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

Short Title: Topical gel containing tranexamic acid

Sara Daneshmand[1], Farideh Shiehzadeh[1], Daryosh Mohebi[2], Omid Chavoshian[3]
[1]Department of Pharmaceutics, Faculty of Pharmacy, Zabol University of Medical Sciences, Zabol, Iran
[2]Faculty of Pharmacy, Zabol University of Medical Sciences, Zabol, Iran
[3]Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

Corresponding Author Information
Sara Daneshmand
sdmehrpooya@gmail.com
+985432225402
https://orcid.org/0000-0001-7474-0157
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Abstract
Purpose: Tranexamic acid (TXA) is used systemically to stop bleeding, but it can lead to thromboembolism. Trials showed 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.
Methods: To develop a gel containing TXA 3% optimized in terms of viscosity and dispersibility, the Central Composite Design (CCD) based on two factors-three levels (Carbopol 940 and HPMC, 1-1.5% and 1-2% respectively) was applied. The spreadability and viscosity were assessed using the glass slide and rheometer, respectively. To confirm the compatibility of TXA with gel, 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, the IVY human method was performed.
Results: HPMC/carbopol 940 (1:1w/w) gel has shown the highest quality in terms of viscosity and dispersibility (3.982 ± 17.6 and 6.052 ± 3562 respectively). The FTIR absorption spectrum showed that all of 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 hours. In vivo, the bleeding time for the TXA gel was significantly lower than the 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.
Keywords: Tranexamic acid, carbopol940, 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 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 show acceptable effects on the site of action, while it carries 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 the amino acid lysine and inhibits bleeding by fibrinolysis inhibition mechanism. This drug is used systemically to stop various types of bleeding, but its systemic use can lead to risks such as thromboembolism, nausea, etc[3]. The topical anti-inflammatory and anti-melanin-producing properties of tranexamic acid has led it to be extensively considered and commercially available in the field of dermatology, as an off-lable topical treatment for rosacea, urticaria, and post-inflammatory hyperpigmentation. However, Randomized Controlled Trials (RCTs) have shown the positive effects of the TXA topical administration on local hemorrhages, including post-surgery or traumatic mucosal (eg. epistaxis)[4] or cutaneous hemorrhages[5,6].
In these studies, a TXA solution is often used, and a sterile gauze is impregnated by it and placed at the site of bleeding[7]. However, there is still a need for a safe 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 insitu-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, an ideal carbopol940/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 an *ex vivo* environment was evaluated. Finally, the *in vivo* efficacy of the formulation was evaluated by examining the bleeding time in healthy volunteers.
According to the authors' knowledge, this is the first HPMC/carbopol based gel formulation containing TXA, developed for topical application as an anti-coagulant agent and in vitro/ in vivo assessments were performed.

MATERIAL 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 the solvents and chemicals were of analytical grade.

Methods

Preparation of TXA containing carbopol940/HPMC gel formulation

To achieve the topical drug-loaded gel formulation, an aqueous solution of carbopol 940 and HPMC with a known concentration of each polymer (TableI) 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 entirely dissolve and hydrated. 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 the final gel formulation was brought up to 100 ml by deionized distilled water. Prepared gel formulations preserved from the 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

To develop an optimized gel base in terms of viscosity and dispersibility, Central Composite Design (CCD) based on two factors-three levels using Design Expert® software (version: DX7Trial) 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 Response Surface Method (RSM). By analyzing the obtained data, the formulation showing the ideal spreadability and viscosity was chosen for further evaluation.

Rheological evaluations

Spreadability

Investigation of the spreadability potential of the prepared gels was performed based on a published study[12] 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 since 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:

$S = m \times l/t$ (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

The viscosity of prepared gel formulations was measured by using rheometer (AMETEK Brookfield R/S plus, USA) using CC3-14 spindle. While the sample holder was filled with the gel, the spindle was inserted into the sample and rotated at speed of 1/min. The rheological evaluations were performed at RT (n=3)[13].

pH evaluation

To ensure the 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 the pH of the prepared solution was assessed using a calibrated pH meter 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.

In order 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 FT-IR spectroscopy (IRAffinity SHIMADZU, Japan) to clarify the molecular interactions. Each sample was prepared as an individual KBr disc and was scanned in the range of 400-4000 cm^{-1}

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 minutes. After cooling to RT using 10 ml of double-distilled water, the samples were brought to a volume of 10 ml. Finally, using a *UV/Vis* spectrophotometer (CE1021, CECIL, England) the absorbance of the samples was read against a blank solution at 565 nm[14].

Drug encapsulation efficiency of gel preparation

To measure the drug encapsulation efficiency (EE), 1 g of gel formulation was carefully weighed and using deionized distilled water made up to 100 ml. Then following the dilution up to 1:5 and filtration with a 0.45 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)[15]. The 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 prepared gel formulation (run 13) using Franz cells was applied. All the experiments were performed according to the Ethics Committee Acts (approval code. IR.ZBMU.REC.1397.085) 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 hr.

The process of the release test

Each donor compartment was covered by 16 cm^2 skin while the dermal side facing the receiver compartment. This later compartment was filled with well-stirred 29 ml of 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 base were applied on the skins of separated cells, while a 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 hours 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)[16].

In vivo bleeding time assessment

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

Intervention process

The control and treatment interventions were applied on the same volunteers at a fair enough separated period. In the control group no treatment was applied while for the topical gel group, TXA 3%-containing HPMC/carbopol 940 (1:1w/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) were topically applied on the volar aspect of the arm

IVY procedure

After 5 hours of applying the topical interventions, a cuff inflated on the upper arm to 40 mmHg. Three stab wounds (3mm deep) were made by a sterile lancet on the volar aspect of the forearm. The blood was removed every 30 seconds by filter paper till 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 the bleeding time[17].

Statistical analysis

Data were presented as mean ± SD. The statistical analysis was performed using Prism 6.0 software. Statistical significance was evaluated by one-way Analysis of Variance (ANOVA) followed by Tukey-Kramer as a post-test. A p-value equal to or less than 0.05 was considered statistically significant. The response surface method 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 also 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.

According to the author's knowledge, this is the first study on topical TXA gel formulation to prevent superficial dermal and mucosal bleeding. Topical tranexamic acid formulations have previously been developed to treat melasma[18].

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

Based on previous studies[19] 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 the 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 the viscosity and spreadability as the response functions. According to the literature, the concentration range of 1-1.5% and 1-2%

were considered for carbopol 940 and HPMC respectively. The total 13 experiments designed by the software are presented in table I.
In this study, 5 runs (Runs 2,3,5,6, and 12) are 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 in order to improve the precision of the experiment[12].

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[20]. As can be seen in the table 1, only the viscosities of run 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 (Figure1) by the Design-Expert software, the viscosity change is proportional to the concentration of polymers used which has also been shown in previous studies[20,21].
A similar pattern can be seen between the dispersibility factor of gels and the concentration of polymers used in their matrix (Figure2), which is consistent with previous studies[22].
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. Based on this, run 13 (HPMC/carbopol 940 (1:1w/w)) gel has shown the highest quality in terms of dispersibility of the gel.
The statistical studies showed that the change in the concentration of HPMC had a more significant effect on the viscosity than the change in the concentration of the carbopol 940, and in general, the variation in the concentration of the polymers had a more significant effect on the viscosity than on the spreadability.
The results revealed that the gel prepared in run13 possessed the optimized viscosity and dispersibility, 3.982 ± 17.6 and 6.052 ± 3562 respectively (Table 1). This was designed by Expert software with 0.981 desirability.
The gel composition predicted by the 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-mentioned factors were considered as determining responses to choosing the ideal formulation. But, because of the ease 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 apparent quality of topical gels. The relative clarity of prepared gel formulations was inspected visually. 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 shown 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[23]. Investigations showed that all the prepared gels were in this acidity range, Table 3.
Finally, according to the data analysis performed using the 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.
The visual inspection and pH determination of optimized gel (run13) 6 months after preparation showed no significant change indicating desirable stability in the storage condition.

FT-IR spectroscopy
The possible interactions among polymers and active ingredients were investigated by FT-IR spectroscopy. 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 in 1543 cm^{-1} and 3200-2500 cm^{-1} respectively. CH2-N and NH stretch appeared in 1396 cm^{-1} and 1533 cm^{-1} respectively.
In the IR spectrum of carbopol 940/HPMC gel (Figure 5), the C–O of secondary alcohol stretch is recognized in 1100 cm^{-1}. CH3 showed its stretch peak in 1381 cm^{-1} and the broad absorptive peak fell in 3250-3000 cm^{-1} was attributed to O–H stretch
The IR absorption spectrum of the TXA 3%-containing carbopol 940/ HPMC (1:1*w/w*) gel (Figure 6) showed that all of the drug index peaks discussed earlier appeared here without displacement. This indicates that the drug did not

chemically interfere with the structure of the gel and could be entirely released once applied[24]. It also showed the gel base functional groups were free to establish electrostatic bands with the site of action and show mucoadhesive characteristics[25].

Drug content uniformity

Resistance to phase separation is one of the important features of ideal pharmaceutical gels. Phase separation, in addition to the apparent 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 (1:1w/w) gel, 5 different samples were taken from different parts of the gel, and TXA content was quantified. The obtained results showed 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[23].

Ex vivo permeation study

To evaluate the release of the TXA from the TXA 3%-containing carbopol 940/ HPMC (1:1w/w) gel and permeation of it through the skin, Franz cells covered by animal skin were used. This method was done to correlate an *in vitro* to an *in vivo* environment. With all their limitations and sometimes poor correlation to *in vivo* results, still *in vitro* permeation experiments and animal models, provide important options for evaluating drug delivery systems[26]. The plot of cumulative drug release within 5 hours was presented in figure 7. As can be seen, the drug was released and penetrated through the skin model uniformly and cumulative release has reached 90% in 4 hours and 97% in 5 hr. This indicates the ability of the gel to completely release the drug and let it permeate through the skin in a relatively short time when applied topically. This result is in correlation with the FTIR absorptive spectrum which showed no strong interaction between TXA and gel base which led to complete drug release.

Bleeding time assessment

In this study, it was 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 [17] was used to evaluate the efficacy of the topical TXA 3%-containing carbopol 940/ HPMC (1:1w/w) gel on reducing the bleeding time in healthy volunteer individuals.

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 considered cost . The IVY method has been widely used to evaluate coagulation status and platelet function in various conditions such as pathologic conditions, drug-induced, or pre-surgery evaluations. Although more sophisticated and accurate tests are 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 [27].

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 the subjects even in the TXA gel treated group showed normal bleeding time (4-10 minutes[28]).

The results can be explained by the ability of the gel to remain in the site of application and complete release the drug which led to higher efficacy in reducing bleeding time in comparison with the free drug solution. This result has been in line with other studies on topical gels containing anticoagulant in reducing bleeding time in animal models[29].

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[30].

STUDY LIMITATIONS

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

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 the desirable physical quality of this gel as well as 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 the drug permeate through the skin within 5 hours. The results of *in vivo*

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.

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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		Facor 2 HPMC		Response 1 Viscosity		Response 2 Spreadability
	Spreadability (g.cm/sec)	%w/w	17.6	%w/w	18.15	Cps	3.04 g.cm/sec
1		1.00		2.00		4800 ± 6.341	10 ± 2.946
2		1.25		1.50		4305 ± 7.253	11.28 ± 2.203
3		1.25		1.50		4289 ± 6.579	11.89 ± 3.018
4		1.25		2.00		4965 ± 6.922	9.77 ± 2.839
5		1.25		1.50		4350 ± 7.417	11.57 ± 2.582
6		1.25		1.50		4245 ± 7.883	11.89 ± 2.290
7		1.50		2.00		4980 ± 7.421	8.98 ± 2.713
8		1.25		1.00		3836 ± 7.585	16.92 ± 3.374
9		1.00		1.50		4040 ± 8.257	14.19 ± 3.596
10		1.50		1.00		4100 ± 7.433	15.17 ± 2.924
11		1.50		1.50		4270 ± 7.269	10.73 ± 2.561
12		1.25		1.50		4136 ± 6.970	11.28 ± 3.459
13		1.00		1.00		3562 ± 6.052	17.6 ± 3.982

TABLE 2. Acidity of the carbopol 940/HPMC gels containing tranexamic acid 3% prepared based on response surface method, runs 1-13 (Mean±SD, n=3)

Response	Experimental values	Predicted values	% Bias *
Viscosity (Cps)	3562	3607.85	1.271

*% bias: (predicted value - experimental value)/predicted value * 100.

TABLE 3. Percentage of tranexamic acid content measured in 5 different zones of tranexamic acid 3%-containing carbopol 940/ HPMC (1:1w/w) gel, run 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

Table 4. Percentage of tranexamic acid content measured in 5 different zones of tranexamic acid 3%-containing carbopol 940/ HPMC (1:1w/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

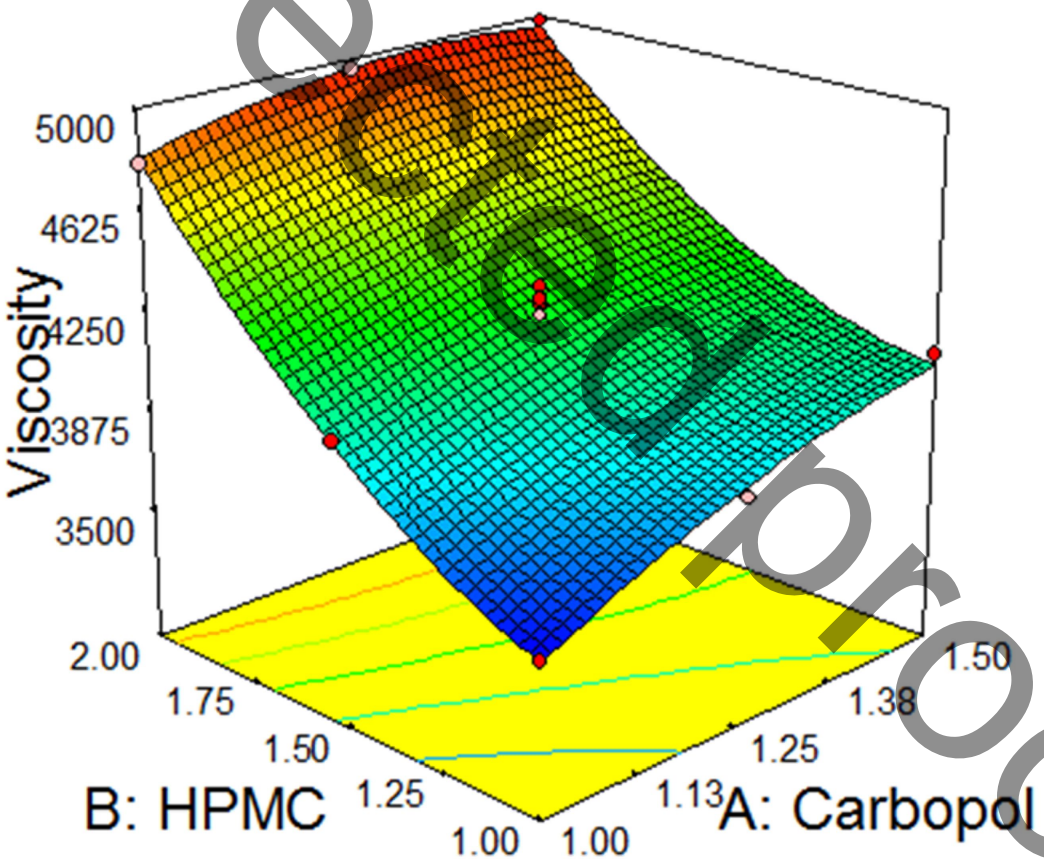

FIGURE 1. The 3D response surface plot showing the influence of HPMC and carbopol 940 concentrations on tranexamic acid 3% gels viscositiy

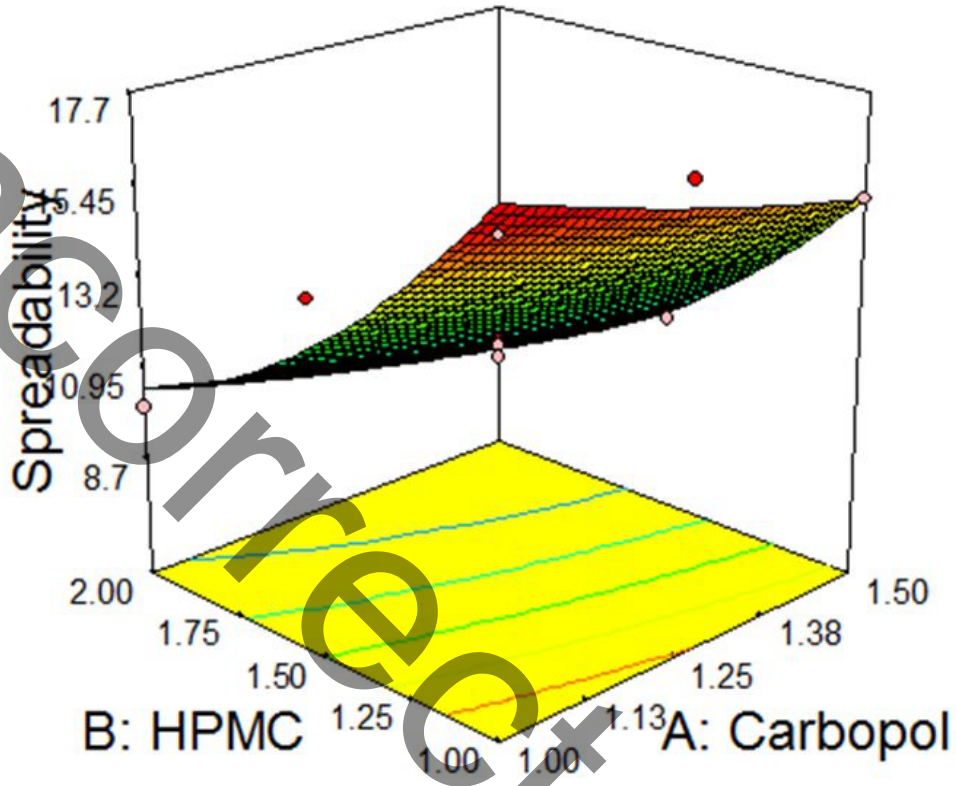

FIGURE 2. 3D response surface plot showing the influence of HPMC and carbopol 940 on tranexamic acid 3% gels spreadibility

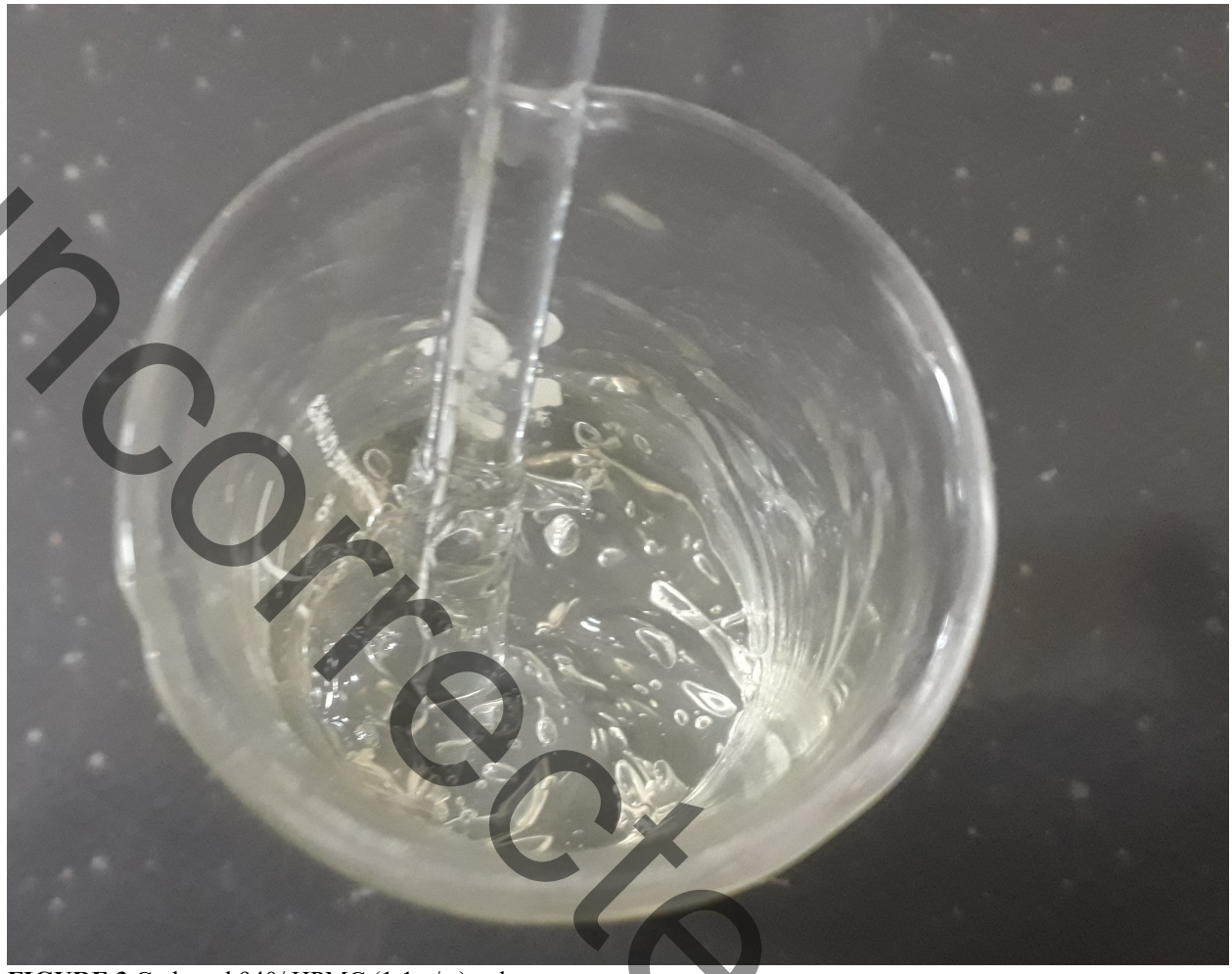

FIGURE 3. Carbopol 940/ HPMC (1:1w/w) gel

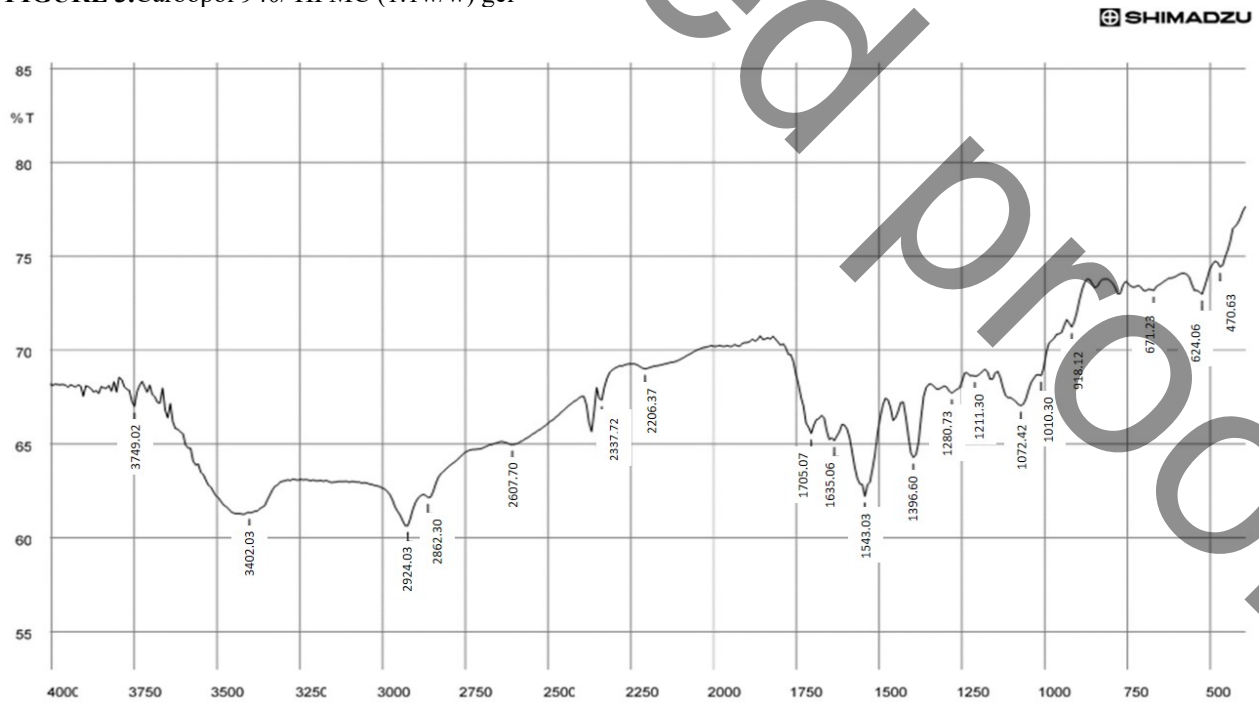

FIGURE 4. Fourier transform-infrared spectroscopy pattern: pure tranexamic acid

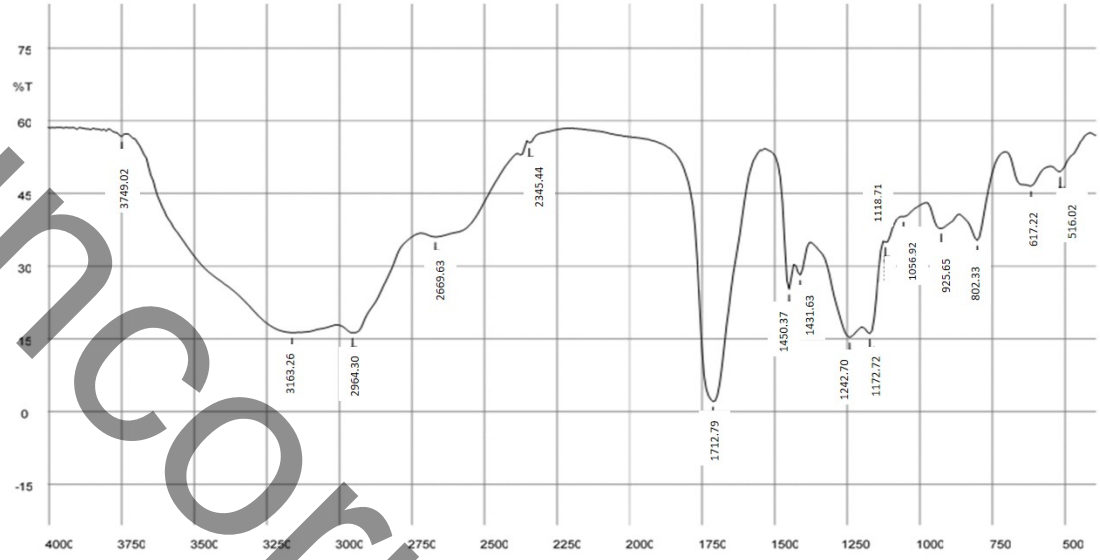

FIGURE 5. Fourier transform-infrared spectroscopy pattern: carbopol 940/ HPMC (1:1w/w) gel

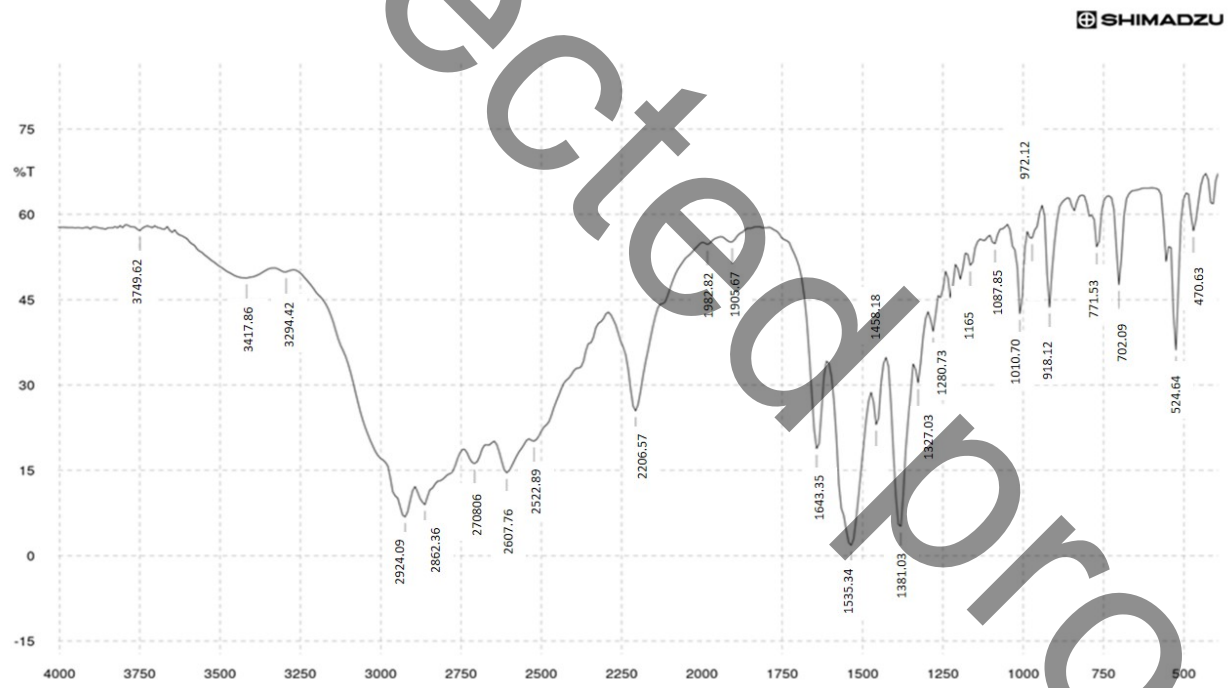

FIGURE 6. Fourier transform-infrared spectroscopy pattern: tranexamic acid 3%-containing carbopol 940/ HPMC (1:1w/w) gel, run 13

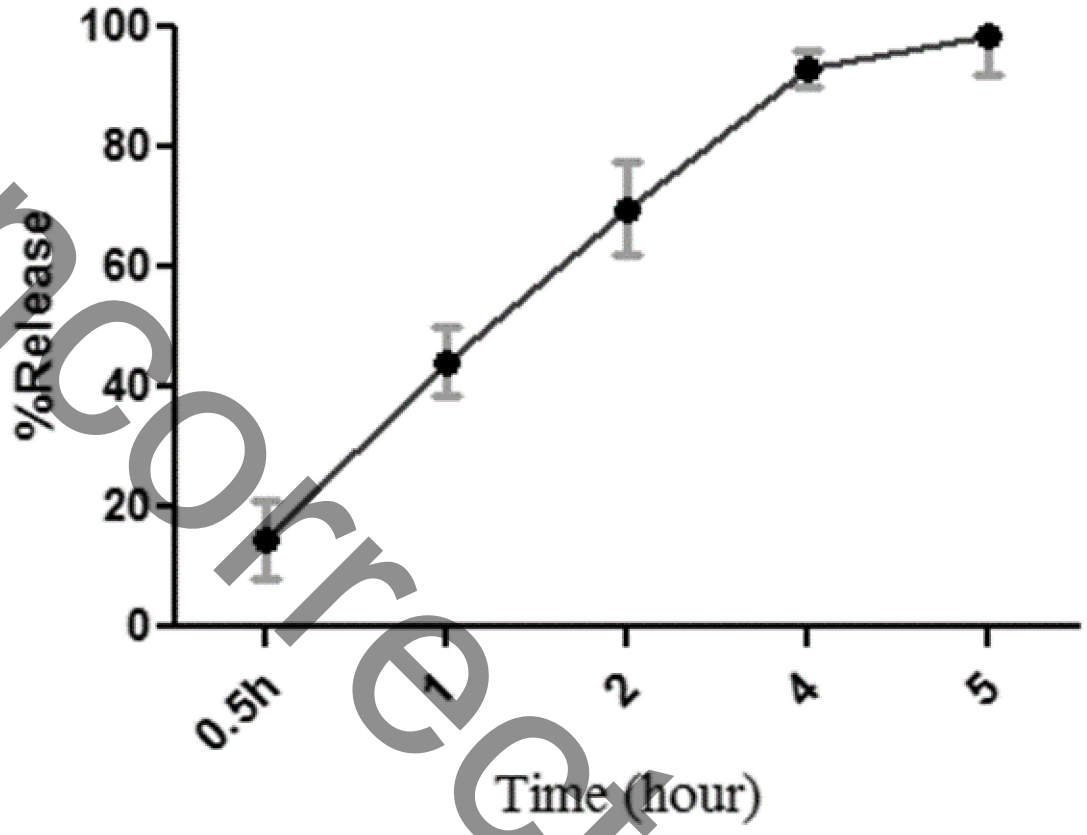

FIGURE 7. Plot of cumulative tranexamic acid release percentage from tranexamic acid 3%-containing carbopol 940/ HPMC (1:1w/w) gel within 5 hours evaluated by an ex vivo permeation study using Franz cell (Mean±SD, n=3)

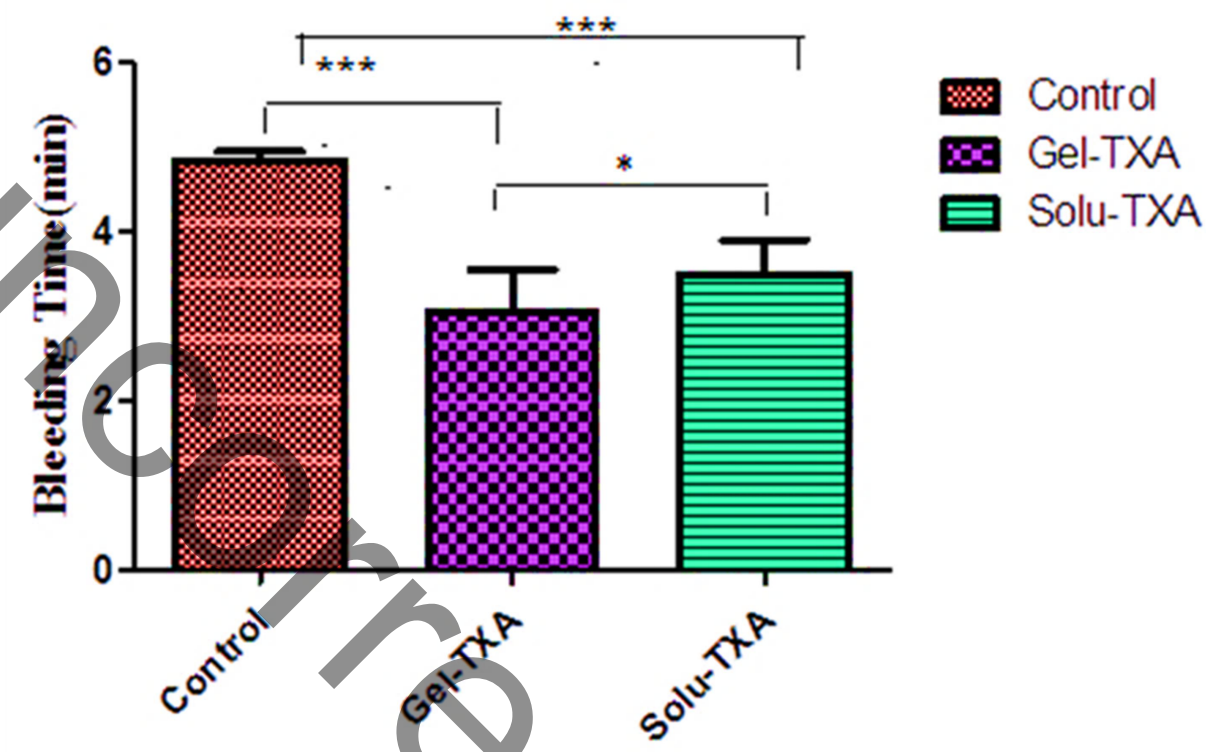

FIGURE 8. Bleeding time (minuts) measured in healthy volunteers receiving control, tranexamic acid 3%-containing carbopol 940/ HPMC (1:1w/w) gel and topical aqueous tranexamic acid solution, * P < .05 and *** P < .001, (Mean±SD, n=3)