Formulation, Characterization, and Optimization of a Topical Gel Containing Tranexamic Acid to Prevent Superficial Bleeding: *In Vivo* and *In Vitro* Evaluations

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ABSTRACT

Objectives: Tranexamic acid (TXA) is used systemically to stop bleeding, but it can lead to thromboembolism. Trials have revealed the efficacy of topical TXA on local hemorrhages. However, there is a need for an efficient delivery system that can keep the drug at the site of action.

Materials and Methods: To develop a gel containing TXA (3%) optimized in terms of viscosity and dispersibility, the central composite design based on two factors-three levels [carbopol 940 and hydroxypropyl methylcellulose (HPMC), 1-1.5% and 1-2%, respectively] was applied. The spreadability and viscosity were assessed using glass slide and rheometer, respectively. To confirm the compatibility of TXA with the gel, fourier transform-infrared (FTIR) spectroscopy was performed. Drug content uniformity was analyzed by a spectroscopy method. An *ex vivo* mice model using Franz cells was applied to evaluate the permeation of TXA through the skin. To investigate the effect of topical TXA gel on bleeding time, IVY human method was performed.

Results: HPMC/carbopol 940 (1:1, *w/w*) gel showed the highest quality in terms of viscosity and dispersibility (3.982 ± 17.6 and 6.052 ± 3.562, respectively). FTIR absorption spectrum showed that all the TXA index peaks appeared without displacement. The complete-encapsulated TXA content was uniformly dispersed throughout the gel. *In vitro* TXA cumulative release reached 90% in 4 h. The bleeding time determined *in vivo* for TXA gel was significantly lower than that for TXA solution and control.

Conclusion: The results confirm the importance of further studies on this formulation as a potential medication to stop acute superficial bleeding.

Key words: Tranexamic acid, hydroxypropyl methylcellulose, gel

INTRODUCTION

In recent years, due to the difficulty, significant costs, and slow pace of discovery and development of new active ingredients, a major part of pharmaceutical science has focused on repositioning or in other words repurposing Food and Drug Administration (FDA)-approved drugs for human use to treat diseases.[1] One type of repositioning is to replace systemically administered formulations with topical ones to cure local pathologic conditions. If this formulation can display acceptable effects on the site of action, while carrying fewer risks than systemic administration due to reduced systemic exposure to the drug, it could find a new position in the treatment schedule.[2]

Tranexamic acid (TXA) was one of these studied medicines. It has a structure similar to amino acid lysine and inhibits

bleeding by fibrinolysis inhibition mechanism. This drug is used systemically to stop various types of bleedings, but its systemic use can lead to risks such as thromboembolism and etc.[3] The topical anti-inflammatory and anti-melanin-producing properties of tranexamic acid have led it to be extensively considered and commercially available in the field of dermatology as an off-label topical treatment for rosacea, urticaria, and post-inflammatory hyperpigmentation. However, randomized controlled trials have shown the positive effects of TXA topical administration on local hemorrhages, including post-surgery or traumatic mucosal (e.g. epistaxis)[4] or cutaneous hemorrhages.[5,6]

In these studies, TXA solution is often used, and sterile gauze is impregnated by it and placed at the site of bleeding.[7] However, there is still a need for a safer and more efficient drug delivery system than the method used, which can keep the drug at the site of action for a certain period and help it to be better absorbed.

Recently, in some studies, nasal spray formulations containing TXA in the form of powder mixed with hyaluronic acid[8] or in situ gel forming chitosan[9] have been considered for epistaxis treatment.

Gels composed of polymers such as poly (acrylic acid) (Carbopol®) and cellulose derivatives are one of suitable bases for prolonged delivery of water-soluble drugs to the dermal and mucosal areas, and their effectiveness for this purpose has been proven in the previous studies.[10]

Therefore, in this study, using the response surface method (RSM), an ideal carbopol 940/hydroxypropyl methylcellulose (HPMC) gel formulation containing TXA 3% was developed in terms of appearance, spreadability, and acidity. Then the uniformity of drug loading, interactions with, and release from the gel base in ex vivo environment was evaluated. Finally, in vitro efficacy of the formulation was evaluated by examining bleeding time in healthy volunteers.

To the best of our knowledge, this is the first HPMC/carbopol-based gel formulation containing TXA developed for topical application as anticoagulant agent, while in vitro/in vivo assessments were performed.

MATERIALS AND METHODS

Materials
TXA, Carbopol® 940, HPMC, ninhydrin, and triethanolamine were purchased from Sigma Aldrich (Germany). Deionized distilled water was freshly prepared. In this study, all solvents and chemicals were of analytical grade.

Methods

Preparation of TXA containing carbopol 940/HPMC gel formulation
To achieve the topical drug-loaded gel formulation, an aqueous solution of carbopol 940 and HPMC with known concentration of each polymer (Table 1) was prepared by dissolving the polymers powder in 100 mL of deionized distilled water under 1100 rpm stirring for 2 hours at room temperature (RT) to completely dissolve and hydrate. A constant volume (0.23 mL) of triethanolamine aqueous solution was then slowly added, while stirring continued. Following the gel base formation, 3% (w/w) of TXA was added and the gel formulation was stirred at 200 rpm overnight to load the drug efficiently. Finally, the volume of final gel formulation was brought up to 100 mL by deionized distilled water. Prepared gel formulations were preserved from air and direct light in sealed amber glass containers and kept at 4 °C before further analysis.[11]

Optimization of the carbopol/HPMC gel formulation by central composite design (CCD)
To develop an optimized gel base in terms of viscosity and dispersibility, CCD-based on two factors-three levels using Design Expert® software (version: DX7 trial) was applied. Carbopol 940 and HPMC concentrations were considered as independent variables while keeping triethanolamine volume constant (0.23 mL). The influence of independent factors on Y1 (spreadability) and Y2 (viscosity) as dependent variables was evaluated by RSM. By analyzing the obtained data, the formulation showing ideal spreadability and viscosity was chosen for further evaluation.

Rheological evaluations

Spreadability
The investigation of spreadability potential of the prepared gels was performed on the basis of a published study[11] with some slight modifications.

In this method, 2 g of gel was placed on a standard glass slide in the center between two lines with a distance of 4 cm. Then, the second glass slide weighing 110 g was gently placed on the gel. The dispersion time was calculated from the moment the second glass slide was placed until the gel was completely dispersed between the two lines. The experiment was repeated three times, and the meantime was calculated. The following equation (Equation 1) was used to calculate the dispersibility:

$$S = m \times l/t \text{ (Equation 1)}$$

Where, S= spreadability, m= weight of the upper slide (110 g), l- the distance of two lines (4 cm), t- time is taken in sec.

Viscosity
Viscosity of the prepared gel formulations was measured using a rheometer (AMETEK Brookfield R/S plus, USA) using a CC3-14 spindle. While the sample holder was filled with the gel, the spindle was inserted into the sample and rotated at a speed of 1/min. Rheological evaluations were performed at RT (n: 3).[12]

pH evaluation
To ensure acidity of the gel bases place in the standard range of 4.5-5.5, 1 g of each gel was diluted in 100 mL of double distilled water, and pH of the prepared solution was assessed using a calibrated pHmeter at RT (827 PH Lab, Metrohm, Switzerland).

Visual inspection
To evaluate the relative apparent transparency, suspended particles, and uniformity of the gel structures as a common

method of gel base quality control, each gel base was visually inspected by the naked eye using an illuminated dark background.

To assess the physical stability of the gel after 6 months of preparation, all the above items and pH were re-examined in the final optimized gel. The gel was preserved in an opaque sealed bottle at ambient temperature.

Fourier transform-infrared spectroscopy (FTIR)

FTIR spectroscopy was performed to confirm the compatibility of the active ingredient with the gel base chemical structure. Carbopol/HPMC (1:1 *w/w*) gel (run 13), TXA 3%-containing HPMC/carbopol 940 (1:1 *w/w*) gel, and TXA powder were analyzed by FTIR spectroscopy (IRAffinity SHIMADZU, Japan) to clarify the molecular interactions. Each sample was prepared as individual KBr disk and was scanned in the range of 400-4000 cm^{-1}.

The TXA quantification method

To 1 mL of different dilutions of the drug solution (10-100 μg/mL), 1 mL of phosphate buffer (pH: 8) and 2 mL of the methanolic solution of 0.2% ninhydrin as the reagent were added (derivatization process). The samples were then heated with liquid paraffin oil at 90 °C for 20 min. After cooling to RT using 10 mL of double distilled water, the samples were brought to a volume of 10 mL. Finally, using an ultraviolet/visible spectrophotometer (CE1021, CECIL, England) the absorbance of the samples was read against a blank solution at 565 nm.[13]

Drug encapsulation efficiency (EE) of gel preparation

To measure the drug EE, 1 g of gel formulation was carefully weighed using deionized distilled water made up to 100 mL. Then, following the dilution up to 1:5 and filtration with a 0.45 μm filter, the derivatization process was performed according to standard samples (mentioned earlier) and its absorption was read against a blank at 565 nm (n: 3).[14] EE percentage was calculated using the following equation (Equation 2):

EE% = 100 × (detected drug content in gel)/ (primary drug content added into the gel (Equation 2)

TXA content uniformity of gel formulation

To check the uniformity of the content of the gel formulation, 72 hours after preparation, samples were taken from five different points of HPMC/carbopol 940 (1:1, *w/w*) gel and the amount of drug was determined according to the quantification method.

Ex vivo permeation study

An *ex vivo* animal model evaluating permeation of TXA through natural skin following topical application of the prepared gel formulation (run 13) using Franz cells was applied. All experiments were performed according to the Ethics Committee Acts (approval code: IR.ZBMU.REC.1397.085, date: 20.11.2018) of Zabol University of Medical Sciences, Iran and complied with the ARRIVE guidelines and in accordance with the guide for the care and use of laboratory animals proposed by the National Institutes of Health (NIH).

Skin preparation

To obtain a suitable skin to cover each Franze cell, the shaved abdominal BALB/c mice skin was excited under systemic ether-induced anesthesia. The subcutaneous appendixes were eliminated from the skin by soaking the dermal side in normal saline for 1 h.

Process of the release test

Each donor compartment was covered by 16 cm^2 skin for the dermal side faced the receiver compartment. This later compartment was filled with well-stirred 29 mL phosphate buffer pH 7.4 and the entire system was circulated by a 37 °C water jacket.

Drug-loaded gel formulation and respective blank gel bases were applied on the skins of separated cells, while control cell remained untreated. The donor compartment was sealed by parafilm during the process. The samples were taken from the receiver compartment at 0.5, 1, 2, 4, and 5 h and replaced with the fresh PBS, which had been maintained at 37 °C. Finally, the drug content of each sample was analyzed using the aforementioned spectrophotometric method and the release profile was determined in terms of the cumulative release percentage of TXA (n: 3).[15]

In vivo bleeding time assessment

The time taken for a standard small wound to heal is called bleeding time. To investigate the effect of topical TXA gel on bleeding time compared with TXA topical solution, a known *in vivo* assessment called IVY method was performed on 10 healthy men aged 25 to 45 years on the same health conditions. All experiments were performed according to the Ethics Committee Acts (approval code: IR.ZBMU.REC.1397.085) of Zabol University of Medical Sciences, Iran.

The intervention process

Control and treatment interventions were applied to the same volunteers at a sufficiently separated period. In the control group, no treatment was applied, while for the topical gel group, TXA 3%-containing HPMC/carbopol 940 (1:1 *w/w*) gel and for the solution group, a TXA 3% aqueous solution (a gas was soaked by and placed on the site of the action) was topically applied on the volar aspect of the arm.

IVY procedure

After 5 h of applying the topical interventions, a cuff was inflated on the upper arm to 40 mmHg. Three stab wounds (3 mm deep) were made using a sterile lancet on the volar aspect of the forearm. The blood was removed every 30 seconds by filter paper untill no blood residue remained on the filter paper. The times were recorded and the average time of the 3 incisions was reported. The statistical analysis was done to clarify the significance of treatment influence on reducing bleeding time.[16]

Statistical analysis

Data are presented as mean ± standard deviation. The statistical analysis was performed using Prism 6.0 software. Statistical significance was evaluated by one-way ANOVA followed by

Tukey-Kramer as a *post hoc*-test. A *p* value equal to or less than 0.05 was considered statistically significant. RSM was used to optimize the formulations *via* Design-Expert software (version: DX7Trial).

RESULTS AND DISCUSSION

Gel formulation design

Due to the increasing need for efficient drug formulations and the cost-effectiveness of changing and optimizing the formulations of drugs available in the pharmaceutical market instead of discovering new drugs, in the present study, a new topical gel formulation of TXA was developed.

To best of our knowledge, this is the first study on topical TXA gel formulation to prevent superficial dermal and mucosal bleeding. Topical TXA formulations have been previously developed to treat melasma.[17]

In a limited number of studies, nasal spray powder formulations containing TXA mixed with hyaluronic acid or *in situ* gel forming chitosan have been considered for epistaxis treatment. While they have shown promising *in vitro* results, extensive *in vivo* and human assessments are still lacking.[8,9]

Based on previous studies,[18] carbopol 940 (as the gelling agent) in combination with HPMC (as a viscosity enhancer) were considered to form the gel matrix. The formulations were prepared using various concentrations of carbopol 940 and HPMC.

The formulation was first designed using experimental design method. RSM based on CCD was used to evaluate and optimize the effect of carbopol 940 and HPMC concentrations as independent variables on viscosity and spreadability as response functions. According to the literature, the concentration ranges

of 1-1.5% and 1-2% were considered for carbopol 940 and HPMC, respectively. Experiments (13 in total) designed by the software are presented in Table 1.

In this study, 5 runs (runs 2, 3, 5, 6, and 12) were carried out as center points. The experiment runs, in which repetitions in the independent parameters occurred, are center points. The values of each factor are the medians of the values used in the factorial portion. These points are replicated to improve the precision of the experiment.[11]

Rheological characterizations

Due to the importance of viscosity of topical pharmaceutical products in their ease of use and patient compliance (difficulty of handling, application, and delayed drug release in case of high viscosity and quick removal from the application site in case of low viscosity) the viscosity of the gel should be within the appropriate range. In previous studies, viscosities lower than 4000 centipoises (Cps) have been considered suitable for topical gel products.[19] As can be seen in Table 1, only the viscosities of runs 8 and 13 showed viscosities lower than the upper limit, while they were not too low.

According to the three-dimensional plot obtained from the data analysis (Figure 1) by Design-Expert software, viscosity change is proportional to the concentration of polymers used, which has also been reported in previous studies.[19,20]

A similar pattern can be seen between the dispersibility factor of gels and the concentration of polymers used in their matrix (Figure 2), which is consistent with previous studies.[21]

In the dispersibility calculation, weight and distance parameters are constant, so, the quicker the gel dispersion at the specified distance takes place, the better the dispersibility and consequently the higher the quality of the gel would obtain.

Table 1. Independent variables (carbopol 940 and HPMC concentrations) and their respective responses (viscosity and spreadibility) for different runs of carbopol 940/HPMC gel preparation containing tranexamic acid 3%, (mean ± SD, n: 3)

Run	Factor 1 carbopol 940 (%, *w/w*)	Factor 2 HPMC (%, *w/w*)	Response 1 viscosity Cps	Response 2 spreadability g.cm/sec
1	1.00	2.00	4.800 ± 6.341	10 ± 2.946
2	1.25	1.50	4.305 ± 7.253	11.28 ± 2.203
3	1.25	1.50	4.289 ± 6.579	11.89 ± 3.018
4	1.25	2.00	4.965 ± 6.922	9.77 ± 2.839
5	1.25	1.50	4.350 ± 7.417	11.57 ± 2.582
6	1.25	1.50	4.245 ± 7.883	11.89 ± 2.290
7	1.50	2.00	4.980 ± 7.421	8.98 ± 2.713
8	1.25	1.00	3.836 ± 7.585	16.92 ± 3.374
9	1.00	1.50	4.040 ± 8.257	14.19 ± 3.596
10	1.50	1.00	4.100 ± 7.433	15.17 ± 2.924
11	1.50	1.50	4.270 ± 7.269	10.73 ± 2.561
12	1.25	1.50	4.136 ± 6.970	11.28 ± 3.459
13	1.00	1.00	3.562 ± 6.052	17.6 ± 3.982

HPMC: Hydroxypropyl methylcellulose, SD: Standard deviation, Cps: Centipoise

Based on this, run 13 [HPMC/carbopol 940 (1:1, *w/w*)] gel has shown the highest quality in terms of dispersibility of the gel.

The statistical studies revealed that the change in the concentration of HPMC had a more significant effect on the viscosity than the change in the concentration of carbopol 940. In general, the variation in the concentration of the polymers had a more significant effect on the viscosity than on the spreadability.

The results disclosed that the gel prepared in run 13 possessed the optimized viscosity and dispersibility, 3.982 ± 17.6 and 6.052 ± 3.562, respectively (Table 1). This was designed by Design-Expert software with 0.981 desirability.

The gel composition predicted by mathematical model was prepared in triplicate to validate the prediction. The predicted theoretical values were very close to the responses produced by the experiments, indicating that the experimental design employed in the current study was robust (Table 2).

Visual inspection and determination of pH

The above factors were considered determining responses to choose the ideal formulation. However, due to easiness of evaluating the apparent clarity and acidity and their important role in the apparent quality of the gel, these two parameters were also analyzed for all the runs.

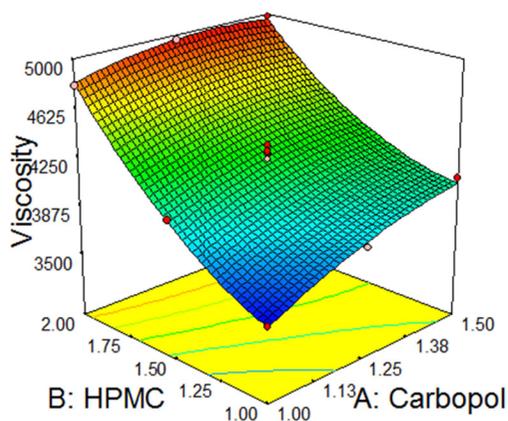Uniformity, transparency, and being free of suspended particles determine the obvious quality of topical gels. The relative clarity of the prepared gel formulations was visually inspected. Out of 13 runs, gels of runs 1, 2, 3, 4, 5, 6, 10, 12, and 13 showed higher transparency than the rest. Run 13 gel is presented in Figure 3.

Another factor to consider when preparing topical gel products is their acidity. If that exceeds the normal pH of the skin (4.1-5.8) and mucosal membrane (5.5-6.5), it will lead to local irritation. In addition, extreme pHs can cause gel formulation instability.[22] Investigations showed that the prepared gels were in this acidity range, Table 3.

Finally, according to the data analysis performed using Design-Expert software, the formulation of run 13 [HPMC/carbopol 940 (1:1, *w/w*)] was considered the best gel formulation both in terms of viscosity and dispersibility and was subjected to further analysis.
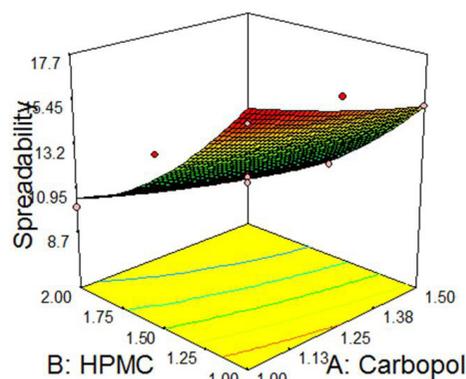

Figure 2. 3D response surface plot showing the influence of HPMC and carbopol 940 on tranexamic acid 3% gels spreadibility

HPMC: Hydroxypropyl methylcellulose

Figure 1. 3D response surface plot showing the influence of HPMC and carbopol 940 concentrations on tranexamic acid 3% gels viscositiy

HPMC: Hydroxypropyl methylcellulose

Table 2. Comparison between predicted and experimental values in the formulation prepared under predicted optimum conditions

Response	Experimental values	Predicted values	% Bias*
Viscosity (Cps)	3562	3607.85	1.271
Spreadability (g.cm/sec)	17.6	18.15	3.04

*% Bias: (predicted value-experimental value)/predicted value 100, Cps: Centipoise

Table 3. Acidity of the carbopol 940/HPMC gels containing tranexamic acid (3%) prepared based on response surface method, runs 1-13 (mean ± SD, n: 3)

Run	pH
1	5.42 ± 1.231
2	5.44 ± 2.455
3	5.45 ± 1.783
4	5.41 ± 2.359
5	5.43 ± 1.561
6	5.46 ± 2.213
7	5.30 ± 1.954
8	5.32 ± 2.323
9	5.46 ± 2.586
10	5.40 ± 1.440
11	5.37 ± 1.575
12	5.47 ± 2.691
13	5.39 ± 1.876

HPMC: Hydroxypropyl methylcellulose, SD: Standard deviation

The visual inspection and pH determination of optimized gel (run 13) 6 months after preparation showed no significant change indicating desirable stability in storage conditions.

FTIR spectroscopy

The possible interactions among polymers and active ingredients were investigated by FTIR spectroscopy. The IR spectra of pure TXA (Figure 4) showed the distinguished, strong, wide bands of carbonyl stretch (C=O) and the O-H stretch of the carboxylic acid at 1543 cm^{-1} and 3200-2500 cm^{-1} respectively. CH2-N and NH stretch appeared at 1396 cm^{-1} and 1533 cm^{-1}, respectively.

In the IR spectrum of carbopol 940/HPMC gel (Figure 5), C-O of secondary alcohol stretch is recognized at 1100 cm^{-1}. CH$_3$ presented a stretch peak at 1381 cm^{-1} and the broad absorptive peak fell in 3250-3000 cm^{-1}, which was attributed to O-H stretch.
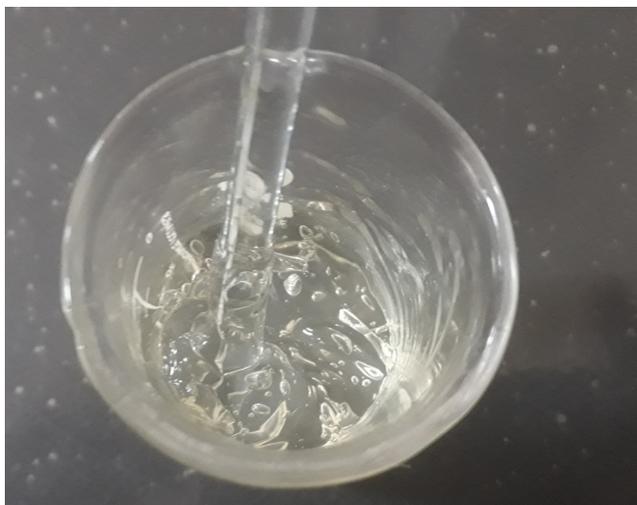
IR absorption spectrum of TXA (3%)-containing carbopol 940/HPMC (1:1, *w/w*) gel (Figure 6) exhibited that all of the drug index peaks discussed earlier appeared without displacement. This indicates that the drug did not chemically interfere with the structure of gel and could be entirely released once applied.[23] It also indicated that the gel base functional groups were free to establish electrostatic bands with the site of action and show mucoadhesive characteristics.[24]

Drug content uniformity

Resistance to phase separation is one of the important features of ideal pharmaceutical gels. Phase separation in addition to the obvious quality of the gel will change the consistency of the drug throughout the gel. Therefore, to evaluate the content uniformity of the TXA (3%)-containing carbopol 940/HPMC (1:1, *w/w*) gel, 5 different samples were taken from different parts of the gel and TXA content was quantified. The obtained results indicated that the TXA content of all the samples falls into the range of 97.3-102.6% (Table 4) of the expected drug content, which implied drug content uniformity and phase consistency of the gel.[22]

Figure 3. Carbopol 940/HPMC (1:1, w/w) gel

Table 4. Percentage of tranexamic acid content measured in 5 different zones of tranexamic acid (3%)-containing carbopol 940/HPMC (1:1, w/w) gel, run 13, (mean ± SD, n: 3)

	Drug content %
1	97.324 ± 2.237
2	98.394 ± 2.668
3	99.158 ± 2.924
4	101.6 ± 3.421
5	102.675 ± 2.806

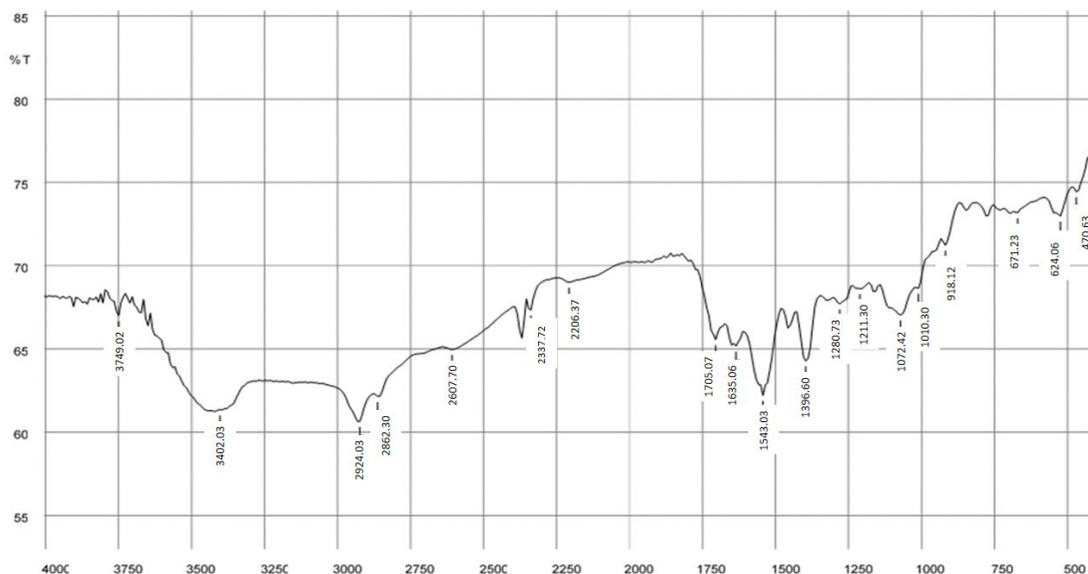HPMC: Hydroxypropyl methylcellulose, SD: Standard deviation

Figure 4. Fourier transform-infrared spectroscopy pattern: pure tranexamic acid

Ex vivo permeation study

In order to evaluate the release of TXA from TXA (3%)-containing carbopol 940/HPMC (1:1, *w/w*) gel and its permeation through the skin, Franz cells covered by animal skin were used. This method was used to correlate an *in vitro* environment with an *in vivo* environment. With all their limitations and sometimes poor correlation to *in vivo* results, *in vitro* permeation experiments and animal models still provide important options for evaluating drug delivery systems.[25] The plot of cumulative drug release within 5 h is presented in Figure 7. As can be seen, the drug was released and penetrated through the skin model uniformly and cumulative release reached 90% in 4 h and 97% in 5 h. This indicates ability of the gel to completely release the drug

and let it permeate through the skin in relatively short time, when applied topically. This result is in correlation with the FTIR absorptive spectrum, which showed no strong interaction between TXA and the gel base, which led to complete drug release.

Bleeding time assessment

In this study, we focused on the preparation of topical formulations containing TXA, an anticoagulant medicine. In order to check its efficiency in stopping bleeding, a common fast screening method without the need for paraclinical evaluation called IVY method[16] was used to evaluate the efficacy of topical TXA (3%)-containing carbopol 940/HPMC (1:1, *w/w*) gel on reducing the bleeding time in healthy volunteer individuals.
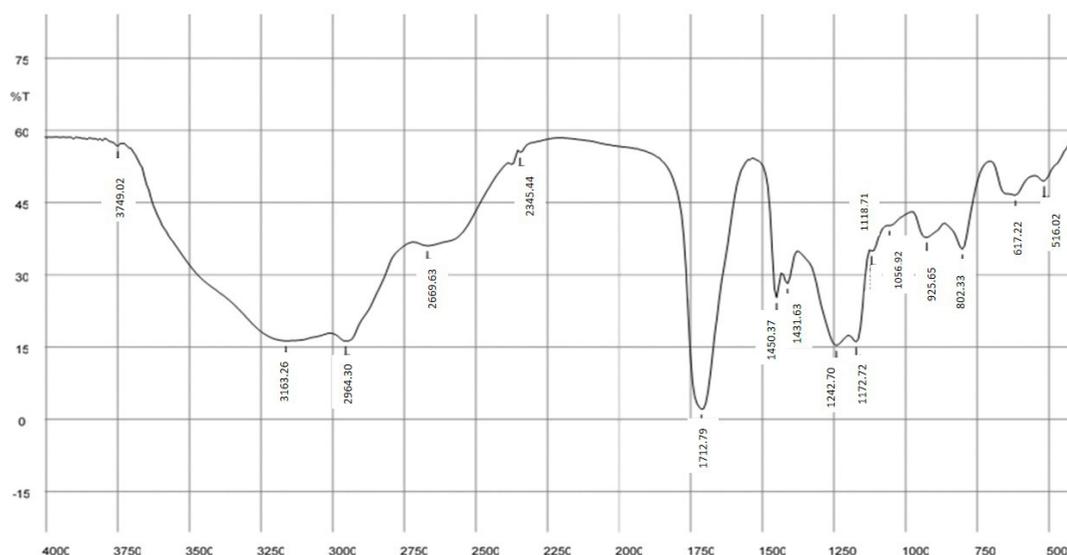

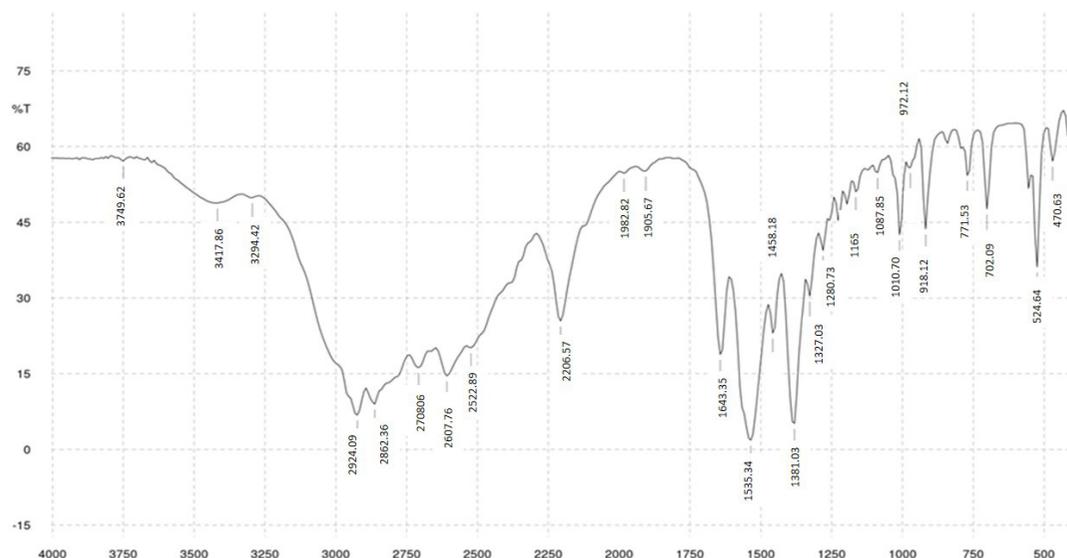**Figure 5.** Fourier transform-infrared spectroscopy pattern: carbopol 940/ HPMC (1:1, w/w) gel

Figure 6. Fourier transform-infrared spectroscopy pattern: tranexamic acid 3%-containing carbopol 940/HPMC (1:1, w/w) gel, run 13

HPMC: Hydroxypropyl methylcellulose

The process of coagulation has different stages and different methods have been developed to evaluate the status of each of these stages in hemostasis. The choice of each of these methods to evaluate blood coagulation depends on various factors, including the mechanism considered in the coagulation process, the speed of operation, and the cost. IVY method has been widely used to evaluate coagulation status and platelet function in various conditions such as pathologic conditions, drug-induced or presurgery evaluations. Although more sophisticated and accurate tests have been applied for this purpose recently, the bleeding time test is still used both in animal studies and in human studies, especially in the early stages of evaluation.[26]

Based on the bleeding times of groups presented in Figure 8, bleeding time in the control group was significantly higher than the other two groups ($p < 0.001$). Similarly, bleeding time in the TXA solution treated group was significantly higher than in the
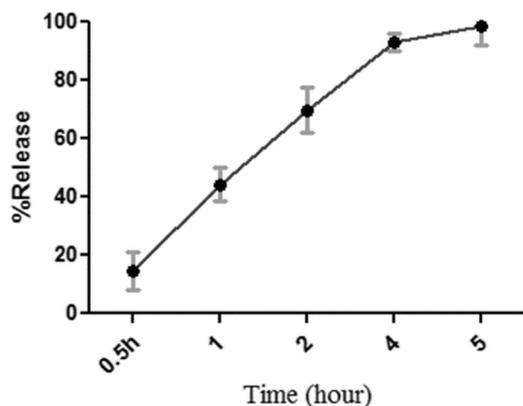
TXA gel group ($p < 0.05$). All subjects even in the TXA gel-treated group displayed normal bleeding times (4-10 minutes).[27]

The results can be explained by the ability of the gel to remain in the site of application and complete release of the drug, which led to higher efficacy in reducing bleeding time compared with the free drug solution. This result has been in line with other studies on topical gels containing anticoagulants in reducing bleeding time in animal models.[28]

The absence of abnormal bleeding time in all three groups indicates the safety of topical use of TXA and topical products containing it. Meanwhile, the decrease in the bleeding time of the gel group compared to the soluble drug indicates the short-term and transient effects of this drug in reducing the bleeding time, which can find its place in cases such as acute nosebleeds.[29]

Study limitations

In this study, the impact of topical use of TXA gel on plasma coagulation factors has not been evaluated.

Figure 7. Plot of cumulative tranexamic acid release percentage from tranexamic acid 3%-containing carbopol 940/HPMC (1:1, w/w) gel within 5 hours evaluated by an *ex vivo* permeation study using Franz cell (mean ± SD, n: 3)

HPMC: Hydroxypropyl methylcellulose, SD: Standard deviation

CONCLUSION

In this study, an optimal carbopol 940/HPMC gel formulation containing TXA (3%) was designed and fabricated using the experimental design method. *In vitro* studies showed desirable physical quality of this gel and non-chemical interactions with the loaded drug. In the *ex vivo* permeation test, it was shown that the gel was capable to completely release its loaded drug and drug permeate through the skin within 5 h. The results of *in vitro* evaluation of the gel on reducing bleeding time led to promising results that could confirm the importance of further studies on this formulation as a potential medication to stop acute superficial bleeding.

Ethics

Ethics Committee Approval: This study has been registered in the Ethics Committee of Zabul University of Medical Sciences and the code of ethics is the same as the study registration code given in the text of the manuscript (approval code. IR.ZBMU. REC.1397.085, date: 20.11.2018).

Informed Consent: The informed consent form was signed by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: D.M., Concept: O.C., S.D., F.S., Design: O.C., S.D., Data Collection or Processing: D.M., S.D., O.C., Analysis or Interpretation: S.D., F.S., Literature Search: F.S., Writing: F.S., S.D., O.C.

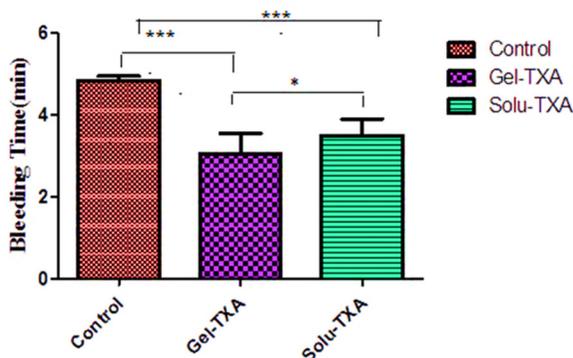**Conflict of Interest:** No conflict of interest was declared by the authors.

Figure 8. Bleeding time (minutes) measured in healthy volunteers receiving control, tranexamic acid (3%)-containing carbopol 940/ HPMC (1:1 w/w) gel and topical aqueous tranexamic acid solution, *$p < 0.05$ and ***$p < 0.001$, (mean ± SD, n: 3)

HPMC: Hydroxypropyl methylcellulose, SD: Standard deviation

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