stand for 1, 2 and 3 minutes, 20 mL preheated (37.5°C) distilled water was gently dripped down the inside edge of the beaker to prevent disruption of the clotted blood (Figure 20). Erythrocytes (RBCs) which are not clotted by the haemostatic gauze are haemolysed in the doubled distilled water and release haemoglobin. Once the doubled distilled water was added to the blood soaked haemostatic gauze, 2ml samples were gently withdrawn from the solution (not close to the gauze strip) and the UV absorbance of the hemoglobin in solution was determined at 540nm (wavelength of maximum absorbance) using a UV-VIS spectrophotometer (Lambda 25, Perkin Elmer, USA). (Cassano *et al.,* 2017) The absorbance of 0.25 ml whole human blood diluted to 20 ml doubled distilled water was used as a blank (no clotting). Clotting 'extent' was calculated from equation 2.

Clotting extent = Ab Haem blank – Ab Haem gauze ---------- Equation 2.

Where, Ab Haem blank is the absorbance at 540nm of haemoglobin in the blank solution, and

Ab Haem gauze is the absorbance at 540nm of the haemoglobin in the haemostatic gauze strip solution.

The rate of clotting was then calculated from the slope of the clotting extent versus time graph. Absorbance from Quikclot© gauze was also measured to compare the clotting ability with the various CS-TA haemostatic gauze formulations.

3.3.5 Gauze sterility test of haemostatic gauze formulations

Each haemostatic gauze strip was tested for sterility after storage in polycap vacuum tubes in the dark for 4 weeks at room temperature. Impregnated gauze strips were cut into 0.5 x 1 cm^2 and tests were done in duplicates in Oxoid media. Each square strip was tested for growth.

1. Test a was done with 10 ml triptone soya broth incubated at 37.5°C for 48 hours to check bacterial contamination.
2. Test b was done with 10 ml triptone soya broth incubated at 25°C for 7 days to see any fungal contamination.
3. Test c was done with 10 ml Thio gylcolate incubated at 37.5°C 48 hours to check anaerobic growth.
4. Duplicates of (control a) blank gauze that was only gauze without formulation were also tested.
5. Duplicates of (control b) blank test without any sample were also done.
6. Growth and non-growth were determined visually by looking for turbidity and compared with each control.

3.3.6 Statistical analysis

In order to determine the effect of the various concentrations of TA and CS on the physicochemical properties of the haemostatic gauze, the release and dissolution of TA from SBF, as well as on the final rate of clotting, the results were subjected to a single tail 95% confidence limit Analysis of Variance (ANOVA) test using the Solver function on Microsoft Excel 2016 software. P values below 0.05 were regarded as significant.

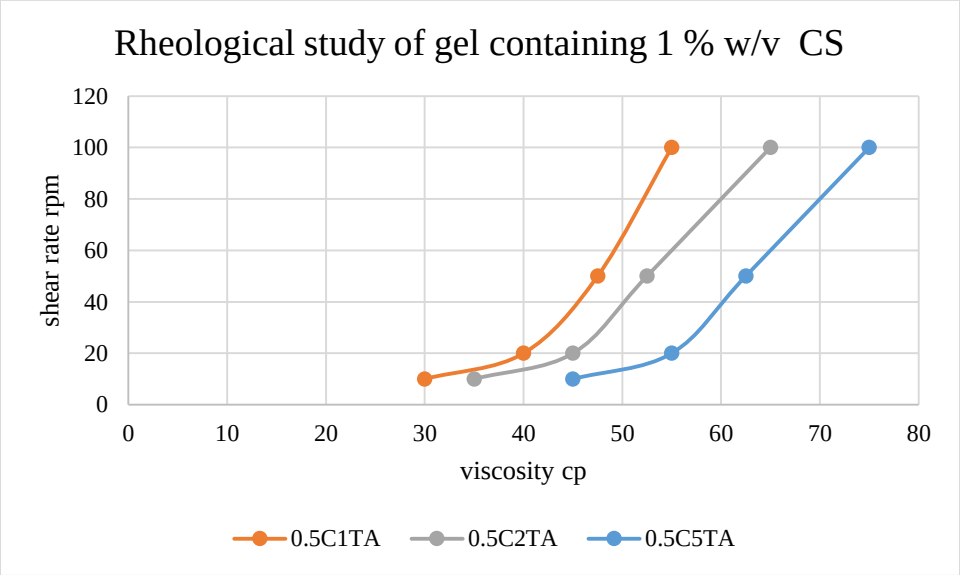
Chapter 4 Results

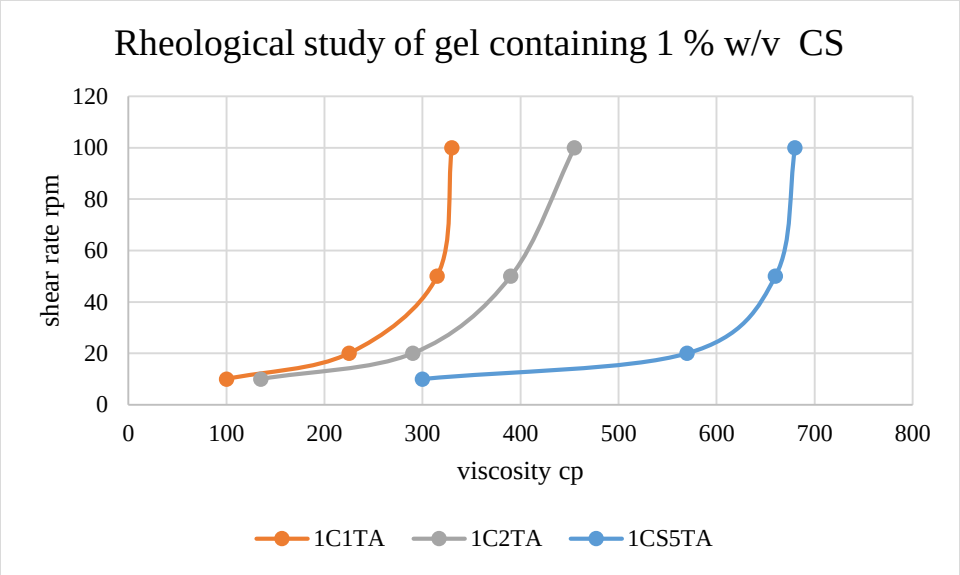
4.1 Physicochemical properties

4.1.1 Rheological properties of the haemostatic gauze formulations

The viscosity results of the investigation indicated that the CS-TA gels exhibited non-Newtonian shear thinning thixotropic flow (Figure 5). As the shear rate increased from 10 rpm to 20 rpm viscosity initially increased quicker and after about 20 rpm viscosity gradually increased for each gel formulations

From the graphs below which represent typical plots found in the rheological study and it can predict that different concentrations of CS-TA gel follow non-Newtonian shear thinning, thixotropic behavior.





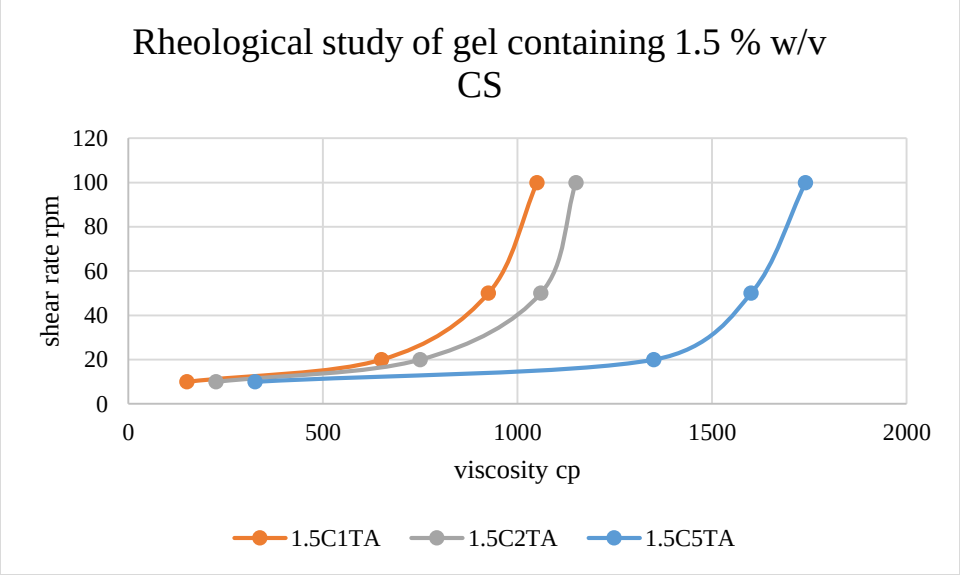


Figure 5 : Rheology of CS-TA gel formulations by grouping with CS % $^{w}/_{v}$ quantity.

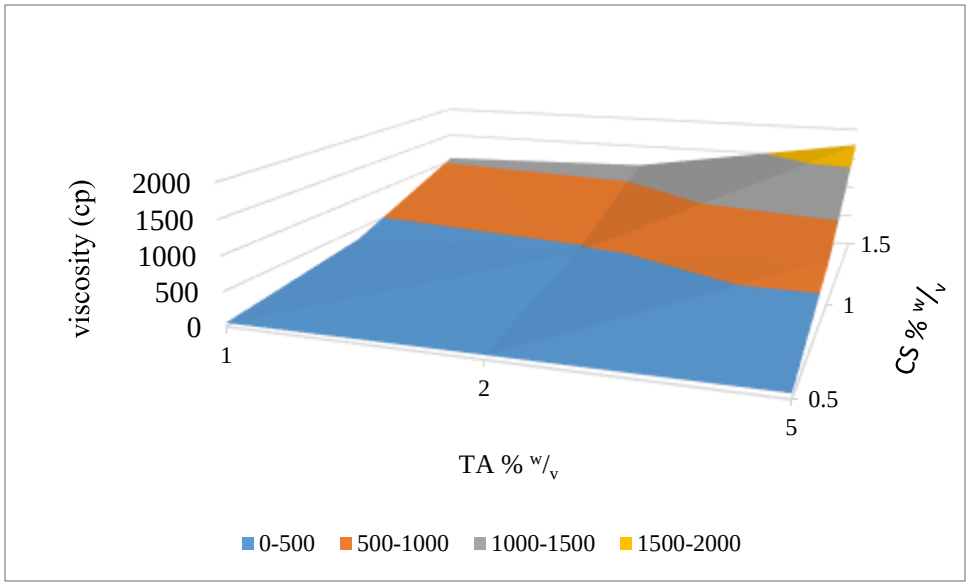


Figure 6 : 3D plot of the effect of TA % $^{w}/_{v}$ and CS % $^{w}/_{v}$ on viscosity of haemostatic gel formulations.

As can be observed from Figure 6, as the CS % $^{w}/_{v}$ increased from 0.5% $^{w}/_{v}$ CS to 1.5 % $^{w}/_{v}$ CS the viscosity of gel also increased. As the % $^{w}/_{v}$ TA increased from 1 % $^{w}/_{v}$ TA to 5 % $^{w}/_{v}$ TA the viscosity also increased. All gels were found to be easily pourable onto the gauze strips.

From the ANOVA (Table 4) it can be observed that the effect of CS on the viscosity of the haemostatic gauze strip formulations is significant with a p-value of 1.07×10^{-7}. However, the effect of TA on the viscosity of the haemostatic gauze strip formulations (Table 5) was not significant (P = 0.5584).

Table 4 : ANOVA result of the effect of CS % $^{w}/_{v}$ on the viscosity.

Anova test result: Single Factor						
SUMMARY						
CS Groups	*Count*	*Sum*	*Average*	*Variance*		
0.5% $^{w}/_{v}$ CS	6	390	65	110		
1% $^{w}/_{v}$ CS	6	2930	488.3333	25256.67		
1.5% $^{w}/_{v}$ CS	6	7970	1328.333	106896.7		
ANOVA RESULT						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between CS Groups	4961644	2	2480822	56.270	1.07E-07	3.68
Within CS Groups	661316.7	15	44087.78			
Total	5622961	17				

Table 5 : ANOVA result of the effect of TA % $^{w}/_{v}$ on the viscosity.

Anova test result: Single Factor						
SUMMARY						
TA Groups	*Count*	*Sum*	*Average*	*Variance*		
1% $^{w}/_{v}$ TA	6	2870	478.3333	212256.7		
2% $^{w}/_{v}$ TA	6	3340	556.6667	242666.7		
5% $^{w}/_{v}$ TA	6	4990	831.6667	568296.7		
ANOVA RESULT						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between TA Groups	413211.1	2	206605.6	0.605751	0.558483	3.68232
Within TA Groups	5116100	15	341073.3			
Total	5529311	17				

4.1.2. Weight uniformity of haemostatic gauze formulations.

Weight variations of haemostatic gauze strip were found to vary between 0.388 ± 0.026 g and 0.615 ± 0.074 g as shown in the 3D plot in Figure 7.

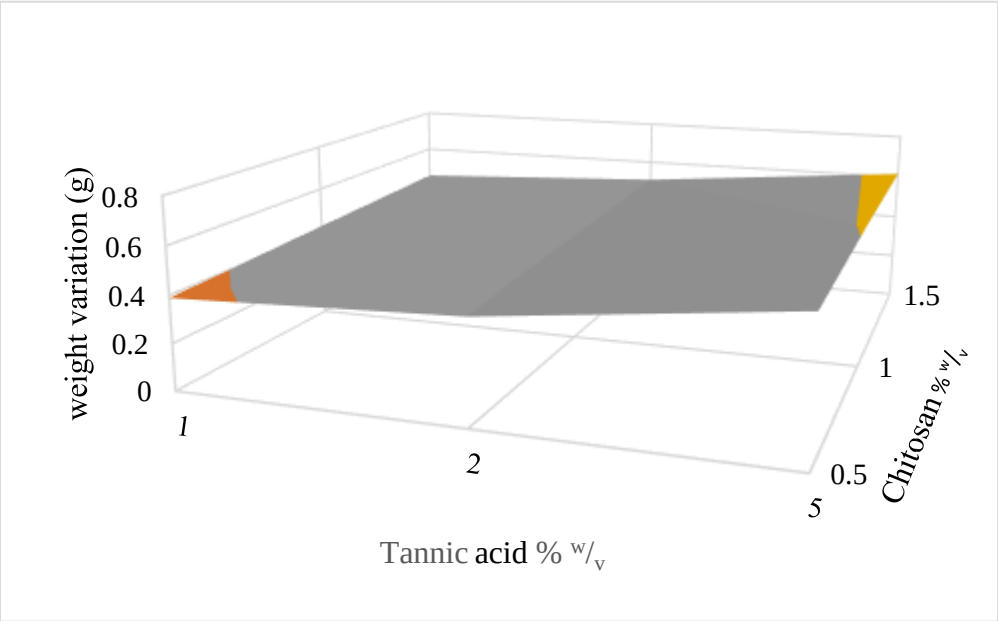


Figure 7 : 3D plot of the effect of TA % $^{w}/_{v}$ and CS % $^{w}/_{v}$ on weight uniformity of haemostatic gauze formulations.

As shown in Figure 7, and collected data as the % $^{w}/_{v}$ of CS, so does the increase in weight. This is obviously due to pourability of the viscous gel. However, the one-way ANOVA results for the effect of CS % $^{w}/_{v}$ on the variance of weight of the haemostatic gauzes presented in Table 6 shows no significant difference (P = 0.8960).

Table 6 : ANOVA result of the effect of % $^{w}/_{v}$ CS on weight uniformity of haemostatic gauze formulations.

Anova test result: Single Factor							
SUMMARY							
CS Groups	*Count*	*Sum*	*Average*	*Variance*			
0.5% $^{w}/_{v}$ CS	18	0.201490662	0.011193926	0.00015036			
1% $^{w}/_{v}$ CS	18	0.228312404	0.012684022	0.00010665			
1.5% $^{w}/_{v}$ CS	18	0.206994358	0.011499687	4.69522E-05			
ANOVA Result							
Source of Variation	*SS*	*df*	*MS*	*F*	*Pvalue*	*F crit*	
Between CS Groups	2.2299E-05	2	1.11496E-05	0.1100	0.8960	3.1787	
Within CS Groups	0.00516747	51	0.000101323				
Total	0.00518977	53					

Table 7 : ANOVA result of the effect of % $^{w}/_{v}$ TA on weight uniformity of haemostatic gauze formulations.

Anova test result: Single Factor						
SUMMARY						
TA Groups	*Count*	*Sum*	*Average*	*Variance*		
1% $^{w}/_{v}$ TA	18	0.17573538	0.009763077	0.000105426		
2% $^{w}/_{v}$ TA	18	0.170585436	0.009476969	3.97225E-05		
5% $^{w}/_{v}$ TA	18	0.290476608	0.016137589	0.000130104		
ANOVA RESULT						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between TA Groups	0.00051048	2	0.00025524	2.7818	0.0713	3.1787
Within TA Groups	0.00467929	51	9.17508E-05			
Total	0.00518977	53				

The one-way ANOVA (Table 7) of the weight uniformity between the different formulations due to TA shows no significant difference. The resultant p value = 0.0713 is more than 0.05 so there is no significance difference found.

4.1.3 Thickness variation of the haemostatic gauze formulations

Variations of thickness within each haemostatic gauze formulation (represented by the SD of the three points measured) were compared between the various haemostatic gauze formulations tested. Figure 8 is a 3D plot of the effect of CS % $^{w}/_{v}$ and TA % $^{w}/_{v}$ on the thickness variation of the haemostatic gauze formulations.

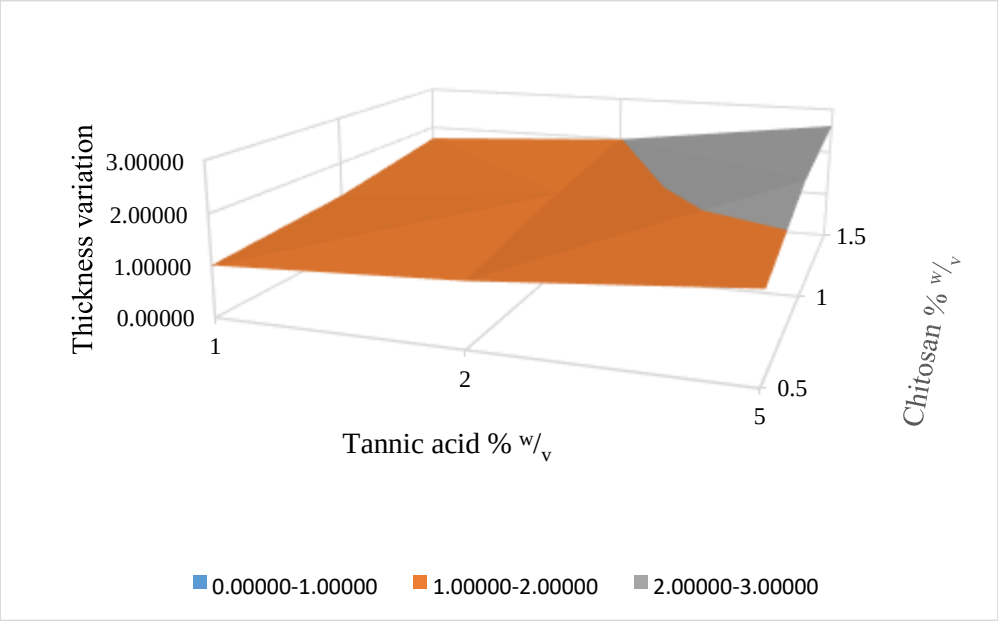


Figure 8 : 3D plot of the effect of CS % $^{w}/_{v}$ and TA % $^{w}/_{v}$ on the thickness variation of haemostatic gauze formulations.

As can be observed from Figure 8, as the concentration of CS increases from 0.5 to 1.5%$^{w}/_{v}$, so does the variation in thickness increase. It can also be observed that as the concentration of TA % $^{w}/_{v}$ increases from 1 to 5%$^{w}/_{v}$, so does the variation in thickness increase (very slightly).

Table 8 : ANOVA result of the effect of CS % $^{w}/_{v}$ on thickness variations.

Anova test result: Single Factor						
SUMMARY						
CS Groups	*Count*	*Sum*	*Average*	*Variance*		
0.5 % $^{w}/_{v}$ CS	18	2.921517494	0.162306527	0.0048097		
1 % $^{w}/_{v}$ CS	18	3.871869286	0.215103849	0.0030937		
1.5 % $^{w}/_{v}$ CS	18	5.222849373	0.290158299	0.0084891		
ANOVA result						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between CS Groups	0.14860	2	0.07430040	13.5976	1.84897E-05	3.178
Within CS Groups	0.27867	51	0.00546420			
Total	0.42727	53				

The ANOVA results in Table 8 for thickness variation shows that CS affect significantly (P= 1.85 x 10^{-5}) where P value has been found very < then 0.05.

However, the results in Table 9 shows that there is not a significant difference found for the different formulations of TA on the thickness variation of the haemostatic gauze strips (P = 0.0732).

Table 9 : ANOVA result of the effect of % $^{w}/_{v}$ TA on thickness variations.

Anova test result: Single Factor						
SUMMARY						
TA Groups	*Count*	*Sum*	*Average*	*Variance*		
1% $^{w}/_{v}$ TA	18	3.419602221	0.189977901	0.00854796		
2% $^{w}/_{v}$ TA	18	3.955986171	0.21977701	0.00979053		
5% $^{w}/_{v}$ TA	18	4.640647762	0.257813765	0.00434717		
ANOVA RESULT						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between TA Groups	0.04161891	2	0.02080	2.751	0.0732	3.1787
Within TA Groups	0.38565635	51	0.00756			
Total	0.42727526	53				

4.1.4 Swellability of the haemostatic gauze formulations

Figure 9 shows 3D presentation of effect of CS and TA on swellability. From Figure 9, it can be seen that the swellability % increased as the CS % $^{w}/_{v}$ increased in the haemostatic gauze.

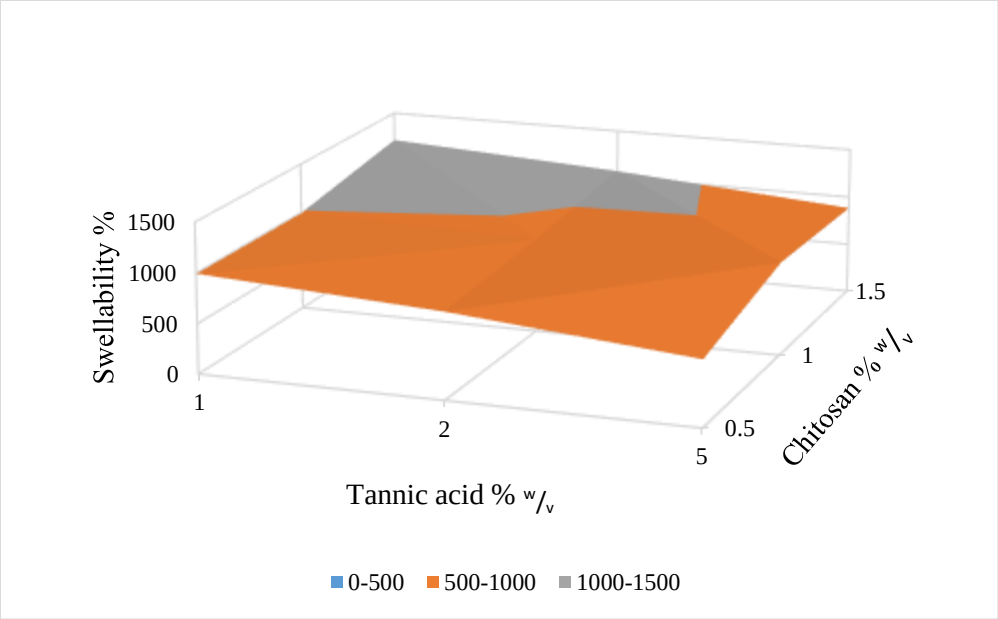


Figure 9 : 3D plot of the effect of % $^{w}/_{v}$ CS and % $^{w}/_{v}$ TA on the swelling % of haemostatic gauze formulations.

Table 10 : ANOVA result of the effect of % $^{w}/_{v}$ CS on Swellability.

Anova test result: Single Factor

SUMMARY

CS Groups	*Count*	*Sum*	*Average*	*Variance*
0.5 % $^{w}/_{v}$ CS	18	15072.0873	837.3381834	28800.0580
1% $^{w}/_{v}$ CS	18	17206.7602	955.9311227	4595.93506
1.5 %$^{w}/_{v}$ CS	18	18931.9448	1051.774711	33644.2897

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between CS Groups	415399.819	2	207699.90	9.29440	0.00036	3.1787
Within CS Groups	1139684.81	51	22346.76			
Total	1555084.63	53				

This effect of CS on the swellability % was significant (P=0.00036) as indicated in Table 10. However, as the concentration of TA increased in the haemostatic gauze, the swellability % decreased. This is due to the crosslinking of CS with TA. The effect TA % $^{w}/_{v}$ is also significant with a P = 0.00003 (Table 11).

Table 11 : ANOVA result of the effect of % $^{w}/_{v}$ TA on Swellability.

Anova test result: Single Factor						
SUMMARY						
TA Groups	*Count*	*Sum*	*Average*	*Variance*		
1% $^{w}/_{v}$ TA	18	19154.29	1064.1275	22229.843		
2% $^{w}/_{v}$ TA	18	17226.55	957.03106	13792.719		
5% $^{w}/_{v}$ TA	18	14829.93	823.88543	24777.590		
ANOVA RESULT						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*Fcrit*
Between TA Groups	521482.021	2	260741.01	12.8654	2.99426E-05	3.17
Within TA Groups	1033602.61	51	20266.71			
Total	1555084.63	53				

4.1.5 Tensile strength of the haemostatic gauze formulations

Tensile strength is one of the important mechanical properties for haemostatic gauze dressing applications. In all 9 formulations of the haemostatic gauze, the tensile strength was found to be more than 0.57 kg/cm^2 (which is far stronger than is needed in any manufacturing and packing procedure). Figure 10 shows a typical force versus time profile of the haemostatic gauze formulation. Most of the haemostatic gauze strips did not tear at the maximum setting (Table 3) on the Texture Analyzer.

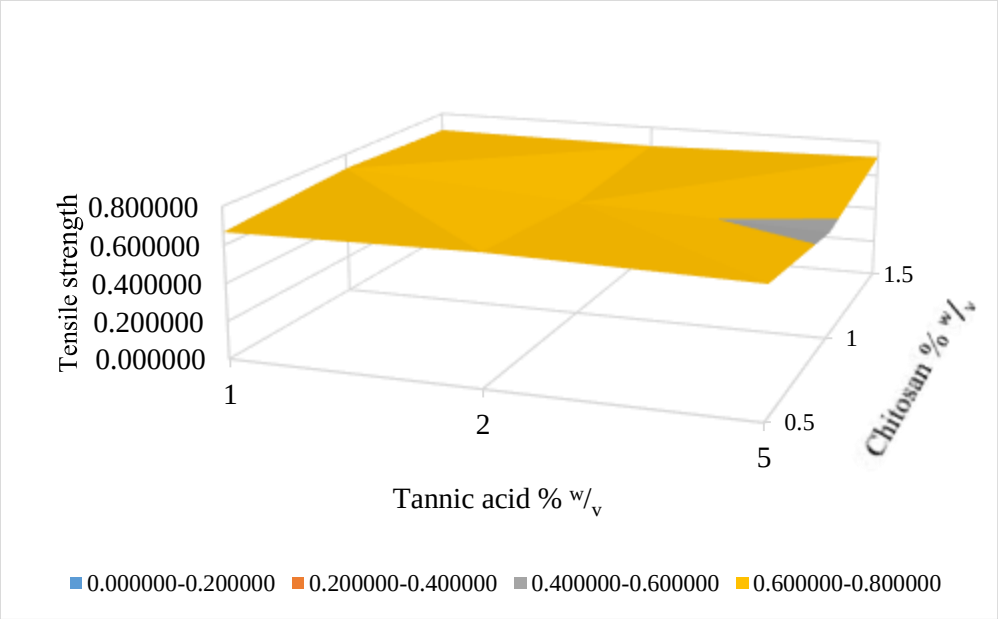


Figure 10 : 3D plot of the effect of % $^{w}/_{v}$ CS and % $^{w}/_{v}$ TA on the tensile strength of haemostatic gauze formulations.

As can be seen from Figure 10, as the % $^{w}/_{v}$ of CS and TA increased there was no significant change (P = 0.6884) for CS (Table 12) and P = 0.7925 for TA (Table 13) in the tensile strength of the different haemostatic gauze formulations has been found. This was because most of the tensile strength values were higher than the maximum set on the Texture Analyser (Figure 11).

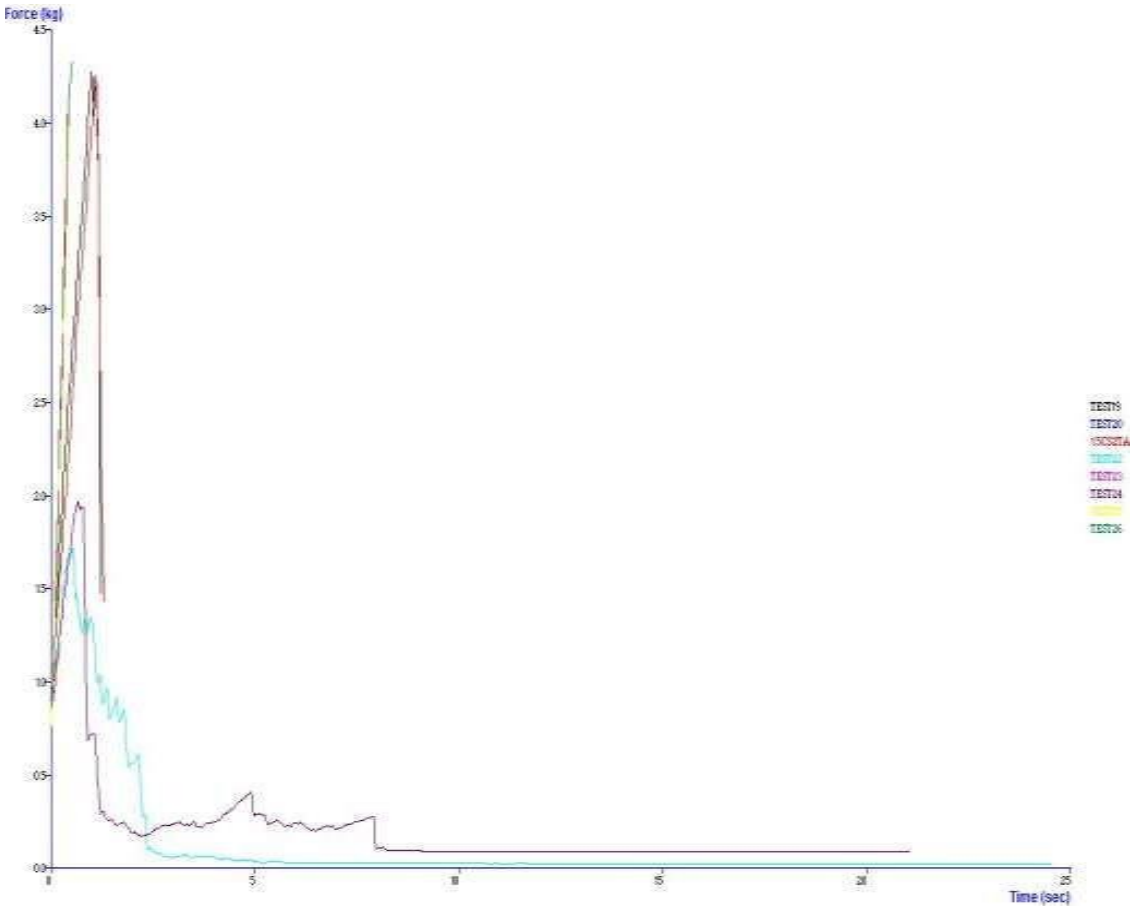


Figure 11 : Typical tensile strength measurements for different haemostatic gauze formulations.

Where test 19 = 0.5C1TA, test 20 = 0.5C5TA, test 21=1.5C2TA, test 22 = 1C1TA, test 23 =

1C5TA, test 24 =0.5C5TA, test 25 = 1.5C5TA, test 26 = 0.5C2TA.

Table 12 : ANOVA result of the effect of % $^{w}/_{v}$ CS on Tensile strength.

Anova test result: Single Factor						
SUMMARY						
CS Groups	*Count*	*Sum*	*Average*	*Variance*		
0.5% $^{w}/_{v}$ CS	18	12.10658	0.67258	0.037398		
1% $^{w}/_{v}$ CS	18	11.5492	0.64162	0.028931		
1.5% $^{w}/_{v}$ CS	18	12.53242	0.69624	0.0413801		
ANOVA RESULT						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between CS Groups	0.0270	2	0.01350	0.3761	0.6883	3.1787
Within CS Groups	1.8310	51	0.03590			
Total	1.8580	53				

Table 13 : ANOVA result of the effect of % $^{w}/_{v}$ TA on Tensile.

Anova test result: Single Factor						
SUMMARY						
TA Groups	*Count*	*Sum*	*Average*	*Variance*		
1% $^{w}/_{v}$ TA	18	12.48138	0.69341	0.027025484		
2% $^{w}/_{v}$ TA	18	11.9961	0.66645	0.029691439		
5% $^{w}/_{v}$ TA	18	11.71072	0.650595	0.051590674		
ANOVA RESULT						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between TA Groups	0.016867	2	0.008433	0.2336	0.7925	3.1787
Within TA Groups	1.841229	51	0.036102			
Total	1.858096	53				

4.1.6 Release and dissolution of TA % $^{w}/_{v}$ of haemostatic gauze formulations into SBF

4.1.6.1 Calibration curve for TA

The calibration curve for TA in SBF is presented in Figure 12. The correlation coefficient R^2 = 0.991 means curve reflects that there is linear relationship between TA concentration and absorbance in the concentration range studied.

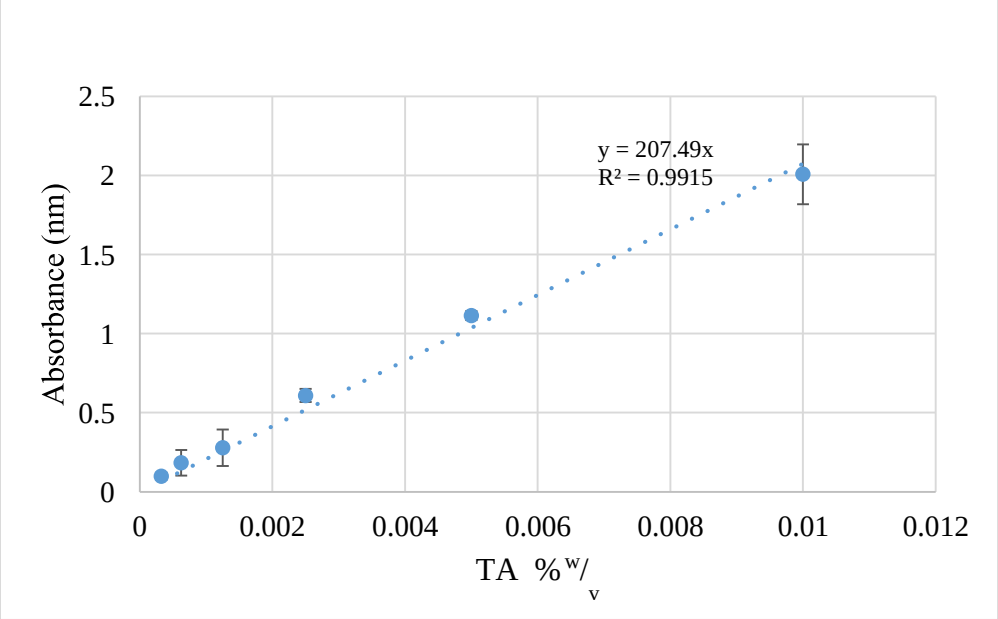


Figure 12 : Calibration curve for TA % $^{w}/_{v}$ acid of haemostatic gauze formulations in SBF.

4.1.7 Drug content uniformity of the haemostatic gauze formulations

Drug content uniformity varied from 95% to 99% (Figure 13). Mean ± SD percentile of drug content has been given in Table 16.

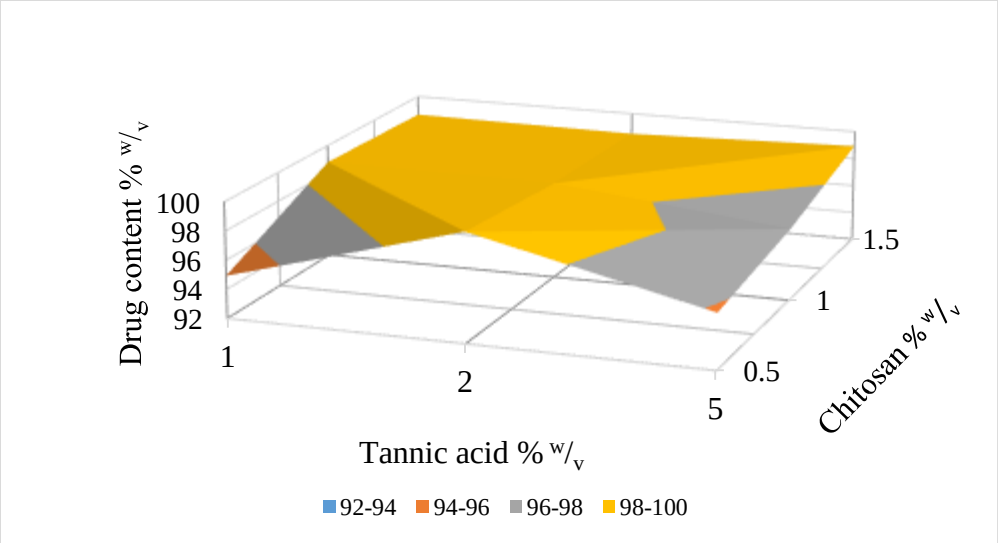


Figure 13 : 3D plot of the effect of CS % $^{w}/_{v}$ and TA % $^{w}/_{v}$ on drug content percentage of haemostatic gauze formulations.

Homogeneous content of drug is a very crucial property for gauze formulation. In each 9 formulations, from 3D plot (Figure 13) of drug content Vs %$^{w}/_{v}$ TA and %$^{w}/_{v}$ CS shows no variability. Drug content percentile found to be above 94 %.

The one-way ANOVA of the TA and CS content (Table 14 and 15) indicate there was no significant difference found on the effect of TA and CS on the TA uniformity of the haemostatic gauze formulations (P values 0.9350 and 0.9455) respectively.

Table 14 : ANOVA result of the effect of CS % $^{w}/_{v}$ on content uniformity of haemostatic gauze formulations.

Anova test result: Single Factor						
SUMMARY						
CS Groups	*Count*	*Sum*	*Average*	*Variance*		
0.5% $^{w}/_{v}$ CS	18	1741.67	96.75968	179.7496517		
1% $^{w}/_{v}$ CS	18	1767.12	98.17340	243.5126135		
1.5% $^{w}/_{v}$ CS	18	1776.08	98.67142	366.0790194		
ANOVA RESULT						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between CS Groups	35.408261	2	17.70413	0.0672	0.9350	3.178
Within CS Groups	13418.801	51	263.1137			
Total	13454.210	53				

Table 15 : ANOVA result of the effect of TA % $^{w}/_{v}$ on content uniformity of haemostatic gauze formulations.

Anova test result: Single Factor						
SUMMARY						
TA Groups	*Count*	*Sum*	*Average*	*Variance*		
1% $^{w}/_{v}$ TA	18	1754.10863	97.45047954	113.3433811		
2% $^{w}/_{v}$ TA	18	1780.32676	98.90704237	221.7984458		
5% $^{w}/_{v}$ TA	18	1750.44580	97.24698915	454.5462636		
ANOVA RESULT						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between TA Groups	29.5125621	2	14.756281	0.0560	0.9455	3.1787
Within TA Groups	13424.6975	51	263.22936			
Total	13454.2101	53				

4.1.8 Summery of characterizations of haemostatic gauze formulations

The summery of data of mean and SD for each of the haemostatic gauze formulations are shown in Table 16 below. It has been found that viscosity increased as TA-CS content increased. Weight varied from 0.3884 ± 0.02 to 0.6151 ± 0.07 g, thickness variation found between 1.023 ± 0.12 and 2.615 ± 0.29 mm. Weight and thickness also found to be increased as TA-CS content increased. Swellability has been found above 654.43 ± 45.22 % and thickness more than 0.5782 ± 0.22 (kg/cm^2). The drug content was found to be uniform throughout the formulated haemostatic gauzes with the SD values varying ± 0.05 to ± 0.15 more than 95%.

Table 16 : Summary of characterizations of the haemostatic gauze formulations.

Formulations % w/v	Viscosity cp of gel at 100 rpm speed Mean ± SD (n=6)	Weight variations (g) Mean ± SD (n=6)	Thickness variations (mm) Mean ± SD (n=6)	Swellability % increase. Mean ± SD (n=6)	Tensile strength (kg/cm^2) Mean ± SD (n=6)	Drug content uniformity (% of total added) Mean ± SD (n=6)
0.5C1TA	55 ± 5	0.3884 ± 0.02	1.023 ± 0.12	995.61 ± 141.18	0.6679 ± 0.21	94.94 ± 05.69
0.5C2TA	65 ± 5	0.4357 ± 0.00	1.251 ± 0.14	861.98 ± 22.59	0.6870 ± 0.15	99.52 ± 20.77
0.5C5TA	75 ± 5	0.5785 ± 0.02	1.657 ± 0.22	654.43 ± 45.22	0.6628 ± 0.20	95.81 ± 05.79
1C1TA	330 ± 10	0.4213 ± 0.01	1.261 ± 0.17	993.06 ± 61.55	0.7182 ± 0.06	98.80 ± 14.32
1CS2TA	455 ± 5	0.4732 ± 0.01	1.697 ± 0.21	941.49 ± 73.57	0.6284 ± 0.14	98.72 ± 10.76
1CS5TA	680 ± 10	0.6034 ± 0.04	2.370 ± 0.25	933.24 ± 41.42	0.5782 ± 0.22	97.00 ± 19.14
1.5C1TA	1050 ± 50	0.4516 ± 0.01	1.714 ± 0.27	1203.71 ± 100.2	0.6941 ± 0.17	98.61 ± 08.60
1.5C2TA	1150 ± 50	0.5064 ± 0.02	1.993 ± 0.30	1067.62 ± 107.9	0.6839 ± 0.20	98.48 ± 08.96
1.5C5TA	1740 ± 0	0.6151 ± 0.07	2.615 ± 0.29	883.99 ± 148.84	0.7107 ± 0.22	98.93 ± 29.71

4.1.9 Dissolution of TA % $^{w}/_{v}$ of the haemostatic gauze formulations in SBF

From the graphs (Figure 14-16) of the dissolution profiles of the various haemostatic gauze formulations, the results indicate that there is an initial rapid release of TA from the surface of the haemostatic gauze and then a slower dissolution rate of TA after 30 seconds. The dissolution of TA should follow a first-order kinetic rate because the TA is releasing from an increasing solvent path over time.

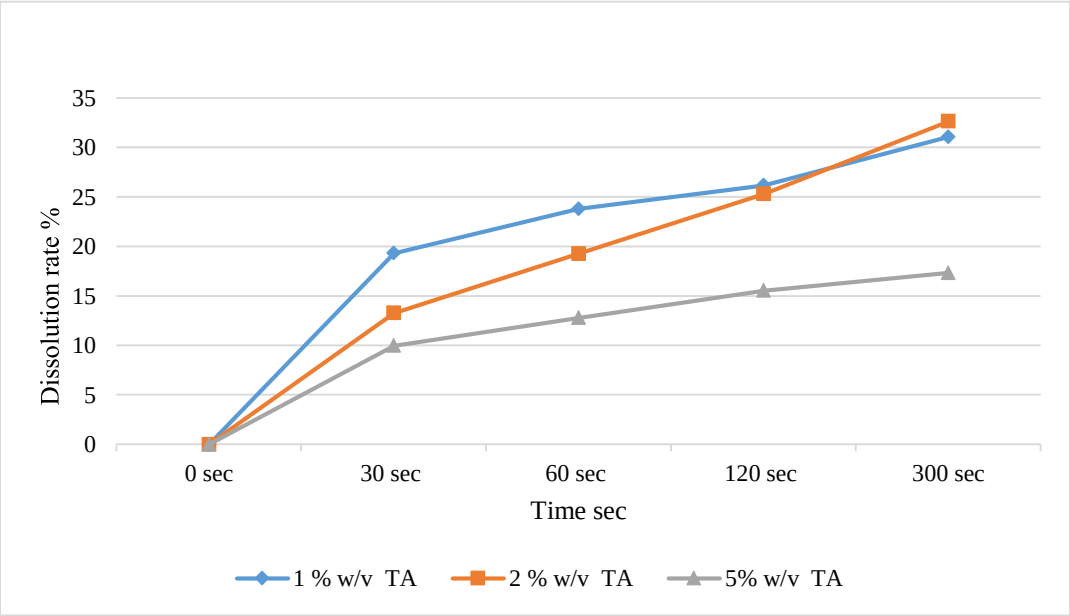


Figure 14 : Effect of 0.5 % $^{w}/_{v}$ CS on dissolution profile of haemostatic gauze formulations.

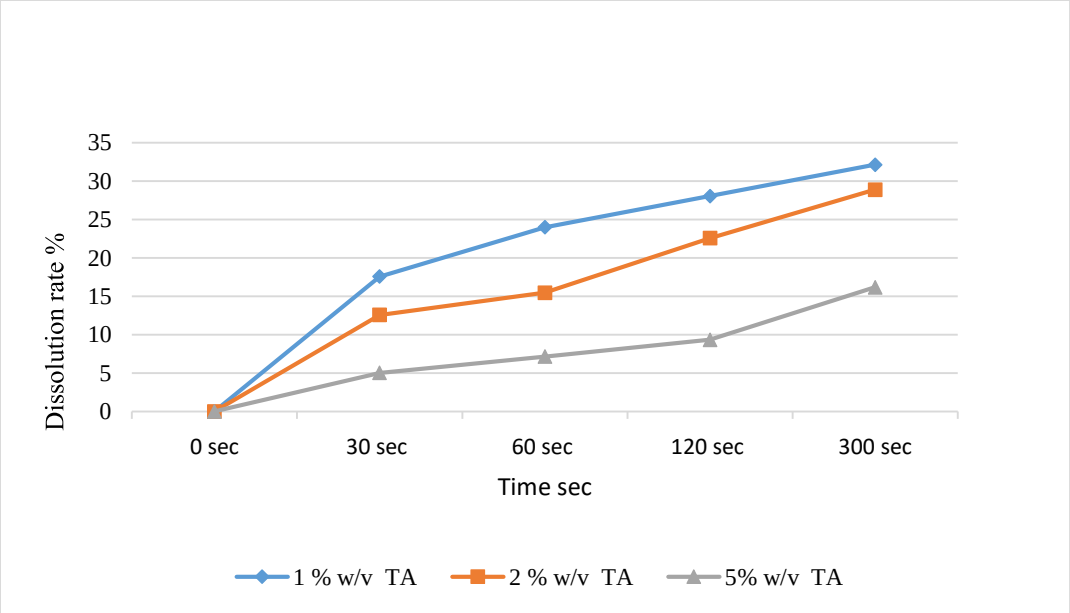


Figure 15 : Effect of 1 % $^{w}/_{v}$ CS on dissolution profile of haemostatic gauze formulations.

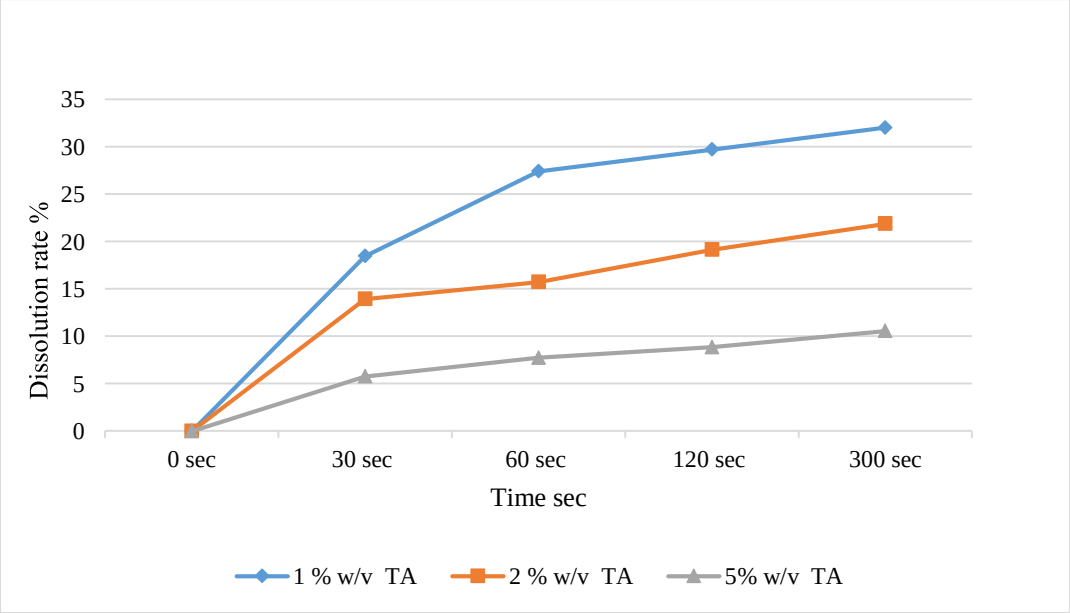


Figure 16 : Effect of 1.5 % $^{w}/_{v}$ CS on dissolution profile of haemostatic gauze formulations.

Although theoretically it is a first order kinetic rate of release, the results from the calculation of the Pearson correlation coefficient indicate that it is closer to a zero-order model than the first

order model. Therefore, it can predict that the release and dissolution of TA from the haemostatic gauze formulations followed zero-order kinetics for the dissolution.

When comparing the amount of TA in solution at 300 sec, as the %TA increased from 1 to 5%$^{w}/_{v}$ in the formulation, the %TA in solution at 300 sec decreased. This effect is because of crosslinking of CS and TA. It was also found that as % $^{w}/_{v}$ of CS increased from 0.5 to 1.5%$^{w}/_{v}$, the % TA in solution at 300 sec remained relatively constant (Figure 17).

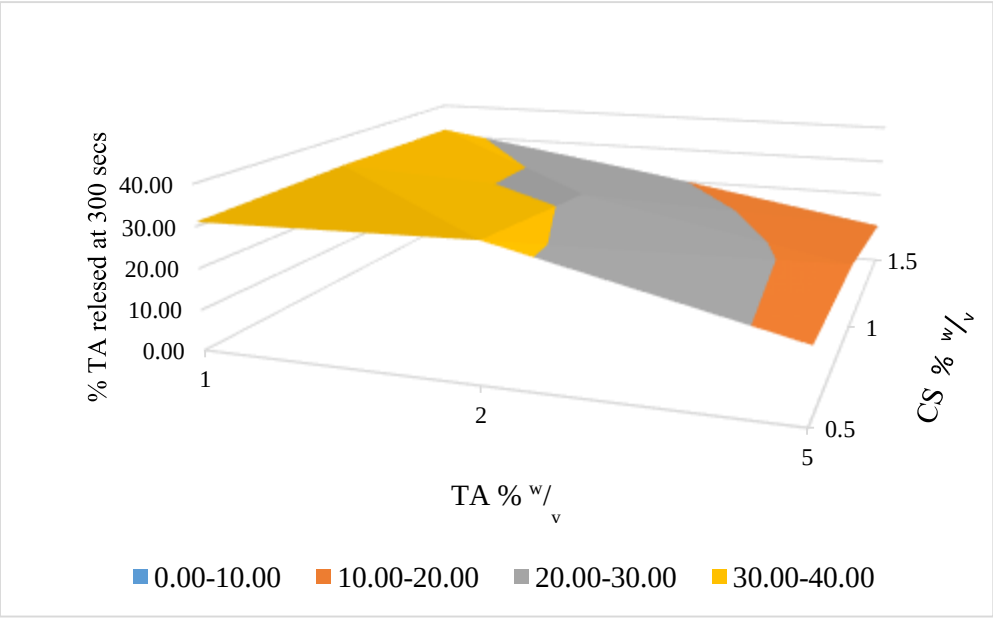


Figure 17 : 3D plot of the effect of TA and CS on the dissolution of TA % $^{w}/_{v}$ from haemostatic gauze formulations at 300 sec.

The highest dissolution % (µg/ml) found at 300 seconds, was for formulation 0.5C2TA (32.63 %) while the lowest dissolution % found at 300 seconds, was for formulation 1.5C5TA (10.57 %) (Table 17).

Table 17 : Dissolution rate % of haemostatic gauze formulation.

Formulation	% conc at 60 sec Mean ± SD	% conc at 120 sec Mean ± SD	% conc at 180 sec Mean ± SD	% conc at 300 sec Mean ± SD	Zero order	Corr coeff	First order	Corr coeff
0.5C1TA	19.31 ± 6.16	23.79 ± 6.20	26.15 ± 6.20	31.05 ± 7.96	0.038	0.942		
log conc	1.29 ± 0.79	1.38 ± 0.79	1.42 ± 0.79	1.49 ± 0.90			0.0006	0.912
0.5C2TA	13.27 ± 13.12	19.25 ± 18.8	25.28 ± 23.45	32.63 ± 20.91	0.064	0.946		
log conc	1.12 ± 1.12	1.28 ± 1.27	1.40 ± 1.37	1.51 ± 1.32			0.0012	0.896
0.5C5TA	9.97 ± 27.76	12.79 ± 25.2	15.54 ± 22.38	17.32 ± 16.87	0.023	0.887		
log conc	1.00 ±	1.11 ± 1.40	1.19 ± 1.35	1.24 ± 1.23			0.0007	0.854
1C1TA	17.57 ± 5.91	23.99 ± 7.25	15.54 ± 6.60	32.12 ± 9.33	0.045	0.887		
log conc	1.24 ± 0.77	1.38 ± 0.86	1.45 ± 0.82	1.51 ± 0.97			0.0008	0.845
1C2TA	12.56 ± 10.17	15.46 ± 13.3	22.56 ± 15.90	28.87 ± 19.13	0.057	0.956		
log conc	1.10 ± 1.01	1.19 ± 1.12	1.35 ± 1.20	1.46 ± 1.28			0.0012	0.924
1C5TA	5.01 ± 8.84	7.14 ± 15.82	9.34 ± 11.88	16.16 ± 26.77	0.039	0.996		
log conc	0.70 ±_0.95	0.85 ± 1.20	0.97 ± 1.07	1.21 ± 1.43			0.0017	0.968
1.5C1TA	18.48 ± 11.88	27.44 ± 10.6	29.74 ± 10.92	32.05 ± 10.93	0.037	0.766		
log conc	1.27 ± 1.07	1.44 ± 1.02	1.47 ± 1.04	1.51 ± 1.04			0.0006	0.727
1.5C2TA	13.96 ± 14.91	15.75 ± 16.7	19.16 ± 19.83	21.90 ± 24.40	0.027	0.942		
log conc	1.14 ± 1.17	1.20 ± 1.22	1.28 ± 1.30	1.34 ± 1.39			0.0007	0.923
1.5C5TA	5.78 ± 10.99	7.76 ± 14.93	8.87 ± 18.73	10.57 ± 18.64	0.015	0.918		
log	0.76 ± 1.04	0.89 ± 1.17	0.95 ± 1.27	1.02 ± 1.27			0.0008	0.876

From ANOVA single factor calculation, Table 18 and 19, it can be predicted that no significant differences (P= 0.0719) was found in the dissolution of TA at 300 sec as CS% $^{w}/_{v}$ changed. However, a significant difference (0.0379) was found as TA% $^{w}/_{v}$ changed between the groups on dissolution of TA at 300 sec. The rate of release increased as the %TA w/v changed from 1.0 to 2.0%. However, the rate of release decreased when the %TA w/v changed from 2.0% to 5.0 % (Table 17). This is could be due to the crosslinking effect of TA on the CS gel.

Table 18 : ANOVA result of the effect of CS % $^{w}/_{v}$ on dissolution profile of haemostatic gauze formulations.

Anova test result: Single Factor						
SUMMARY						
CS Groups	*Count*	*Sum*	*Average*	*Variance*		
0.5% CS	18	0.7593893	0.04218829	0.00092838		
1% CS	18	0.85993115	0.04777395	0.00099104		
1.5% CS	18	0.48308058	0.02683781	0.00036935		
ANOVA RESULT						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between CS Groups	0.00423095	2	0.002115	2.7728	0.0719	3.1787
Within CS Groups	0.03890924	51	0.000762			
Total	0.0431402	53				

Table 19 : ANOVA result of the effect of TA % $^{w}/_{v}$ on dissolution profile of haemostatic gauze formulations.

Anova test result: Single Factor						
SUMMARY						
TA Groups	*Count*	*Sum*	*Average*	*Variance*		
1% TA	18	0.72779626	0.04043313	0.00055893		
2% TA	18	0.90222977	0.05012388	0.00139105		
5% TA	18	0.472375	0.02624306	0.00028218		
ANOVA RESULT						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between TA Groups	0.00519337	2	0.002596	3.4899	0.0379	3.1787
Within TA Groups	0.03794682	51	0.000744			
Total	0.0431402	53				

4.2 Blood clotting ability of the haemostatic gauze formulations

The absorbance of the resultant hemoglobin-containing (released from non-clotted blood) solution was determined, where a low absorbance value indicates a higher clotting amount. A few photographs of the clotting ability test are shown in Figure 18.

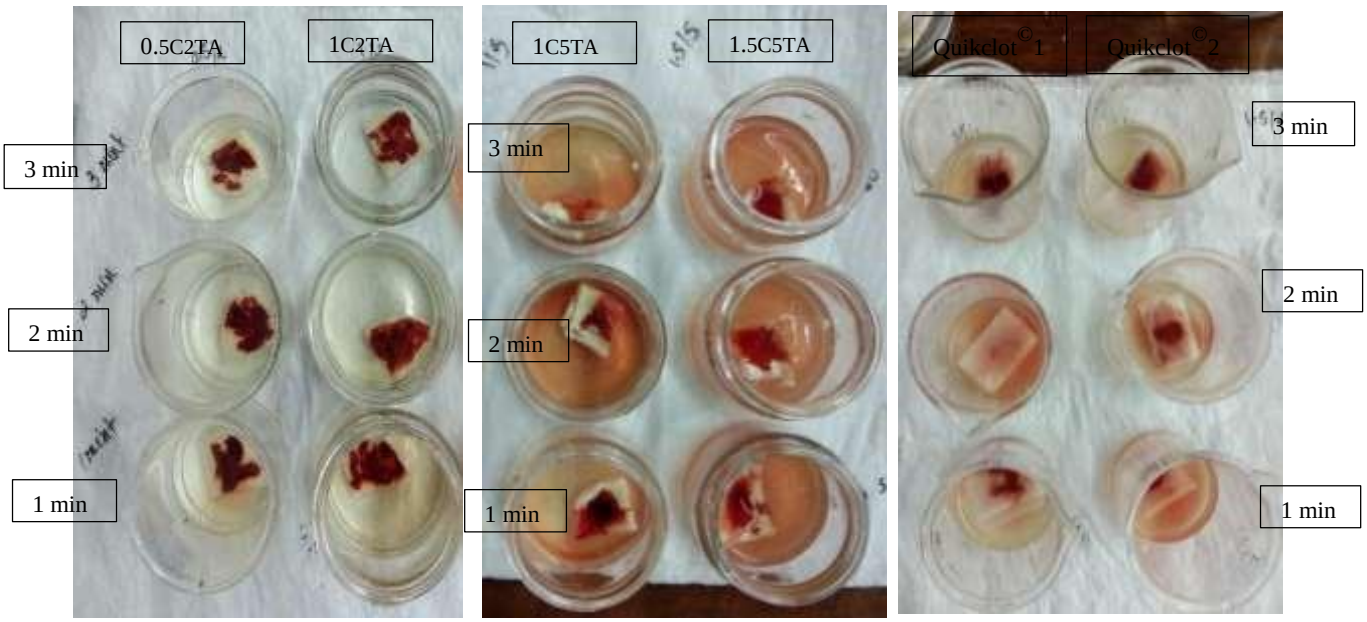


Figure 18 : 0.5C2TA % $^{w}/_{v}$, 1C2TA % $^{w}/_{v}$, 1C5TA % $^{w}/_{v}$, 1.5C5TA % $^{w}/_{v}$ and two samples of Quikclot© clotting ability at 1, 2 and 3 minutes.

The results of the clotting ability of the various haemostatic gauze formulations prepared in this research as well as that for Quikclot© is presented in Table 20. The clotting rate was calculated from the slope of the clotting extent versus time for the haemostatic gauze formulations. Formulation 0.5C5TA had the greatest rate of clotting (0.0056 extent/sec). However, when comparing the extent of clotting after 180 sec, formulation 0.5C1TA had the highest extent of

clotting (1.3648). The rate of clotting and extent of clotting at 180 sec for all of the haemostatic formulations were greater than that of Quikclot© (0.0021 extent/sec and 0.6855).

Table 20 : Clotting rate of each haemostatic gauze formulation and Quikclot©.

Time (sec)	**Formulations (n=6)**									
	0.5C1TA Mean ± SD	**0.5C2TA Mean ± SD**	**0.5C5TA Mean ± SD**	**1C1TA Mean ± SD**	**1C2TA Mean ± SD**	**1C5TA Mean ± SD**	**1.5C1TA Mean ± SD**	**1.5C2TA Mean ± SD**	**1.5C5TA Mean ± SD**	**Quikclot© Mean ± SD**
60	0.9972 ± 0.4292	0.7283 ± 0.4897	0.3360 ± 0.3176	0.9668 ± 0.3829	0.9110 ± 0.2844	0.37483 ± 0.23883	0.8018 ± 0.0546	0.5970 ± 0.4278	0.3752 ± 0.2958	0.4290 ± 0.3013
120	1.2083 ± 0.3724	1.0773 ± 0.4035	0.5797 ± 0.4527	1.1257 ± 0.2944	1.2172 ± 0.1682	0.58083 ± 0.26395	0.9990 ± 0.1379	0.7633 ± 0.4595	0.6118 ± 0.2248	0.5803 ± 0.3404
180	1.3648 ± 0.3141	1.2660 ± 0.2760	1.0053 ± 0.3475	1.3572 ± 0.1572	1.3220 ± 0.1319	0.9045 ± 0.16404	1.1673 ± 0.0955	0.9363 ± 0.3162	0.7593 ± 0.1553	0.6855 ± 0.3786
Slope	*0.0031*	*0.0045*	*0.0056*	*0.0033*	*0.0034*	0.00441	*0.0030*	*0.0028*	*0.0032*	*0.0021*
Pearson	*0.9963*	*0.9855*	*0.9879*	*0.9943*	*0.9623*	*0.9918*	*0.9990*	*0.9999*	*0.9911*	*0.9946*

[1] calculated from equation 2

From Figure 19 it can be seen that clotting extent value for the CS-TA % $^{w}/_{v}$ gauze were found different within the group. It can be predicted that as % $^{w}/_{v}$ CS 0.5 to 1.5 % $^{w}/_{v}$ increased, clotting extent decreased and as % $^{w}/_{v}$ of TA increased from 1 to 5 % $^{w}/_{v}$, clotting extent decreased.

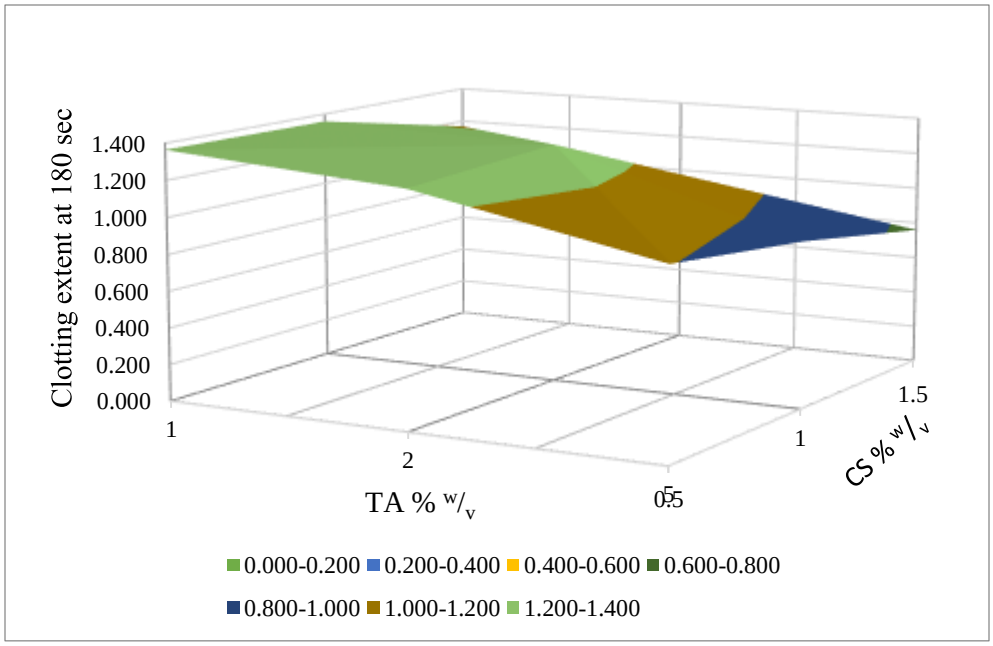


Figure 19 : 3D plot of effect of CS % $^{w}/_{v}$ and TA % $^{w}/_{v}$ at 180 sec on blood clotting ability of haemostatic gauze formulations.

As shown in Figure 20, formulated patches showed a higher clotting extent value compared to the reference sample Quikclot© rolled gauze. Also, the clotting extent value of the patches increased significantly over time (1 to 3 minutes) when incubated with human whole blood. The results show that absorbance of haemoglobin decreased and clotting extent increased as time increased. Excellent blood clotting has been found for 0.5C1TA and 1C1TA formulations (Figure 20). For 1C2TA, 0.5C2TA blood clotting seems better. For the formulations 1.5C2TA,

1.5C5TA slowest clotting extent have been found in compare with in the group. (Table 20)

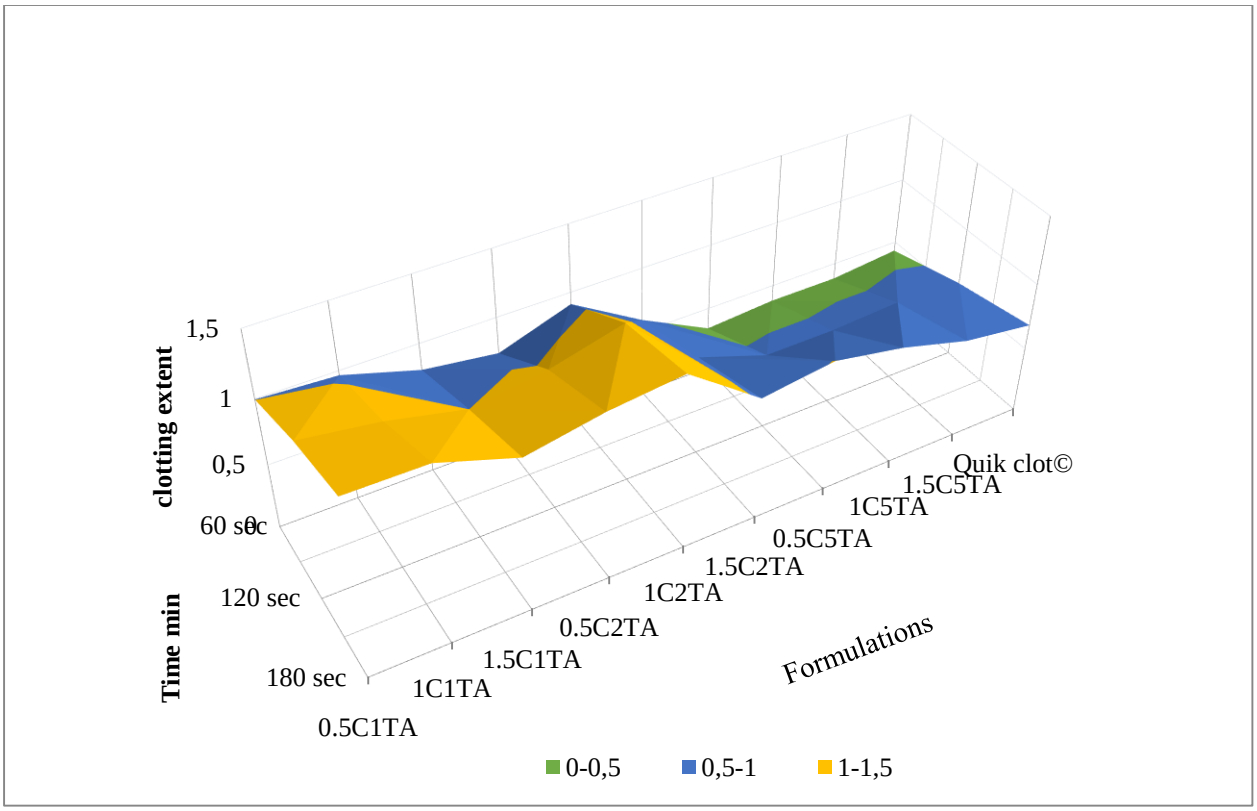


Figure 20 : 3D plot for blood clotting extent of haemostatic gauze formulations compared with Quikclot© at 60, 120 and 180 sec.

Table 21 : ANOVA result of the effect of % $^{w}/_{v}$ CS at 180 sec on clotting ability of haemostatic gauze formulations.

Anova test result: Single Factor						
SUMMARY						
CS Groups	*Count*	*Sum*	*Average*	*Variance*		
0.5% $^{w}/_{v}$ CS	18	21.817	1.212056	0.128684		
1% $^{w}/_{v}$ CS	18	21.502	1.194556	0.069114		
1.5% $^{w}/_{v}$ CS	18	17.178	0.954333	0.07658		
ANOVA RESULT						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between CS Groups	0.746602	2	0.373301	4.081601	0.022684	3.178799
Within CS Groups	4.664433	51	0.091459			
Total	5.411036	53				

From Table 21 and 22, it can be seen that CS % $^{w}/_{v}$ and TA % $^{w}/_{v}$ both significantly affect the clotting ability, as p values are 0.0226 and 0.00016 respectively.

Table 22 : ANOVA result of the effect of % $^{w}/_{v}$ TA at 180 sec on clotting ability of haemostatic gauze formulations.

Anova test result: Single Factor						
SUMMARY						
TA Groups	*Count*	*Sum*	*Average*	*Variance*		
1% $^{w}/_{v}$ TA	18	23.336	1.296444	0.055606		
2% $^{w}/_{v}$ TA	18	21.146	1.174778	0.098981		
5% $^{w}/_{v}$ TA	18	16.015	0.889722	0.071422		
ANOVA RESULT						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between TA Groups	1.568894	2	0.784447	10.41263	0.000161	3.178799
Within TA Groups	3.842141	51	0.075336			
Total	5.411036	53				

The extent of clotting results followed those of the dissolution results, with the clotting extent decreasing as the %TA in the formulations increased (Figure 21 below). The TA needs to be released before it can clot blood.

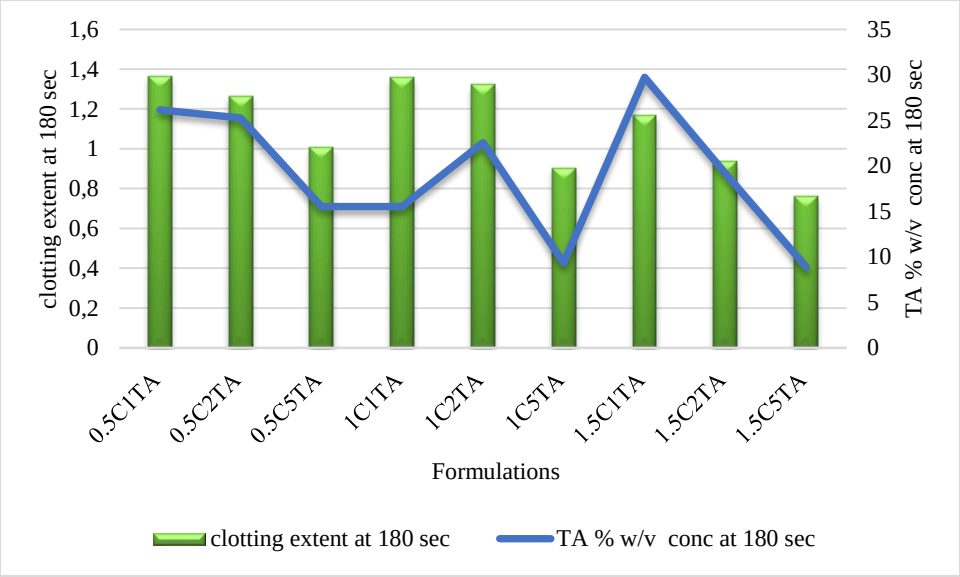


Figure 21 : Correlation between % $^{w}/_{v}$ TA dissolution and clotting extent at 180 seconds.

4.3 Sterility test of the haemostatic gauze formulations

Most of the haemostatic gauze formulations after 4 weeks storage in closed containers in a dark cupboard at room temperature, showed growth (Table 23). This could be because the formulation was prepared in a normal, nonaseptic laboratory environment. There has been growth found for gauze without formulation too. Anaerobic growth was found to be higher than fungal and bacterial growth for each of the formulations.

Table 23 : Sterility test results of the haemostatic gauze formulations.

No.	formulations	Test a1	Test a2	Test b1	Test b2	Test c1	Test c2
1	**0.5C1TA**	Ng	Ng	G	G	G	G
2	**0.5C2TA**	Ng	G	Ng	Ng	Ng	G
3	**0.5C5TA**	G	G	G	G	G	G
4	**1C1TA**	G	G	G	Ng	G	G
5	**1C2TA**	G	G	G	G	G	G
6	**1C5TA**	G	G	G	Ng	G	G
7	**1.5C1TA**	Ng	Ng	Ng	Ng	Ng	G
8	**1.5C2TA**	G	G	Ng	G	G	G
9	**1.5C5TA**	Ng	Ng	G	G	G	G
10	**Control a**	G	Ng	Ng	G	G	Ng
11	**Control b**	Ng	Ng	Ng	Ng	Ng	Ng

Test a = TSB 37 deg 48 hrs. Test b = TSB 25 deg 7 days. Test c = Thio 37 deg 48 hrs.

G = growth and Ng = no growth. Control a is blank gauze without formulation. Control b is only media.

Chapter 5 Conclusion and recommendation

5.1 Conclusion

The main aim of this research was to synthesize and evaluate a new haemostatic gauze containing TA and CS that has a high absorptivity of blood and effective blood coagulation. The casting method of impregnating standard gauze strips and then lyophilizing it, is a simple process and could lend itself for mass-production at a relatively low cost as compared to importing such haemostatic products.

Besides the local production advantage, the CS and TA haemostatic gauze formulations in this study have been found effective in *in-vitro* blood clotting and superior to commercially available

Quikclot© From the rheological study, it has been found that CS-TA gel follows a non-Newtonian shear thinning, thixotropic behavior. Viscosity and thickness were significantly affected by the CS % $^{w}/_{v}$ in the formulations tested.

The excellent swelling property (very helpful in blood clotting) of the CS-TA haemostatic gauze formulations tested in this research - was found to be more than 654.43%. Swellability was significantly affected as CS % $^{w}/_{v}$ and TA % $^{w}/_{v}$ content changed. As the swelling increased, the CS-TA haemostatic gauze formulations absorbed more blood.

Haemostatic impregnated haemostatic gauze requires a certain amount of friability to withstand handling and mechanical shocks when packaging as well as at the time of application to a wound. The tensile strength of all the haemostatic gauze formulations were found to be more than 0.57 kg/cm^2 which is crucial.

Homogeneous and equal active pharmaceutical ingredient (API) distribution is one of the important characteristics of all pharmaceutical formulations. Determination of drug content shows the drug is equally cross-linked in gel and impregnated on gauze.

In-vitro TA dissolution data analysis show that dissolution rate from impregnated gauze varied significantly as the CS-TA ratio changed. However, only TA % $^{w}/_{v}$ demonstrated a significant difference, while CS % $^{w}/_{v}$ concentrations did not significantly affect the dissolution rate. The results indicate that a 0.5C2TA, 1CS1TA give rise to best release and dissolution while 1.5C5TA give lowest dissolution of TA from the various haemostatic gauze formulations.

From the results of blood clotting analysis, best clotting rate has been found for the 1C2TA formulation. Excellent and quick clotting extent have been found for 0.5C1TA (1.36 ± 0.31), 1C1TA (1.36 ± 0.16) and 1C2TA (1.32 ± 0.13) formulations while 1.5C5TA was found to have the lowest clotting extent within the CS-TA formulations group. It also provides evidence that CS and TA combination shows efficacious and better blood clotting when compared with Quikclot© commercial gauze. Furthermore, blood clotting extent increases within a few minutes when exposed the haemostatic gauze formulation.

The sterility test results show that there was growth found for both blank gauze and the haemostatic gauze formulations. In future the haemostatic gauze formulation in this study would need to be prepared under aseptic conditions. More growth found for anaerobic bacteria in comparison to nonaerobic bacteria and fungi. Preservatives could also be added to the formulation to prevent further topical infection from gauze to wound. However, one would need to determine the effect of the preservative on the clotting ability of the haemostatic gauze.

5.2 Recommendation

The present research report provides information and statistical data regarding successful synthesis of a haemostatic gauze drug delivery system for blood clotting. Further work to validate large-scale production process is recommended. Even though there were no difficulties found on a small scale in laboratory production except sterility. Hence, it is recommended that a preservative should be added to the haemostatic gauze drug delivery system or it should be formulated under an aseptic environment.

For detailed surface morphology, porosity and microstructure of crosslinking between TA and CS, scanning electron microscopy is also recommended.

Moreover, the concept of a haemostatic gauze formulation using CS polymer can be used to deliver other bioactive, nontoxic and potential haemostatic drugs for topical application and can be used to synthesize new formulations.

The ultimate goal for development of this haemostatic gauze that is locally available and as effective as the commercially available Celox® gauze has been achieved.

Chapter 6 References

Abd El-Mabood, El-Sayed A, Hazeme A, and Nasser Z. 2014. "Topical Tannic Acid Application Decreases Posthemorrhoidectomy Pain: A Novel Idea." *The Egyptian Journal of Surgery* 33 (4): 213.

Aelenei, N., Marcel I.P., Ovidiu N., Gabriela L., and Lacramioara B., 2009. "Tannic Acid Incorporation in Chitosan-Based Microparticles and in *Vitro* Controlled Release." *Journal of Materials Science: Materials in Medicine* 20 (5): 1095–1102.

Ahmed, S., and Saiqa I. 2016. "Chitosan Based Scaffolds and Their Applications in Wound Healing." *Achievements in the Life Sciences* 10 (1): 27–37.

Al-Hijazeen, M., Eun L., Aubrey M., and Dong A., 2016. "Effects of Tannic Acid on Lipid and Protein Oxidation, Color, and Volatiles of Raw and Cooked Chicken Breast Meat during Storage." *Antioxidants* 5 (2): 19.

Aranaz, I., María G., María F., Francisco, I., María C. Gutiérrez, M., and Del Monte, F.,. 2014. "Preparation of Chitosan Nanocompositeswith a Macroporous Structure by Unidirectional Freezing and Subsequent Freeze-Drying." *Marine Drugs* 12 (11): 5619–42.

Archana, D., Dutta J., and Dutta, P.K., 2013. "Evaluation of Chitosan Nano Dressing for Wound Healing: Characterization, in *Vitro* and in Vivo Studies." *International Journal of Biological Macromolecules* 57 (June): 193–203.

Azeredo, H., and Waldron K., 2016. "Crosslinking in Polysaccharide and Protein Films and Coatings for Food Contact – A Review." *Trends in Food Science & Technology* 52 (November): 109–22.

Balan, V., and Verestiuc L., 2014. “Strategies to Improve Chitosan Hemocompatibility: A Review.” *European polymer journal* 53: 171–88.

Bano, I., Arshad M., Yasin T., and Afzal M., Ghauri. 2018. “Preparation, Characterization and Evaluation of Glycerol Plasticized Chitosan/PVA Blends for Burn Wounds.” *International Journal of Biological Macromolecules* 124: 155-162.

Bano, I., Arshad M., Yasin T., Afzal M., Ghauri, and Younus M.. 2017. “Chitosan: A Potential Biopolymer for Wound Management.” *International Journal of Biological Macromolecules* 102: 380–83.

Barrett, K., Barman S., Boitano S., and Reckelhoff J., 2017. “Blood as a Circulatory Fluid & the Dynamics of Blood & Lymph Flow.” In *Ganong’s Medical Physiology Examination & Board Review*. New York, NY: McGraw-Hill Education 26e Eds.

Başaran, E., and Yasemin Y., 2012. “Ocular Application of Chitosan.” *Expert Opinion on Drug Delivery* 9 (6): 701–12.

Bennett, B., and Littlejohn, L.,. 2014. “Review of New Topical Haemostatic Dressings for Combat Casualty Care.” *Military Medicine* 179 (5): 497–514.

Bennett, B., Littlejohn, L., 2017. “Bleeding Control Using Haemostatic Dressings: Lessons Learned.” *Wilderness & Environmental Medicine* 28 (2): S39–49.

Bharkatiya, M., Nema R., and Bhatnagar, M., 2010. “Development and Characterization of Transdermal Patches of Metoprolol Tartrate.” *Asian Journal of Pharmaceutical and Clinical Research* 3 (2): 130–34.

Bilck, A.P., Müller C.M.O., Olivato J.B., Mali S., Grossmann, M.V.E., Yamashita, F., 2015. “Using Glycerol Produced from Biodiesel as a Plasticiser in Extruded Biodegradable Films.” *Polímeros* 25 (4): 331–35.

Boateng, J., and Catanzano , O., 2015. “Advanced Therapeutic Dressings for Effective Wound Healing--A Review.” *Journal of Pharmaceutical Sciences* 104 (11): 3653–80.

Booth, B.W., Inskeep B.D., Shah, H., Park, J.P., Hay, E.J., and Burg,K.J.L., 2013. “Tannic Acid Preferentially Targets Estrogen Receptor-Positive Breast Cancer.” *International Journal of Breast Cancer* 2013: 1–9.

Breeze, J., Penn-Barwell, J.G., Keene D., O’Reilly, D., Jeyasankar, J., and Mahoney, P..F., 2017. *Ballistic Trauma: A Practical Guide*. 4th ed. Springer. Kumar, M. N. V. Ravi, R. A. A. Muzzarelli, C. Muzzarelli, H. Sashiwa, and A. J. Domb. 2004. “Chitosan Chemistry and Pharmaceutical Perspectives.” *Chemical Reviews* 104 (12): 6017–84.

Carraway, J.W., Kent, D., Young, K., Cole, A., Friedman R.,, and Ward. K.R., 2008. “Comparison of a New Mineral Based Haemostatic Agent to a Commercially Available Granular Zeolite Agent for Haemostasis in a Swine Model of Lethal Extremity Arterial Hemorrhage.” *Resuscitation* 78: 230–35.

Cassano, R., Luisa M., Gioia, S., Mellace, S., Picci N., and Trombino S.,. 2017. “Hemostatic Gauze Based on Chitosan and Hydroquinone: Preparation, Characterization and Blood

Coagulation Evaluation." *Journal of Materials Science. Materials in Medicine* 28 (12): 190.

Chan, L.W., Kim, C.H., Wang, X., Pun, S.H., White, N.J., and Kim, T.H., 2016. "PolySTAT-Modified Chitosan Gauzes for Improved Haemostasis in External Hemorrhage." *Acta Biomaterialia* 31 (February): 178–85.

Cipriano-Salazar, M., Rojas-Hernández, S., Olivares-Pérez, J., Jiménez-Guillén, R., Cruz-Lagunas, B., Camacho-Díaz, L.M., and Ugbogu, A.E., 2018. "Antibacterial Activities of Tannic Acid against Isolated Ruminal Bacteria from Sheep." *Microbial Pathogenesis* 117 (April): 255–58.

Crispo, J.A.G., Ansell, D.R., Piche, M., Eibl, J.K., Khaper, N., Ross, G.M, and Tai, T.C., 2010. "Protective Effects of Polyphenolic Compounds on Oxidative Stress-Induced Cytotoxicity in PC12 Cells." *Canadian Journal of Physiology & Pharmacology* 88 (4): 429–38.

Croisier, F., and Jérôme, C. 2013. "Chitosan-Based Biomaterials for Tissue Engineering." *European Polymer Journal* 49 (4): 780–92.

Dai, C., Liu, C., Wei, J., Hong, H., and Zhao Q. 2010. "Molecular Imprinted Macroporous Chitosan Coated Mesoporous Silica Xerogels for Hemorrhage Control." *Biomaterials* 31 (30): 7620–30.

Dash, M., Chiellini, F., Ottenbrite, R.M., and Chiellini, E,. 2011. "Chitosan"A Versatile Semi-Synthetic Polymer in Biomedical Applications." *Progress in Polymer Science* 36: 981–10

Davoren, J.B., and Hsu, G. 2019. “Blood Disorders.” In *Pathophysiology of Disease: An Introduction to Clinical Medicine, 8e,* edited by Gary D Hammer and Stephen J McPhee. New York, NY: McGraw-Hill Education.

Desbri, J., Martinez, C., and Rinaudo, M 1996. “Biological Macromolecules Hydrophobic Derivatives of Chitosan: Characterization and Rheological Behaviour.” *International Journal of Biological Macromolecules* 19:21-28.

Deville, S. 2006. “Freezing as a Path to Build Complex Composites.” *Science* 311 (5760): 515–18.

Escárcega-Galaz, A.A., Sánchez-Machado, D.I., López-Cervantes, J., Sanches-Silva, A., Madera-Santana, T.J., and Paseiro-Losada, P. 2018. “Mechanical, Structural and Physical Aspects of Chitosan-Based Films as Antimicrobial Dressings.” *International Journal of Biological Macromolecules* 116: 472–81.

Freeman, C., and Wilkinson, A. 2018. “Hemorrhage Control.” In *Reichman’s Emergency Medicine Procedures,* edited by Eric F Reichman, 3rd ed. New York, NY: McGraw-Hill Education.

Gale, A.J. 2011. “Continuing Education Course #2: Current Understanding of Haemostasis.” *Toxicologic Pathology* 39 (1): 273–80.

Gegel, B.T, Austin, P.N., and Johnson, A.D., 2013. “An Evidence-Based Review of the Use of a Combat Gauze (QuikClot) for Hemorrhage Control.” *AANA Journal* 81 (6): 453–58.

Glick, J.B., Kaur, R.R., and Siegel, D. 2013. “Achieving Haemostasis in Dermatology-Part II:

Topical Haemostatic Agents." *Indian Dermatology Online Journal* 4 (3): 172–76.

Gruen, R.L., Brohi, K., Schreiber, M., Balogh, Z.J., Pitt, V., Narayan M., and Maier, R.V. 2012. "Haemorrhage Control in Severely Injured Patients." *The Lancet* 380 (9847): 1099–1108.

Gülçin, İ., Huyut,Z., Elmastaş, M., and Aboul-Enein, H.Y., 2010. "Radical Scavenging and Antioxidant Activity of Tannic Acid." *Arabian Journal of Chemistry* 3 (1): 43–53.

Güven, H.E. 2017. "Topical Haemostatics for Bleeding Control in Pre-Hospital Setting: Then and Now." *Turkish Journal of Trauma and Emergency Surgery* 23(5):357-361.

Hangge, P., Stone, J., Albadawi, H., Zhang, Y.S., Khademhosseini, A., and Oklu, R. 2017. *Cardiovascular Diagnosis and Therapy. Cardiovascular Diagnosis and Therapy*. 7(3): S267-S275.

Hasan, S.S., The, K.M., Ahmed, S.I., Chong, D.W.K., Ong, H.C., and Naina, B. 2015. "Quality of Life (QoL) and International Normalized Ratio (INR) Control of Patients Attending Anticoagulation Clinics." *Public Health* 129 (7): 954–62.

Heise, K., Hobisch, M., Sacarescu, L., Maver, U., Hobisch, J., Reichelt, T., Sega, M., Fischer, S., and Spirk, S. 2018. "Low-Molecular-Weight Sulfonated Chitosan as Template for Anticoagulant Nanoparticles." *International Journal of Nanomedicine* 13 (August): 4881–94.

Hess, J.R., Brohi, K., Dutton, R.P., Hauser, C.J., Holcomb, J.B., Kluger, Y., Mackway-Jones,

K., Parr, M.J., Rizoli, S.B., Yukioka, T., Hoyt, D.B., and Bouillon, B. 2008. "The Coagulopathy of Trauma: A Review of Mechanisms." *The Journal of Trauma: Injury, Infection, and Critical Care* 65 (4): 748–54.

Ho, J., and Hruza, G. 2007. "Hydrophilic Polymers with Potassium Salt and Microporous Polysaccharides for Use as Haemostatic Agents." *Dermatologic Surgery: Official Publication for American Society for Dermatologic Surgery [et al.]* 33 (12): 1430–33.

Howe, N., and Cherpelis, B. 2013. "Obtaining Rapid and Effective Haemostasis: Part I. Update and Review of Topical Haemostatic Agents." *Journal of the American Academy of Dermatology* 69 (5): 659.e1-659.e17.

Huang, X, Jia, J., Wang, Z., and Hu, Q.L. 2015. "A Novel Chitosan-Based Sponge Coated with Self-Assembled Thrombin/Tannic Acid Multilayer Films as a Haemostatic Dressing." *Chinese Journal of Polymer Science* 33 (2): 284–90.

Inaba, K., Siboni, S., Resnick, S., Zhu, J., Wong, M.D., Haltmeier, T., Benjamin, E., and Demetriades, D. 2015. "Tourniquet Use for Civilian Extremity Trauma." *Journal of Trauma and Acute Care Surgery* 79 (2): 232–37.

Kamble, S.S. 2018. "Chitin and Chitosan Polymer: A Review of Recent Advances and Prospective Applications." *Paintindia* 68 (7): 69–78.

Kamoh, A., and Swantek. J. 2012. "Haemostasis in Oral Surgery." *Dental Clinics of North America* 56 (1): 17–23, vii.

Kang, P.L., Chang, S.J., Manousakas, I., Lee, C.W., Yao, C.H., Lin, F.H., and Kuo, S.M. 2011. "Development and Assessment of Haemostasis Chitosan Dressings." *Carbohydrate Polymers* 85 (3): 565–70.

Karki, S., Kim, H., Na, S.J., Shin, D., Jo, K., and Lee. J., 2016. "Thin Films as an Emerging Platform for Drug Delivery." *Asian Journal of Pharmaceutical Sciences* 11 (5): 559–74.

Kean, T., and Thanou. M. 2010. "Biodegradation, Biodistribution and Toxicity of Chitosan." *Advanced Drug Delivery Reviews* 62 (1): 3–11.

Khoshmohabat, H., Paydar, S., Kazemi, H.M., and Dalfardi, B. 2016. "Overview of Agents Used for Emergency Haemostasis." *Trauma Monthly* 21 (1): E26023.

Kibble, J.D., and Halsey, C.R. 2015. "Blood." In *Medical Physiology: The Big Picture*. New York, NY: McGraw-Hill Education.

Kleiner, L.W., Wright, J.C., and Wang, Y. 2014. "Evolution of Implantable and Insertable Drug Delivery Systems." *Journal of Controlled Release* 181 (6): 1–10.

Knidri, H.E., Belaabed, R., Addaou, A., Laajeb, A., and Lahsini, A., 2018. "Extraction, Chemical Modification and Characterization of Chitin and Chitosan." *International Journal of Biological Macromolecules* 120 (12): 1181–89.

Koksal, O., Ozdemir, F., Etoz, B.C., Buyukcoskun, N.I., and Sigirli, D. 2011. "Haemostatic Effect of a Chitosan Linear Polymer (Celox®) in a Severe Femoral Artery Bleeding Rat

Model under Hypothermia or Warfarin Therapy." *Turkish Journal of Trauma and Emergency Surgery* 17 (3): 199–204.

Kokubo, T., and Takadama, H. 2006. "How Useful Is SBF in Predicting in Vivo Bone Bioactivity?" *Biomaterials* 27 (15): 2907–15.

Konkle, B.A. 2018. "Bleeding and Thrombosis." In *Harrison's Principles of Internal Medicine, 20e*, edited by J Larry Jameson, Anthony S Fauci, Dennis L Kasper, Stephen L Hauser, Dan L Longo, and Joseph Loscalzo. New York, NY: McGraw-Hill Education.

Kozen, B.G., Kircher, S.J., Henao, J., Godinez, F.S., and Johnson, A.S. 2008. "An Alternative Haemostatic Dressing: Comparison of Celox, HemCon, and QuikClot." *Academic Emergency Medicine* 15 (1): 74–81.

Kumar, M.. Ravi, N. V,. Muzzarelli, R. A. A., Muzzarelli, C., Sashiwa, H., and Domb. A. J. 2004. "Chitosan Chemistry and Pharmaceutical Perspectives." *Chemical Reviews* 104 (12): 6017–84.

Kumar, S. 2016. "Local haemostatic agents in the management of bleeding in oral surgery" *Asian Journal of Pharmaceutical and Clinical research* 9 (3): 7.

Kumari, S., Rath, P., Kumar, S.H., and Tiwari, T.N. 2015. "Extraction and Characterization of Chitin and Chitosan from Fishery Waste by Chemical Method." *Environmental Technology & Innovation* 3 (4): 77–85.

Leal A., Hellen L., Leal D., Silva D., Cunha L., and Arimateia J., 2015. "Incorporation of Tannic Acid in Formulations for Topical Use in Wound Healing: A Technological Prospecting." *African Journal of Pharmacy and Pharmacology* 9 (26): 662–74.

Leceta, I., Guerrero, P., Ibarburu, I., Dueñas, M.T., and Caba. K. de la., 2013. "Characterization and Antimicrobial Analysis of Chitosan-Based Films." *Journal of Food Engineering* 116 (4): 889–99.

Lier, H., Krep, H., Schroeder, S., and Stuber, F. 2008. "Preconditions of Haemostasis in Trauma: A Review. The Influence of Acidosis, Hypocalcemia, Anemia, and Hypothermia on Functional Haemostasis in Trauma." *The Journal of Trauma: Injury, Infection, and Critical Care* 65 (4): 951–60.

Lim, S.W. 2012. "Aluminum Potassium Sulfate and Tannic Acid Injection for Hemorrhoids." *Journal of the Korean Society of Coloproctology* 28 (2): 73–77.

Liu, L, Qi, G., Lu, X., and Zhou, H. 2016. "In Situ Forming Hydrogels Based on Chitosan for Drug Delivery and Tissue Regeneration." *Asian Journal of Pharmaceutical Sciences* 11 (6): 673–83.

Makwana, S.B., Patel, V.A., and Parmar, S.J. 2016. "Development and Characterization of In-Situ Gel for Ophthalmic Formulation Containing Ciprofloxacin Hydrochloride." *Pharma Sciences* 6: 1–6.

Mani, M., Ebenezer, V., and Balakrishnan, R. 2015. "Impact of Haemostatic Agents in Oral Surgery." *Biomedical and Pharmacology Journal* 7 (1): 215–19.

Matsvaire, T. 2017. “Synthesis and Characterisation of Chitosan Based Sponges for Wound Dressings,” *Polymer Science* (March).

McGee, K.R., and Lynch, J.J. 2016. “Wounds and Hemorrhage.” In *Cooney’s EMS Medicine*, edited by Derek R Cooney. New York, NY: McGraw-Hill Education.

Mi, F.L., Wu, Y.B., Shyu, S.S., Schoung, J.Y., Huang, Y.B., Tsai, Y.H., and Hao, J.Y. 2002. “Control of Wound Infections Using a Bilayer Chitosan Wound Dressing with Sustainable Antibiotic Delivery.” *Journal of Biomedical Materials Research* 59 (3): 438–49.

Millner, R.W.J., Lockhart, A.S., Bird, H., and Alexiou, C. 2009. “A New Haemostatic Agent: Initial Life-Saving Experience With Celox (Chitosan) in Cardiothoracic Surgery.” *The Annals of Thoracic Surgery* 87 (2): e13–14.

Mohamed, N.A., and Abd El-GhanyN.A.. 2018. “Novel Aminohydrazide Cross-Linked Chitosan Filled with Multi-Walled Carbon Nanotubes as Antimicrobial Agents.” *International Journal of Biological Macromolecules* 115: 651–62.

Mohandas, A., Deepthi, S., Biswas, R., and Jayakumar, R. 2018. “Chitosan Based Metallic Nanocomposite Scaffolds as Antimicrobial Wound Dressings.” *Bioactive Materials* 3 (3): 267–77.

Muxika, A, Etxabide, A., Uranga, j., Guerrero, P., and De La Caba, K. 2017. “Chitosan as a Bioactive Polymer: Processing, Properties and Applications.” *International Journal of Biological Macromolecules* 105: 1358–68.

Nakamura, T., Yoshida, N., Yasoshima, M., and Kojima, Y. 2018. “Effect of Tannic Acid on

Skin Barrier Function." *Experimental Dermatology* 27 (8): 824–26.

Neuffer, M.C., Jonathan, M.D., Rose, D., King, K., Cloonan, C.C., and Vayer, J.S. 2004. "Haemostatic Dressings for the First Responder: A Review." *Military Medicine* 169 (9): 716–20.

Ninan, N., Muthiah M., Park, I.K., Wong, T.W., Thomas, S., and Grohens. Y., 2015. "Natural Polymer/Inorganic Material Based Hybrid Scaffolds for Skin Wound Healing." *Polymer Reviews* 55 (3): 453–90.

Okamoto, Y., Kawakami, K., Miyatake, K., Morimoto, M., Shigemasa, Y., and Minami, S. 2002. "Analgesic Effects of Chitin and Chitosan." *Carbohydrate Polymers* 49 (3): 249–52.

Ong, R.C., Mulvaney, S.W., Lutz, R.H., Surrett, G.W., Pennardt, A.M., and Wedmore, I.S. 2016. "Military Medicine." In *Tintinalli's Emergency Medicine: A Comprehensive Study Guide*, edited by Tintinalli J.E., Stapczynski J.S., Ma O.J.,Yealy D.M., Meckler G.D., and Cline D.M., 8th ed. New York, NY: McGraw-Hill Education.

Oryan, A., and Sahvieh, S. 2017. "Effectiveness of Chitosan Scaffold in Skin, Bone and Cartilage Healing." *International Journal of Biological Macromolecules* 104: 1003–11.

Ouerghemmi, S., Dimassi, S. Tabary, N., Leclercq, L., Degoutin, S., Chai, F., Pierlot, C., Cazaux, F., Ung, A., Staelens, J.N., Blanchemain, N., and Martel, B. 2018. "Synthesis and Characterization of Polyampholytic Aryl-Sulfonated Chitosans and Their in *Vitro*

Anticoagulant Activity." *Carbohydrate Polymers* 196 (September): 8–17.

Oyane, A., Kim, H.M., Furuya, T., Kokubo, T., Miyazaki, T., and Nakamura, T. 2003. "Preparation and Assessment of Revised Simulated Body Fluids." *Journal of Biomedical Materials Research* 65A (2): 188–95.

Palta, S., Saroa, R., and Palta, A. 2014. "Overview of the Coagulation System." *Indian Journal of Anaesthesia* 58 (5): 515.

Parker, W.H., and Wagner, W.H 2010. "Gynecologic Surgery and the Management of Hemorrhage." *Obstetrics and Gynecology Clinics of North America* 37 (3): 427–36.

Porteus, M., and Mantanona, T. 2018. "BLOOD." In *The Big Picture: Medical Biochemistry*, edited by Janson, L.W., and Tischler, M.E. New York, NY: McGraw-Hill Education.

Pozza, M., and Millner, R.W.J. 2011. "Celox (Chitosan) for Haemostasis in Massive Traumatic Bleeding." *European Journal of Emergency Medicine* 18 (1): 31–33.

Pusateri, A.E., McCarthy, S.J., Gregory, K.W., Harris, R.A., Cardenas, L., McManus, A.T., and Goodwin, C.W. 2003. "Effect of a Chitosan-Based Haemostatic Dressing on Blood Loss and Survival in a Model of Severe Venous Hemorrhage and Hepatic Injury in Swine." *The Journal of Trauma* 54 (1): 177–82.

Reddy, B. 2014. "Formulation and Evaluation of Fast Dissolving Oral Films of Zolmitiptan." *American Journal of Advanced Drug Delivery* 2 (April): 153–63.

Reddy, N., Reddy, R., and Jiang, Q. 2015. "Crosslinking Biopolymers for Biomedical

Applications." *Trends in Biotechnology* 33 (6): 362–69.

Rhee, P., Brown, C., Martin, M., Salim, A., Plurad, D., Green, D., Chambers, L., Demetriades, D., Velmahos, G., and Alam, H. 2008. "QuikClot Use in Trauma for Hemorrhage Control: Case Series of 103 Documented Uses." *The Journal of Trauma: Injury, Infection, and Critical Care* 64 (4): 1093–99.

Rinaudo, M. 2006. "Chitin and Chitosan: Properties and Applications." *Progress in Polymer Science* 31 (7): 603–32.

Rivero, S., García, M.A. and Pinotti, A. 2010. "Crosslinking Capacity of Tannic Acid in Plasticized Chitosan Films." *Carbohydrate Polymers* 82 (2): 270–76.

Rubentheren, V., Ward, T.A., Chee, C.Y., and Tang, C.K. 2015. "Processing and Analysis of Chitosan Nanocomposites Reinforced with Chitin Whiskers and Tannic Acid as a Crosslinker." *Carbohydrate Polymers* 115: 379–87.

Ruiz-Caro, R., and Veiga-Ochoa, M.D. 2009. "Characterization and Dissolution Study of Chitosan Freeze-Dried Systems for Drug Controlled Release." *Molecules* 14 (11): 4370–86.

Sagbas, S., Aktas, N., and Sahiner, N. 2015. "Modified Biofunctional p(Tannic Acid) Microgels and Their Antimicrobial Activity." *Applied Surface Science* 354: 306–13.

Sahiner, N., Sagbas, S., and Aktas, N. 2016. "Preparation of Macro-, Micro-, and Nano-Sized Poly(Tannic Acid) Particles with Controllable Degradability and Multiple Biomedical Uses." *Polymer Degradation and Stability* 129: 96–105.

Sahiner, N., Sagbas, S., Aktas, N., and Silan, C. 2016. “Inherently Antioxidant and Antimicrobial Tannic Acid Release from Poly(Tannic Acid) Nanoparticles with Controllable Degradability.” *Colloids and Surfaces B: Biointerfaces* 142: 334–43.

Sahiner, N., Sagbas, S., Sahiner, M., and Silan, C. 2017. “P(TA) Macro-, Micro-, NanoparticleEmbedded Super Porous p(HEMA) Cryogels as Wound Dressing Material.” *Materials Science and Engineering: C* 70: 317–26.

Sajomsang, W., Gonil, P., Saesoo, S., and Ovatlarnporn, C. 2012. “Antifungal Property of Quaternized Chitosan and Its Derivatives.” *International Journal of Biological Macromolecules* 50 (1): 263–69.

Santos, B.C., and Scalbert, A. 2000. “Proanthocyanidins and Tannin-like Compounds – Nature, Occurrence, Dietary Intake and Effects on Nutrition and Health.” *Journal of the Science of Food and Agriculture* 80 (7): 1094–1117.

Saral, S., Indumathi, and Rajarajeswari. 2019. “Mahua Oil-Based Polyurethane/Chitosan/Nano ZnO Composite Films for Biodegradable Food Packaging Applications.” *International Journal of Biological Macromolecules* 124: 163–74.

Schreiber, M.A., and Neveleff, D.J. 2011. “Achieving Haemostasis With Topical Haemostats: Making Clinically and Economically Appropriate Decisions in the Surgical and Trauma Settings.” *AORN Journal* 94 (5): S1–20.

Shariatinia, Z. 2018. “Carboxymethyl Chitosan: Properties and Biomedical Applications.” *International Journal of Biological Macromolecules* 120: 1406–19.

Sionkowska, A., Kaczmarek, B., and Lewandowska. K. 2014. “Modification of Collagen and

Chitosan Mixtures by the Addition of Tannic Acid." *Journal of Molecular Liquids* 199: 318–23.

Sivakumar, S.M. 2016. "Therapeutic Potential of Chitosan Nanoparticles as Antibiotic Delivery System: Challenges to Treat Multiple Drug Resistance." *Asian Journal of Pharmaceutics (AJP): Free Full Text Articles from Asian J Pharm* 10 (2).

Soleiman, P, and Kheiri, F. 2018. "The Effect of Different Levels of Tannic Acid on Some Performance Traits in Holstein Dairy Calves." *Iranian Journal of Applied Animal Science* 8 (1): 19–23.

Sundaram, C.P., and Keenan, A.C. 2010. "Evolution of Haemostatic Agents in Surgical Practice."

Indian Journal of Urology : IJU : Journal of the Urological Society of India 26 (3): 374–78.

Talón, E., Trifkovic, K.T., Nedovic, V.A., Bugarski, B.M., Vargas, M., Chiralt, A., and González-Martínez, C. 2017. "Antioxidant Edible Films Based on Chitosan and Starch Containing Polyphenols from Thyme Extracts." *Carbohydrate Polymers* 157 (2): 1153–61.

Talón, E., Trifkovic, K.T., Vargas, M. Chiralt, A., and González-Martínez, C. 2017. "Release of Polyphenols from Starch-Chitosan Based Films Containing Thyme Extract." *Carbohydrate Polymers* 175 (11): 122–30.

Thornton, P., and Douglas, J. 2010. "Coagulation in Pregnancy." *Best Practice & Research Clinical Obstetrics & Gynaecology* 24 (3): 339–52.

Tomiki, Y., Ono, S., Aoki, J., Takahashi, R, Ishiyama, S., Sugimoto, K., Yaginuma, Y.,

Kojima,Y., Goto, M., Okuzawa, A., and Sakamoto, K. 2015. “Treatment of Internal Hemorrhoids by Endoscopic Sclerotherapy with Aluminum Potassium Sulfate and Tannic Acid.” *Diagnostic & Therapeutic Endoscopy* 2015 (4): 1–7.

Trifković, K., Milašinović, N., Djordjević, V., Zdunić, G., Krušić, M.K., Knežević-Jugović, Z., Šavikin, K., Nedović, V., and Bugarski, B. 2015. “Chitosan Crosslinked Microparticles with Encapsulated Polyphenols: Water Sorption and Release Properties.” *Journal of Biomaterials Applications* 30 (5): 618–31.

Weir, D., and Brindle, C.T. 2015. “Wound Dressings.” In *Text and Atlas of Wound Diagnosis and Treatment*, edited by Rose L Hamm. New York, NY: McGraw-Hill Education.

Xu, F., Weng, B., Gilkerson, R., Materon, L.A., and Lozano, K. 2015. “Development of Tannic Acid/Chitosan/Pullulan Composite Nanofibers from Aqueous Solution for Potential Applications as Wound Dressing.” *Carbohydrate Polymers* 115 (1): 16–24.

Yan, D., Hu, S., Zhou, Z., Shah, Z., Cheng, F., Li, Y., Feng, C., Cheng, X., and Chen, X. 2018. “Different Chemical Groups Modification on the Surface of Chitosan Nonwoven Dressing and the Haemostatic Properties.” *International Journal of Biological Macromolecules* 107 (2): 463–69.

Zboralske, F.F., Phillip A. Harris, Riegelman, S., Rambo, O.N., and Margulis, A.R. 1966. “Toxicity studies on tannic acid administered by enema.” *American Journal of Roentgenology* 96 (2): 505–9.

Zhang, J., Chen, D., Han, D.M., Cheng, Y.H., Dai, C., Wu, X.J., Che, F.Y., and Heng, X.Y. 2018. "Tannic Acid Mediated Induction of Apoptosis in Human Glioma Hs 683 Cells." *Oncology Letters*, March 15(5):6845-6850.

Zhang, Y., Gao B., and Liu, X. 2015. "Topical and Effective Haemostatic Medicines in the Battlefield." *International Journal of Clinical and Experimental Medicine* 8 (1): 10–19.

Zhao, X., Wu, H., Guo, B., Dong, R., Qiu, Y., and Ma, P.X. 2017. "Antibacterial Anti-Oxidant Electroactive Injectable Hydrogel as Self-Healing Wound Dressing with Haemostasis and Adhesiveness for Cutaneous Wound Healing." *Biomaterials* 122: 34–47.

Zielińska, D., Struszczyk, M., Madej-Kiełbik, L., Chmal-Fudali, E., Kucharska, M., Wiśniewska-Wrona, M., and Brzoza-Malczewska, K. 2017. "Design of New-Generation Usable Forms of Topical Haemostatic Agents Containing Chitosan." *Molecules* 22 (12): 1–17.

Appendix

Appendix 1 Approval of title.

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

07 August 2018
Person No: 1562286
PAG

Mrs AA Hafeji
3 Yar Investment Building Commercial Center Road
Azaadville
Mogale City
Mogale city
1739
South Africa

Dear Mrs Hafeji

Master of Science in Medicine: Approval of Title

We have pleasure in advising that your proposal entitled has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix

Appendix 2 WITS HREC approval certificate for the study.

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Mrs Aysha Hafeji

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180565

NAME: (Principal Investigator)	Mrs Aysha Hafeji
DEPARTMENT:	Health Science Department of Pharmacy and Pharmacology Laboratories
PROJECT TITLE:	Formulation of a haemostatic gauze strip for minor wounds and abrasions
DATE CONSIDERED:	25/05/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof M Danckwerts
APPROVED BY:	Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	06/08/2018

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 Research Office Secretary on the Third Floor, Faculty of Health Sciences, Philip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. 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**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **May** and will therefore be due in the month of **May** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 13th Aug 2018

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix
Appendix 3 Turnitin report.

Researchreport.A.Hafeji

ORIGINALITY REPORT

9%	6%	3%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.otal.umd.edu Internet Source	1%
2	unix.eng.ua.edu Internet Source	1%
3	Submitted to University of Auckland Student Paper	1%

Appendix

Submitted to Ventura County Community <1

4 %

College District

Student Paper

<1%

5 Machul, A., D. Miko ajczyk, A. Regiel-Futyra, P. B. Heczko, M. Strus, M. Arruebo, G. Stochel, and A. Kyzio . "Study on inhibitory activity of chitosan-based materials against biofilm producing Pseudomonas aeruginosa strains", Journal of Biomaterials Applications, 2015.
Publication

6 Submitted to Universiti Malaysia Perlis
Student Paper <1%

7 Submitted to De La Salle University - Manila
Student Paper <1%

8 citeseerx.ist.psu.edu
Internet Source <1%

9	Submitted to Napier University Student Paper	<1%
		<1%
10	Lan, Guangqian, Bitao Lu, Tianyou Wang, Lijuan Wang, Jinghao Chen, Kun Yu, Jiawei Liu, Fangying Dai, and Dayang Wu. "Chitosan/gelatin composite sponge is an absorbable surgical haemostatic agent", Colloids and Surfaces B Biointerfaces, 2015. Publication	
11	Submitted to University of Alabama at Birmingham Student Paper	<1%
12	aolp.osa.org Internet Source	<1%
		<1%

Bibaswan Mishra, Jagannath Sahoo, Prasanna Kumar Dixit. "Formulation and process optimization of naproxen nanosuspensions stabilized by hydroxy propyl methyl cellulose", Carbohydrate Polymers, 2015 13
Publication

Submitted to Cranfield University
14 <1%
Student Paper

---

qualitypioneers.ir
15 <1%
Internet Source

---

www.lib.utexas.edu
16 <1%
Internet Source

Submitted to The Robert Gordon University 17 <1%
Student Paper

Submitted to De Montfort University
18 <1%
Student Paper

---

www.mwsales.net

19	Internet Source	<1%
20	Submitted to Taylor's Education Group Student Paper	<1%
21	www.salisbury.edu Internet Source	<1%
22	library.binus.ac.id Internet Source	<1%
23	radkatie.com Internet Source	<1%
24	New Library World, Volume 113, Issue 7-8 (2012-07-07) Publication	<1%

	digitalcommons.brockport.edu 25 Internet Source	<1%
	Submitted to University of Cape Town 26 Student Paper	<1%
	Submitted to Chulalongkorn University 27 Student Paper	<1%
		<1%
	A. K. L. Reyners. "Re-evaluation of the cold 28 face test in humans", European Journal of Applied Physiology, 08/15/2000 Publication	
	Submitted to Universiti Teknologi Malaysia 29 Student Paper	<1%

<1%

30 Masoud Ghorbani, Jafar Ai, Mohammad Reza Nourani, Mahmoud Azami, Batool Hashemi Beni, Shiva Asadpour, Sima Bordbar. "Injectable natural polymer compound for tissue engineering of intervertebral disc: In vitro study", Materials Science and Engineering: C, 2017

Publication

31 eprints.utm.my

<1%

Internet Source

32 www.ideals.illinois.edu

<1%

Internet Source

33 Bogyu Choi, Soyon Kim, Brian Lin, Benjamin M. Wu, Min Lee. "Cartilaginous Extracellular Matrix-Modified Chitosan Hydrogels for Cartilage Tissue Engineering", ACS Applied Materials & Interfaces, 2014

<1%

Publication

34 www.iatdmct2011.de

Internet Source

<1%

<1%

35 Vemula Sateesh Kumar. "Formulation and Evaluation of Transdermal Films of Ondansetron Hydrochloride", MOJ Bioequivalence & Bioavailability, 2017

Publication

36 krishikosh.egranth.ac.in

Internet Source

<1%

37

<1%

Nunzio Denora, Angela Lopedota, Mara Perrone, Valentino Laquintana et al. "Spraydried mucoadhesives for intravesical drug delivery using N -acetylcysteine- and glutathione-glycol chitosan conjugates", Acta Biomaterialia, 2016

Publication

<1%

"EAPS Congress 2016", European Journal of Pediatrics, 2016 38

Publication

scholar.sun.ac.za

39

<1%

Internet Source

Kai Zhang, Jing Su, Xiong Xiong, Xiang Wu,

40 Chenxi Wu, Jiantong Liu. "Microplastic pollution

<1% of lakeshore sediments from remote lakes in

Tibet plateau, China", Environmental Pollution, 2016

Publication

tel.archives-ouvertes.fr 41

<1%

Internet Source

42	etd.lsu.edu Internet Source	<1%
43	dspace.lboro.ac.uk Internet Source	<1%
44	Thi Thi, Junji Sugiyama, Vincent Bulone. "Chapter 10 Bacterial Cellulose-based Biomimetic Composites", InTech, 2010 Publication	<1%
45	etheses.whiterose.ac.uk Internet Source	<1%

46	waset.org Internet Source	<1%
47	Alexander Marin. "Carboxymethylcellulose-Chitosan-coated microneedles with modulated hydration properties", Journal of Applied Polymer Science, 07/05/2011 Publication	<1%
48	Helena Yli-Urpo. "Release of Silica, Calcium, Phosphorus, and Fluoride from Glass Ionomer Cement Containing Bioactive Glass", Journal of Biomaterials Applications, 07/01/2004 Publication	<1%

49 www.measurement.gov.au

Internet Source

<1%

<1%

50 Sara Pourshahrestani, Ehsan Zeimaran, Nahrizul Adib Kadri, Nicola Gargiulo et al. "Potency and Cytotoxicity of a Novel Gallium-Containing Mesoporous Bioactive Glass/Chitosan Composite Scaffold as Haemostatic Agents", ACS Applied Materials & Interfaces, 2017

Publication

<1%

51 Xiu-Ying Xu, Yong Cao, Hao Zhang, Sanabil, Ming-Zhu Zheng, Yu-Zhu Wu, Cheng-Bin Zhao, Jing-Sheng Liu. "Effects of corn starch on the gel properties of black bean protein isolate", Journal of Texture Studies, 2018

Publication

<1%

"Scientific Abstracts", Reproductive Sciences, 52 2012

Publication

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		