Journal of Pharmaceutical Research International

32(14): 109-119, 2020; Article no.JPRI.59897
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Formulation of a Topical Tannic Acid and Chitosan Gel Haemostatic Drug Delivery System for Treatment of Wounds and Abrasions

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Authors' contributions

This work was carried out in collaboration between both authors. Author MPD designed the study and performed the statistical analysis. Author AH wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. Authors MPD and AH managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i1430611
Editor(s):
(1) Dr. Prem K. Ramasamy, Brandeis University, USA.
Reviewers:
(1) Sasikanth Challari, Kannur University, India.
(2) M. Ramesh, Kalaignarkarunanidhi Institute of Technology, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/59897

Original Research Article

Received 28 May 2020
Accepted 05 August 2020
Published 14 August 2020

ABSTRACT

Haemostatic agents accelerate blood clotting and help in wound healing processes for wounds or abrasions. The purpose of this study was to prepare and evaluate a haemostatic gauze impregnated with chitosan and tannic acid as two effective 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 50 mm x 50 mm 8 ply gauze strip and were prepared and evaluated for dissolution into simulated body fluid (SBF) and finally tested for *in vitro* blood clotting ability versus Quikclot© (a commercially imported haemostatic gauze).
There was an initial quick release of tannic acid from the haemostatic gauze strips within 60 secs and thereafter a constant release of tannic acid. The *in vitro* blood clotting ability was found to be better in all the formulated haemostatic gauze strips than that of Quikclot©. Best clotting extent was achieved from 0.5% $^{w}/_{v}$ chitosan and 1% $^{w}/_{v}$ tannic acid gauze strips at 180 secs. In conclusion, the combination of tannic acid and chitosan haemostatic gauze strips developed in this study presents an inexpensive and effective alternative to importing haemostatic gauzes and bandages.

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Keywords: Haemostatic; tannic acid; chitosan; dissolution; bleeding; drug delivery.

1. INTRODUCTION

A haemostatic agent could be a life-saving step to control accidental bleeding in areas where a hospital is not nearby. If used immediately, in a pre-hospital setting, patients will have sufficient time to be stabilized prior to treatment [1]. Minor bleeding from wounds or abrasions also present a day-today problem for patients on anticoagulant therapy. Bleeding times of up to 20 minutes or longer can be observed from a simple surgical wound from a patient on anticoagulant therapy [2].

The quickest and easiest method to stop topical bleeding is the application of direct pressure to the bleeding site [3]. 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 [4]. 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.

Coagulopathy, infection, hypothermia and organ failure are possible complications developing from acute blood loss [5]. Therefore, quick intervention in hemorrhagic control will result in better patient outcomes and a decreased demand for blood products [6]. Thus, topical haemostatic agents can play a major role in the emergency control of hemorrhage and could reduce the associated morbidity and mortality in life-threatening situations [7].

A large number of haemostatic agents have been used to formulate different drug delivery systems. Some examples include Quikclot© clotting sponge which contains kaolin. Advanced Clotting Sponge® which contains zeolite. Celox© haemostatic bandage and gauze which contains chitosan. BloodSTOP® patches which are made up of the natural fibers of 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.

In the biomaterials field, chitosan is a versatile material in the design of a wide range of applications including wound dressings. 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 [8]. This cationic nature provides chitosan the ability to help cell growth, accelerate the blood coagulation and increase the efficacy of chitosan dressings [9,10].

Chitosan based Celox© achieved successful haemostasis in combat gunshot causalities in Afghanistan by all patients in the field [11]. Celox©, achieved haemostasis in 18 of 21 wounds within a minute. The remaining haemostasis required additional application of Celox©, however in the end haemostasis was achieved completely [11].

Tannic acid is also an effective haemostatic agent that has been extensively used [12-14]. In addition, tannic acid has antibacterial properties effective against *methicillin resistant Staphylococcus aureus* [15], inhibits Hepatitis C virus [16] as well as antimitotic and anti-inflammatory properties [17].

Tannic acid as a crosslinking agent for chitosan, has been used to prepare wound dressings. A wound dressing which has an antimicrobial application, has been prepared by crosslinking chitosan, pullulan and tannic acid [18]. Huang et al. [19] formulated a novel haemostatic dressing of porous chitosan sponge coated with self-assembled (thrombin/tannic acid) films, which were based on hydrogen bonding interactions between thrombin and tannic acid at physiologic pH. Their results indicate that the chitosan-based sponge is a promising candidate dressing for uncontrolled hemorrhage due to its storable, bio-safety and highly effective haemostatic properties. However, the addition of the self-assembled thrombin and tannic acid films added a complicated step in the production of the chitosan sponge. It also has a short shelf life (66.9 days) due to the addition of thrombin.

Therefore, the purpose of this research was to prepare a simple gauze strip that is impregnated with tannic acid in a chitosan gel. The process of impregnating a gauze strip with chitosan gel and tannic acid is simple and cost-effective. The

soluble tannic acid is incorporated into a chitosan gel and simply poured onto a strip of gauze which is then lyophilized so that it effectively adsorbs blood plasma and enhances clot formation for minor wounds and abrasions. Addition of tannic acid to the chitosan matrix also leads to a more rigid structure, which increases the tensile strength of the film [20-22].

2. MATERIALS AND METHODS

2.1 Materials

Tannic acid (TA) and medium molecular mass (448877 g/mol) chitosan (CS) were purchased from Sigma-Aldrich, South Africa. Standard 8 ply 50 mm×50 mm gauze (DisChem brand) strips were purchased from a local 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 supplied.

2.2 Methods

2.2.1 Formulation of CS and TA gauze strips

Chitosan (CS) gels containing 0.5% $^{w}/_{v}$, 1.0% $^{w}/_{v}$, and 1.5%$^{w}/_{v}$ were prepared by means of slowly adding the CS powder to 100 ml 0.1 M acetic acid stirred at 200 rpm with an overhead stirrer (Heildolph instruments, Germany) in a water bath (G.F.L.,Germany) set at 50°C until dissolved. Thereafter, 1% $^{w}/_{v}$ 2% $^{w}/_{v}$ and 5% $^{w}/_{v}$ tannic acid (TA) was added to each of the gels to produce the nine different formulations as shown in Table 1. 1.5% $^{w}/_{v}$ chitosan gel with 5% $^{w}/_{v}$ tannic acid where the upper limits to a gel that was still pourable. To increase the plasticity of the gels, 1% $^{v}/_{v}$ glycerol was added to each of the formulations. Five ml of each gel formulation was syringed 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 overnight at a -75°C (Sanyo VIP™ Series, USA). After freezing, the gauze strips were lyophilized in a freeze dryer (FreeZone® 2.5, Labconco®, USA) at -64°C temperature and 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 at ambient temperature until testing.

2.2.2 Assay method for TA

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

2.2.3 Preparation of Simulated Body Fluid (SBF)

SBF was used to measure the TA release and dissolution from the haemostatic gauze formulations. SBF has an ionic concentration similar to that of human blood plasma at pH 7.4.

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	1C1TA
5	1.0	2.0	1.0	1C2TA
6	1.0	5.0	1.0	1C5TA
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

The SBF was buffered with Tris buffer and 1M hydrochloric acid and maintained at 36.5°C. The procedure to prepare SBF was adapted from the method used by Oyane et al. [23] and Kokubo and Takadama [24].

2.2.4 Preparation of TA calibration curve

A 1% $^{w}/_{v}$ TA solution was prepared by dissolving 0.5 g of TA in to 50 ml SBF. This solution was then diluted (5 ml in 50 ml 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%, and 0.000325% $^{w}/_{v}$ TA. The % $^{w}/_{v}$ in solution during the dissolution test was then calculated from the regression equation obtained for the standard curve.

2.2.5 Dissolution of TA

To determine the dissolution rate and release of TA from the haemostatic gauze strips, a Franz diffusion cell (Franz diffusion cell, United Scientific Pty ltd, RSA) was used. Haemostatic gauze strips from each of the formulations (Table 1) were cut into 1 cm^2 squares and then clamped into the Franz diffusion cell (impregnated freeze dried gel side facing down towards the SBF) containing 20 ml SBF and maintained at 36.5°C with continuous magnetic stirring. After predetermined time intervals (60, 120, 180 and 300 secs) 2 ml SBF was removed by a syringe fitted with a 22 µm filter, and then analyzed for TA content. 2 ml SBF (preheated to 36.5°C) was replaced into the diffusion cell to maintain the same total volume. The values were then corrected for a 10% $^{v}/_{v}$ dilution after each sample removal.

2.2.6 *In-vitro* blood clotting ability

When whole blood is exposed to distilled water, water is taken into red blood cells (RBC) due to the osmotic pressure differences between water and RBC and consequently the RBCs burst and haemoglobin is released. Blood that is adsorbed onto the haemostatic gauze is thus not available for hemolysis of cells [25].

Haemostatic gauze strips from each of the nine formulations (n=6) were cut into 1 cm^2 squares. The haemostatic gauze squares were then placed into clean 50 ml glass beakers preheated to 37.5°C. Then, 0.25 ml whole drug free human blood from the same patient for each batch (at 37.5°C) was pippeted onto the surface of the various gauze strips. The blood from the same patient was used for each of the formulations and the blank so as to circumvent any difference in patient's INR and clotting ability. The haemostatic gauze strips were then left to stand for 60, 120 and 180 secs. After allowing the blood soaked haemostatic gauze strips to stand for 60, 120 and 180 secs, 20 mL preheated (37.5°C) double distilled water was gently dripped down the inside edge of the beaker to prevent disruption of the clotted blood (Fig. 3). RBCs which are not entrapped by the haemostatic gauze are hemolyzed in the doubled distilled water and release hemoglobin. Once the doubled distilled water was added to the blood soaked haemostatic gauze strips, 2 ml samples were gently withdrawn from the solution (approximately 2 cm away from the gauze strip) and the UV absorbance of the hemoglobin in solution was determined at 540 nm (wavelength of maximum absorbance of hemoglobin) using a UV-VIS spectrophotometer (Lambda 25, Perkin Elmer, USA) [26]. 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 1.

$$\text{Clotting extent} = \text{Ab Hem blank} - \text{Ab Hem gauze} \quad (1)$$

Where, Ab Hem blank is the absorbance at 540 nm of hemoglobin in the blank solution, and
Ab Hem gauze is the absorbance at 540 nm of the hemoglobin 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. RESULTS

3.1 Dissolution of TA% $^{w}/_{v}$ from the Haemostatic Gauze Formulations in SBF

From the graphs (Fig. 1 a,b,c) 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 the initial 60 secs.

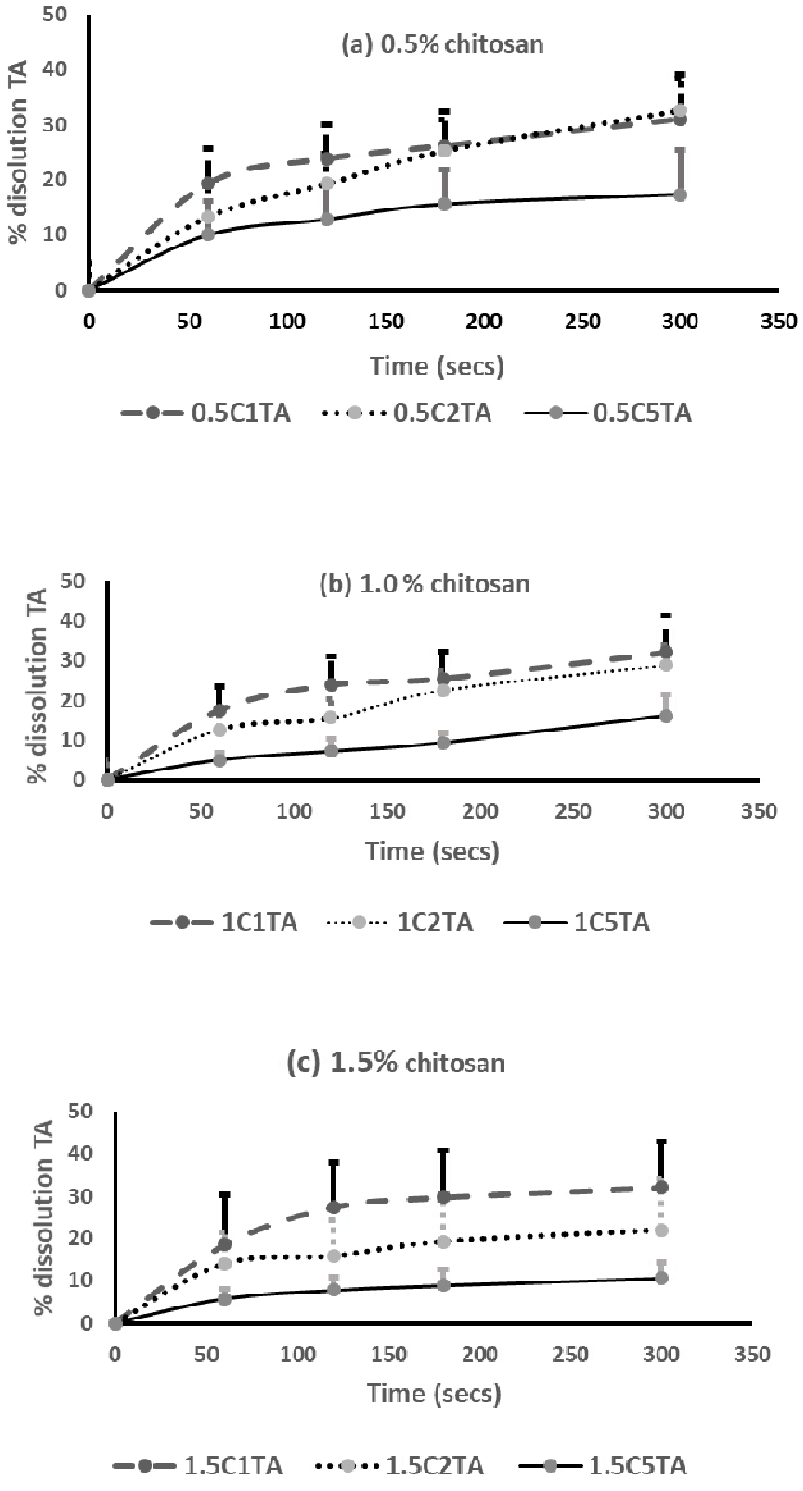

Fig. 1. Effect of (a) 0.5% (b) 1.0% and (c) 1.5% $^{w}/_{v}$ CS on dissolution of TA from haemostatic gauze formulations (n=6). Values are mean ± s.d.

When comparing the amount of TA in solution at 300 secs, as the % $^{w}/_{v}$ TA increased from 1 to 5% in the formulation, the % $^{w}/_{v}$ TA in solution at 300 secs decreased. This effect is probably due to the crosslinking of CS and TA. As the amount of TA in the formulation increased, the greater was the extent of crosslinking of the CS and the more it was held back in the gel. It was also found that as the % $^{w}/_{v}$ of CS increased from 0.5 to 1.5% $^{w}/_{v}$, the % TA in solution at 300 secs remained relatively constant (Fig. 1).

The highest % TA dissolution at 300 secs, was for formulation 0.5C2TA (32.63%) while the lowest % TA dissolution at 300 secs, was for formulation 1.5C5TA (10.57%) (Fig. 2).

The 3D plot the effect of TA and CS shown in figure 2, indicates that there is no significant differences (p= 0.0719) in the amounts of TA in solution when the % $^{w}/_{v}$ of CS was increased from 0.5% to 1.5% in the different haemostatic gauze formulations. However, a significant difference (p =0.0379) was found as the % $^{w}/_{v}$ TA in the haemostatic gauze formulations changed from 1% to 5%, on the dissolution of TA at 300 secs. The % $^{w}/_{v}$ TA in solution at 300 secs increased as the % $^{w}/_{v}$ TA in the gauze strip increased from 1.0% to 5.0% $^{w}/_{v}$. This is could be due to the crosslinking effect of TA on the CS gel.

3.2 Blood Clotting Ability of the Haemostatic Gauze Formulations

A few pertinent photographs of the clotting ability test are shown in Fig. 3.

The results of the clotting ability of the various haemostatic gauze formulations as well as that for Quikclot© are presented in Table 2. 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 secs, formulation 0.5C1TA had the highest extent of clotting (1.3648 extent/sec). The rate of clotting and extent of clotting at 180 secs for all of the haemostatic formulations were greater than that of Quikclot© (0.0021 extent/sec and 0.6855 extent, respectively).

From Fig. 4 it can be seen that as CS% $^{w}/_{v}$ in each formulation increased from 0.5 to 1.5% $^{w}/_{v}$ the clotting extent at 180 secs decreased (p=0.0227) , and as TA% $^{w}/_{v}$ in each formulation increased from 1 to 5% $^{w}/_{v}$, the clotting extent at 180 secs decreased (p=0.0002).

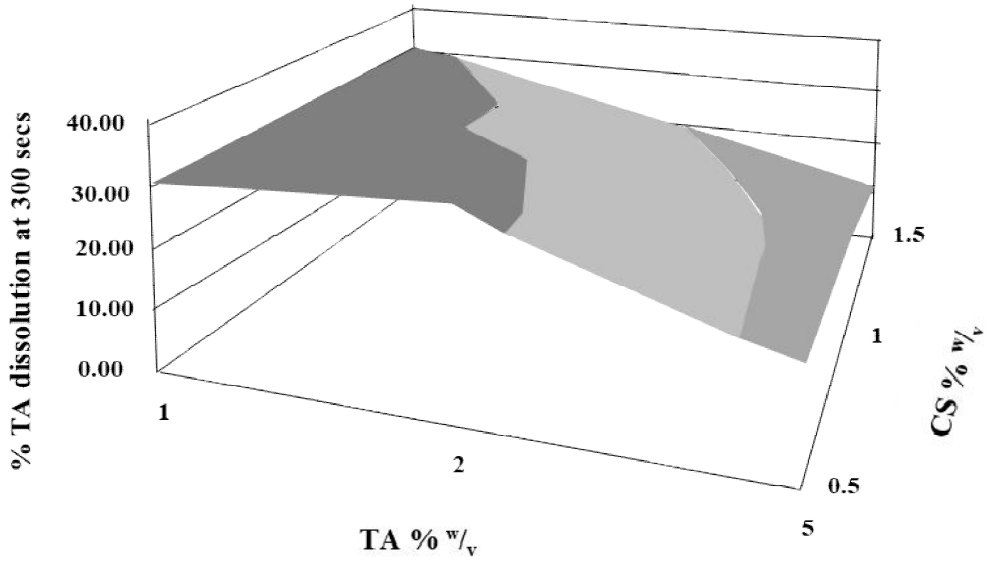

Fig. 2. 3D plot of the effect of TA and CS on the dissolution of TA % $^{w}/_{v}$ from haemostatic gauze formulations at 300 sec

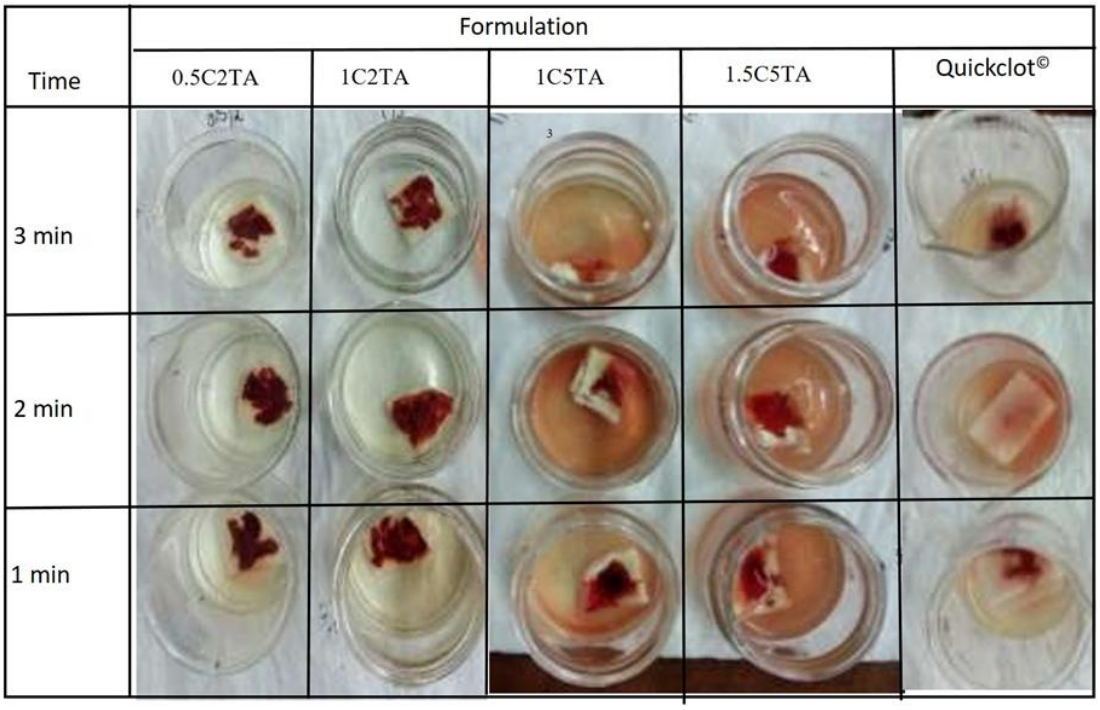

Fig. 3. 0.5C2TA % $^{w}/_{v}$, 1C2TA % $^{w}/_{v}$, 1C5TA % $^{w}/_{v}$, 1.5C5TA % $^{w}/_{v}$ and Quikclot© clotting ability at 1, 2 and 3 minutes

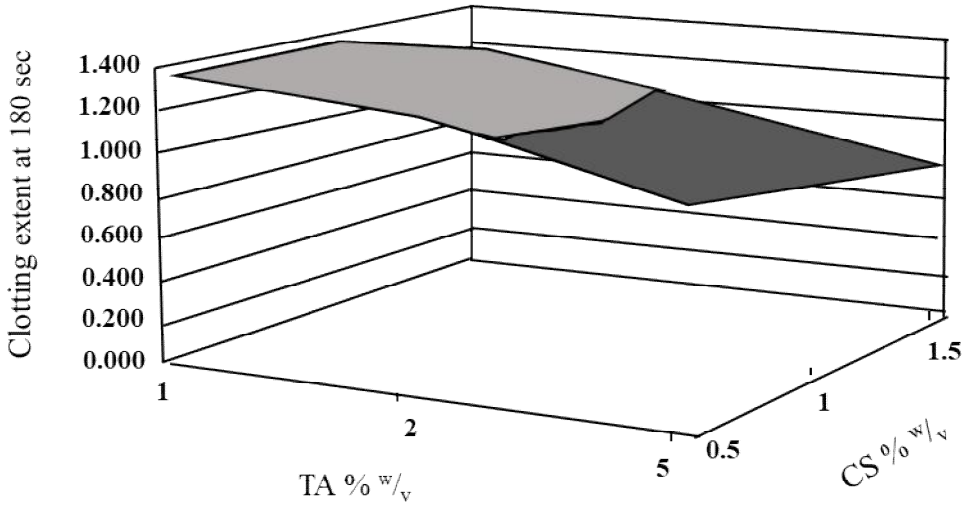

Fig. 4. 3D plot of effect of CS % $^{w}/_{v}$ and TA % $^{w}/_{v}$ at 180 sec on blood clotting ability of haemostatic gauze formulations

As shown in Table 2 the gauze patches formulated in this research showed a higher clotting extent value compared to the reference sample Quikclot© rolled gauze. The clotting extent value of the patches increased over time (60 to 180 secs) when incubated with human whole blood. The results show that absorbance of haemoglobin decreased and clotting extent increased as time increased. Excellent blood clotting was found for 0.5C1TA and 1C1TA formulations (Table 2).

Table 2. Clotting rate of each haemostatic gauze formulation and Quikclot©.

Formulation	Mean extent of clotting[1] ± s.d. (n=6)			slope (secs-1)	Pearson (r)
	60 secs	120 secs	180 secs		
0.5C1TA	0.9972 ± 0.4292	1.2083 ± 0.3724	1.3648 ± 0.3141	*0.0031*	*0.9963*
0.5C2TA	0.7283 ± 0.4897	1.0773 ± 0.4035	1.2660 ± 0.2760	0.0045	0.9855
0.5C5TA	0.3360 ± 0.3176	0.5797 ± 0.4527	1.0053 ± 0.3475	0.0056	0.9879
1C1TA	0.9668 ± 0.3829	1.1257 ± 0.2944	1.3572 ± 0.1572	0.0033	0.9943
1C2TA	0.9110 ± 0.2844	1.2172 ± 0.1682	1.3220 ± 0.1319	0.0034	0.9623
1C5TA	0.3748 ± 0.2388	0.5808 ± 0.2640	0.9045 ± 0.1640	0.0044	0.9918
1.5C1TA	0.8018 ± 0.0546	0.9990 ± 0.1379	1.1673 ± 0.0955	0.0030	0.9990
1.5C2TA	0.5970 ± 0.4278	0.7633 ± 0.4595	0.9363 ± 0.3162	0.0028	0.9999
1.5C5TA	0.3752 ± 0.2958	0.6118 ± 0.2248	0.7593 ± 0.1553	0.0032	0.9911
Quickclot©	0.4290 ± 0.3013	0.5803 ± 0.3404	0.6855 ± 0.3786	0.0021	0.9946

[1] *calculated from equation 2*

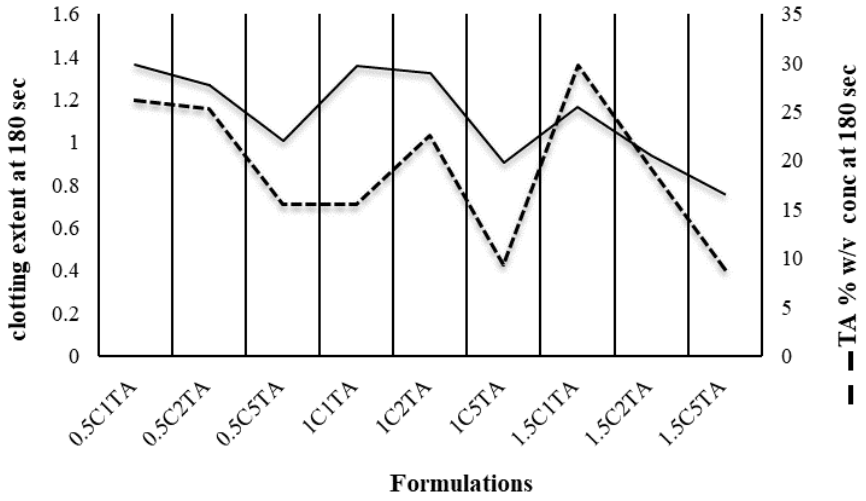

Fig. 5. Correlation between % $^{w}/_{v}$ TA dissolution and clotting extent at 180 seconds

The extent of clotting results followed those of the dissolution results, with the clotting extent decreasing as the TA% $^{w}/_{v}$ in the formulations increased (Fig. 5). Obviously, the TA needs to be released before it can clot blood.

4. CONCLUSION

The main aim of this research was to synthesize and evaluate anew 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©.

In-vitro TA dissolution data analysis show that the dissolution from the impregnated gauze varied significantly as the CS-TA ratio changed. However, only TA% $^{w}/_{v}$ demonstrated a significant difference. The results indicate that a 0.5C2TA, 1C1TA 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, the best clotting rate resulted from the 1C2TA formulation. The 1.5C5TA formulation was found to have the lowest clotting extent within the CS-TA formulations tested. The results also provide evidence that formulations in this research are more effective at blood clotting *in-vitro* when compared to the Quikclot© commercial gauze. Furthermore, blood clotting extent increases within a few minutes when exposed the haemostatic gauze formulation.

5. RECOMMENDATIONS

Further work to validate the large-scale production of TA and CS gauze patches is recommended. Once the large-scale production is ratified, *in-vivo* work needs to be done to confirm the *in-vitro* results obtained in this study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Whole blood was collected from healthy drug free volunteers and ethical approval was provided by the University of the Witwatersrand Human Research Ethics Committee (Clearance number M180565).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zhang Y, Gao B, Liu X. Topical and effective haemostatic medicines in the battlefield. Int J of Clin and Exp Med. 2015;8(1):10–19.
2. Hasan SS, Teh KM, Ahmed SI, et al. Quality of Life (QoL) and International Normalized Ratio (INR) control of patients attending anticoagulation clinics. Public Health. 2015;129(7):954–62.
3. Boulton AJ, Lewis CT, Naumann DN, et al. Prehospital haemostatic dressings for trauma: A systematic review. Emergency Medicine Journal. 2018;35:449-457.
4. Ong RC, Mulvaney SW, Lutz RH, et al. Military medicine. In Tintinalli's Emergency Medicine: A Comprehensive Study Guide, Edited by Tintinalli JE, Stapczynski JS, Ma OJ, Yealy DM, Meckler GD, and Cline DM, 8th Ed. New York, NY: McGraw-Hill Education; 2016.
5. Carraway JW, Kent D, Young K, et al. 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. 2008;78:230–35.
6. Peng HT. Hemostatic agents for prehospital hemorrhage control: A narrative review. Military Med Res. 2020;7(13). Available:https://doi.org/10.1186/s40779-020-00241-z
7. Khoshmohabat H, Paydar S, Kazemi HM, et al. Overview of agents used for emergency haemostasis. Trauma Monthly. 2016;21(1):E26023.
8. Khan M, Mujahid M. A review on recent advances in chitosan based composite for hemostatic dressings. International Journal of Biological Macromolecules. 2018;124(10). DOI: 1016/j.ijbiomac.2018.11.045
9. Dash M, Chiellini F, Ottenbrite RM, et al. Chitosan: A versatile semi-synthetic polymer in biomedical applications. Prog. Polymer Sci. 2011;36:981–10.
10. Archana D, Dutta J, Dutta PK. Evaluation of chitosan nano dressing for wound healing: Characterization, *in vitro* and *in vivo* studies. Int. J. Biol. Macro. 2013;57: 193–203.
11. Pozza M, Millner RWJ. Celox (Chitosan) for haemostasis in massive traumatic bleeding. Eur. J. of Emerg. Med. 2011;18(1):31–33.
12. Tomiki Y, Ono S, Aoki J, et al. Treatment of internal hemorrhoids by endoscopic sclerotherapy with aluminum potassium sulfate and tannic acid. Diag Therap End. 2015;4:1–7.
13. Huang X, Jia J, Wang Z, et al. A novel chitosan-based sponge coated with self-assembled thrombin/tannic acid multilayer films as a haemostatic dressing. Chinese J Poly Sci. 2015;33(2):284–90.
14. Kumar S. Local hemostatic agents in the management of bleeding in oral surgery. Asian J of Pharm Clin Res. 2016;9(3): 7.
15. Cipriano-Salazar M, Rojas-Hernández S, Olivares-Pérez J, et al. Antibacterial activities of tannic acid against isolated ruminal bacteria from sheep. Microbial Pathogenesis. 2018;117(4):255–58.
16. Liu L, Qi G, Lu X, et al. *In situ* forming hydrogels based on chitosan for drug delivery and tissue regeneration. Asian J Pharm Sci. 2016;11(6):673–83.
17. Booth BW, Inskeep BD, Shah H, et al. Tannic acid preferentially targets estrogen receptor-positive breast cancer. Int J Breast Can. 2013;1–9.
18. Xu F, Weng B, Gilkerson R, Materon LA, et al. Development of tannic acid/chitosan/pullulan composite nanofibers from aqueous solution for potential applications as wound dressing. Carbohydrate Polymers. 2015;115(1):16–24.
19. Huang X, Jia J, Wang Z, et al. A novel chitosan-based sponge coated with self-assembled thrombin/tannic acid multilayer films as a hemostatic dressing. Chin J Poly Sci. 2015;33(2):284-290.
20. Rivero S, García MA, Pinotti A. Crosslinking capacity of tannic acid in plasticized chitosan films. Carbohydrate Polymers. 2010;82(2):270–76.
21. Sionkowska A, Kaczmarek B, Lewandowska K. Modification of collagen and chitosan mixtures by the addition of tannic acid. J Mol Liq. 2014;199:318–23.
22. Rubentheren V, Ward TA, Chee CY, et al. Processing and analysis of chitosan nanocomposites reinforced with chitin whiskers and tannic acid as a crosslinker. Carbohydrate Polymers. 2015;115:379–87.
23. Oyane A, Kim HM, Furuya T, et al. Preparation and assessment of revised simulated body fluids. J Biomed Mat Res. 2003;65A(2):188–95.

24. Kokubo T, Takadama H. How useful is SBF in predicting *in vivo* bone bioactivity? Biomaterials. 2006;27(15): 2907–15.
25. Kang PL, Chang SJ, Manousakas I, et al. Development and assessment of haemostasis chitosan dressings. Carbohydrate Polymers. 2011;85(3):565–70.
26. Cassano R, Luisa M, Gioia S, et al. Gauze based on chitosan and hydroquinone: Preparation, characterization and blood coagulation evaluation. J Mat Sci. Mat in Med. 2017;28(12):190.

Peer-review history:
The peer review history for this paper can be accessed here:
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