

A chitosan-based spray for localized tranexamic acid delivery: Formulation development and enhanced hemostatic control

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ABSTRACT

Objective(s): Topical tranexamic acid (TXA) can provide localized antifibrinolytic activity, but rapid drug clearance from bleeding surfaces may limit its effectiveness. Chitosan offers complementary hemostatic properties and may improve local drug retention due to its well-documented mucoadhesive properties. Although previous studies have explored TXA-loaded chitosan-based particulate hemostats, translating this concept into a sprayable, dose-reproducible, bio-adhesive topical delivery system remains insufficiently characterized.

Materials and Methods: A chitosan-based TXA spray was developed as a translational topical delivery platform and characterized for appearance, pH, rheological behavior, spray pattern, dose uniformity, TXA content, and short-term stability. In vivo hemostatic efficacy was evaluated in a standardized rat tail-incision model. Animals received purified water, topical TXA, or the TXA-chitosan spray (n=6/group). Time to hemostasis and blood loss were measured.

Results: The optimized formulation showed a homogeneous appearance and a pH of 6.36 ± 0.01 . Apparent viscosity decreased from 40.5 ± 0.3 to 21.8 ± 0.2 mPa·s as rotational speed increased, indicating shear-thinning behavior. Spray delivery was highly reproducible, with 118.7 ± 0.4 mg delivered per actuation and 0.34% relative standard deviation. TXA content remained 98.2% of the initial value after 30 days at $25 \pm 2^\circ\text{C}$. Both TXA and TXA-chitosan significantly reduced bleeding time versus control ($P \leq 0.005$). Notably, the TXA-chitosan spray reduced blood loss to 16.3 ± 3.9 mg versus 33.2 ± 5.7 mg with TXA alone ($P = 0.02$).

Conclusion: The developed spray provides reproducible topical delivery and substantially enhanced blood-loss control compared with TXA alone, supporting its further development as a localized hemostatic platform.

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Introduction

Uncontrolled hemorrhage remains the leading preventable cause of death after traumatic injury and continues to pose a major challenge for trauma systems (1). Despite substantial advances in trauma resuscitation and damage-control surgery, exsanguination accounts for a large share of early post-traumatic mortality, particularly in the prehospital phase, when definitive surgical intervention is unavailable. Consequently, developing effective, rapidly deployable hemostatic interventions remains a critical priority in contemporary trauma care (2, 3).

Among pharmacological strategies for hemorrhage control, tranexamic acid (TXA) has emerged as one of the most important advances in trauma medicine. TXA is a synthetic lysine analog that inhibits fibrinolysis by competitively blocking lysine-binding sites on plasminogen and plasmin, thereby preventing fibrin degradation and stabilizing newly formed thrombi. The landmark CRASH-2

trial established the clinical value of early intravenous TXA administration, demonstrating a significant reduction in bleeding-related mortality when treatment was initiated within three hours of injury. These findings led to the widespread adoption of TXA in trauma guidelines worldwide (4, 5).

Despite its proven efficacy, systemic TXA administration has important practical and clinical limitations. Intravenous delivery requires vascular access, trained personnel, sterile equipment, and timely administration, conditions that may not be readily achievable in prehospital emergencies or resource-constrained settings (6, 7). Furthermore, systemic exposure may increase concerns regarding adverse neurological and thromboembolic effects in selected patient populations, particularly when high doses are administered or repeated treatment is required. These limitations have stimulated growing interest in localized approaches that deliver TXA directly to the bleeding site while minimizing

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systemic exposure (6, 8).

Topical TXA is an attractive alternative because it enables high local drug concentrations at the wound interface with minimal systemic absorption. Clinical studies across orthopedic (9), cardiothoracic (10), dental (11), and soft-tissue surgical procedures (12) have reported reduced blood loss, transfusion requirements, and postoperative hematoma formation after local TXA application. However, the therapeutic effectiveness of conventional topical formulations remains inconsistent. Active bleeding, wound exudate, and tissue fluid dynamics can rapidly remove dissolved TXA from the injury site, resulting in insufficient local residence time and suboptimal antifibrinolytic activity. In addition, repeated administration or the use of highly concentrated solutions to compensate for drug washout may adversely affect tissue repair and wound healing. Therefore, achieving sustained local retention and controlled release of TXA remains a major challenge for topical hemostatic therapy (13-15).

Advanced bio-adhesive drug-delivery systems offer a promising strategy to overcome these limitations. By prolonging drug residence at the wound surface and protecting the therapeutic agent from rapid dilution and clearance, bio-adhesive matrices may substantially enhance the local efficacy of antifibrinolytic therapy (16). Among available biomaterials, chitosan has attracted considerable attention for its unique combination of biocompatibility, biodegradability, bio-adhesiveness, and intrinsic hemostatic activity. As a positively charged polysaccharide, chitosan can promote erythrocyte and platelet aggregation through electrostatic interactions, facilitate clot formation independently of the classical coagulation cascade, and absorb wound exudate, thereby concentrating endogenous coagulation components at the injury site. Furthermore, its mucoadhesive and film-forming properties enable prolonged contact with wet biological tissues, making it an attractive carrier for localized drug delivery (17, 18).

Integrating TXA into a chitosan-based matrix, known for its potential mucoadhesive and intrinsic hemostatic properties, may provide synergistic therapeutic advantages by combining pharmacological inhibition of fibrinolysis with the polymer's intrinsic hemostatic and adhesive properties. This platform is expected to prolong drug residence at the wound surface, reduce premature drug washout, provide controlled local release, reinforce clot stability, and improve overall hemostatic performance. Nevertheless, despite growing interest in topical TXA, few studies have systematically developed bioadhesive chitosan-based spray formulations and comprehensively evaluated their physicochemical characteristics alongside their biological and hemostatic performance (19). In particular, the relationships between formulation properties, drug-release behavior, bioadhesion, and *in vivo* hemostatic efficacy remain insufficiently characterized.

Although previous studies have explored chitosan-TXA combinations as hemostatic adducts or particulate systems, their translation into a sprayable, dose-reproducible topical dosage form with comprehensive pharmaceutical characterization remains insufficiently characterized. In particular, researchers have not systematically evaluated the relationships between formulation attributes, spray performance, and *in vivo* hemostatic outcomes. This study aimed to address this gap by developing a bioadhesive, chitosan-based spray for localized TXA delivery, designed to overcome the rapid washout and limited residence time of conventional topical TXA solutions. The rationale is that chitosan's intrinsic mucoadhesive and hemostatic properties

may synergize with TXA's antifibrinolytic activity to enhance local hemostatic performance; however, the present work did not evaluate direct evidence of prolonged tissue retention, controlled release, or reduced systemic exposure, which future studies should confirm. The specific objectives were to: (i) develop and optimize a bio-adhesive chitosan-TXA spray formulation; (ii) comprehensively characterize its physicochemical properties, including rheological behavior, spray pattern, delivered dose uniformity, drug content, and short-term stability; and (iii) evaluate its *in vivo* hemostatic efficacy in a standardized rat tail-incision model compared with topical TXA alone and untreated control. Through this systematic approach, the study seeks to establish a reproducible topical hemostatic platform that integrates therapeutic activity with pharmaceutical performance.

Materials and Methods

Materials

Tranexamic acid (TXA) injection (500 mg/5 ml) was obtained from Raha Pharmaceutical Co., Iran, and used as the active pharmaceutical ingredient. The same commercial TXA injection (500 mg/5 ml, 10% w/v) was also used to prepare the 5% w/v topical TXA solution for the positive-control group in the *in vivo* hemostatic evaluation, by dilution with an equal volume of purified water. High-molecular-weight chitosan (molecular weight: 310-375 kDa; degree of deacetylation: >75%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Polyvinylpyrrolidone K30 (PVP K30; pharmaceutical grade) was obtained from Merck KGaA, Germany, and used as a film-forming and viscosity-modifying polymer. Glacial acetic acid (ACS reagent grade, ≥99.7%) was purchased from Dr. Mojallali Pharmaceutical Chemical Industries Complex, Iran, and used to dissolve chitosan. Sodium hydroxide pellets (ACS reagent grade, ≥97%) were obtained from Merck KGaA, Germany, and used to prepare 1 M sodium hydroxide solution for pH adjustment. Polyethylene glycol 400 (PEG 400; pharmaceutical grade) and glycerin (USP grade, ≥99.5%) were purchased from Shazand Petrochemical Company, Iran, and incorporated as co-solvents and humectants, respectively. Polysorbate 80 (Tween 80; pharmaceutical grade) was obtained from Merck KGaA, Germany, and served as the nonionic surfactant. Sodium benzoate (ACS reagent grade, ≥99%) was purchased from Merck KGaA, Germany, and incorporated as the antimicrobial preservative. Purified water (double-distilled, conductivity <1.0 µS/cm) was used throughout the study. Unless otherwise stated, all chemicals and reagents were of analytical or pharmaceutical grade and were used as received without further purification.

Pre-formulation solubility and formulation design

The formulation was designed according to previously reported chitosan-based topical hemostatic formulations (19, 20). High-molecular-weight chitosan (310-375 kDa; degree of deacetylation >75%) was used at a concentration of 1% w/v (equivalent to 0.5 g in a final volume of 50 ml). Chitosan was gradually dissolved in dilute glacial acetic acid (2% v/v; 1 ml in a 50 ml final volume) under continuous magnetic stirring to obtain a homogeneous polymeric solution. The pH of the chitosan polymeric solution was initially adjusted to pH 6.2 using 1 M sodium hydroxide (1% v/v; 0.5 ml per 50 ml final volume). Subsequently, TXA (5% w/w) was incorporated into the adjusted polymeric solution under continuous stirring until complete dissolution and formation of a homogeneous formulation. The final volume was adjusted to 50 ml with purified water, and the

Table 1. Preparation of a reference-based bio-adhesive formulation

Excipient (% w/v)	Formulation		
	F1	F2	F3 (TXA)
Tranexamic acid	5%	5%	5%
Chitosan	1% (MV: 625 kDa; DDA: >75%)	1% (Commercial grade; specifications as per patent reference 20)	1% (MV: 310 375 kDa; DDA: >75%)
PVP K30	-	0.25%	0.25%
PEG 400	-	5%	5%
Tween 80	-	0.2%	0.5%
Glycerol	-	-	1%
Glacial acetic acid	To pH 6.2	1%	2% (To pH 6.2)
Sodium hydroxide (1 M)	-	-	1% (To pH 6.2)
Acetate buffer	To pH 6.2	-	-
Purified water	q.s. to 50 ml	q.s. to 50 ml	q.s. to 50 ml
F1	Best formulation from Antonyuk <i>et al.</i> (2022) (10 tested formulations) (19).		
F2	Optimal formulation from WO 2019/053579 (5 tested formulations) (20).		
F3 (TXA)	Reproduced and optimized formulation for physicochemical quality and efficacy.		

final pH of the optimized formulation was measured as 6.36 ± 0.01 at room temperature. The prepared formulation was visually evaluated for clarity, homogeneity, phase separation, polymer aggregation, and visible precipitation before subsequent physicochemical characterization and biological evaluation. Table 1 summarizes the complete formulation composition.

Preparation of the chitosan-TXA complex

The bioadhesive chitosan-TXA spray was prepared by sequential mixing under continuous magnetic stirring. Initially, three different formulations (F1, F2, and F3) were prepared and compared with previously published chitosan-based hemostatic formulations. Table 1 presents the complete formulation composition.

F1 was adapted from the best-performing formulation reported by Antonyuk *et al.* (2022), which evaluated 10 different chitosan-TXA combinations (19). F2 was based on the optimal formulation disclosed in patent WO 2019/053579, which tested 5 different topical TXA spray formulations (20). F3 (TXA) is the reproduced and optimized formulation selected for all subsequent experiments in the present study, including comprehensive physicochemical characterization, stability assessment, and *in vivo* hemostatic evaluation. F1 and F2 served solely as reference formulations to guide the rational design of F3, and were not subjected to further experimental testing.

The polymeric phase was prepared by gradually dispersing chitosan and PVP K30 in purified water containing glacial acetic acid under continuous stirring until complete hydration and formation of a uniform polymeric solution. TXA was incorporated into the polymeric phase under continuous stirring, followed by sequential addition of PEG 400, glycerin, Tween 80, and sodium benzoate based on their physicochemical compatibility and solubility characteristics.

The formulation pH was measured with a calibrated digital pH meter and adjusted to the predetermined value using 1 M sodium hydroxide. Magnetic stirring was continued until a homogeneous formulation was achieved. The final formulation (F3) was transferred to sterile spray containers and used for subsequent physicochemical characterization, stability studies, and biological evaluation.

Physicochemical characterization

Macroscopic appearance

The prepared formulation was visually inspected for

color, clarity, homogeneity, phase separation, polymer aggregation, and visible precipitation immediately after preparation and throughout the study period.

pH determination

The pH of the formulation was measured at $25 \pm 1^\circ\text{C}$ using a calibrated digital pH meter (Labtron Co., UK). Measurements were performed in triplicate, and results were expressed as mean $\pm$ standard deviation (SD).

Viscosity Measurement

Rheological measurements were performed using a rotational viscometer (ROTAVISC io-vi, IKA-Werke GmbH & Co. KG, Germany) equipped with an ELVAS-SP coaxial cylinder spindle at $25 \pm 1^\circ\text{C}$. Approximately 16 ml of each formulation was transferred into the sample container, and viscosity was determined over a rotational speed range of 10–100 rpm. Samples were equilibrated for 60 sec at each speed before recording the viscosity. All measurements were performed in triplicate, and results were expressed as mean $\pm$ SD.

Spray performance

Spray pattern

Spray characteristics were evaluated using manually actuated spray bottles. Three independent spray bottles were prepared and assessed for spray pattern. For each bottle, five consecutive actuations were performed, each directed onto a separate piece of filter paper from a fixed distance of 10 cm. The filter papers were placed on a flat, non-absorbent surface to ensure consistent spray deposition.

Quantitative spray-pattern analysis was performed as follows: Immediately after each actuation, the spray pattern deposited on the filter paper was photographed with a digital camera positioned perpendicular to the filter paper at a standardized distance of 30 cm. Camera settings (resolution, zoom, and lighting) were kept constant throughout the assessment to ensure consistency.

The resulting images were analyzed using ImageJ software (version 1.54, National Institutes of Health, USA) to quantitatively measure two key parameters: (1) the overall spray diameter (defined as the outermost boundary of the spray deposition), and (2) the diameter of the central deposition zone (defined as the region of highest spray density). The scale was calibrated using a reference ruler included in each photograph.

Spray output was determined gravimetrically by measuring the amount of formulation delivered after repeated actuations, and the average output per actuation was calculated. All measurements were performed in triplicate, and results were expressed as mean $\pm$ standard deviation (SD) across all measurements ($n=15$ total measurements, representing 3 bottles $\times$ 5 actuations per bottle).

Delivered dose uniformity and TXA content per actuation

The delivered dose per actuation was determined gravimetrically by weighing the spray container before and after ten consecutive actuations on an analytical balance (± 0.1 mg accuracy). The mass difference was divided by the number of actuations to calculate the mean delivered dose per actuation. Results were reported as mean $\pm$ SD.

Since the formulation contained 5% (w/w) TXA, the amount of drug delivered per actuation was calculated from the measured delivered dose according to the following equation:

$$\text{TXA (mg/actuation)} = \text{Delivered dose (mg)} \times 0.05$$

The relative standard deviation (RSD) of the delivered dose was calculated to evaluate actuation-to-actuation dose uniformity.

Drug content assay

The TXA content of the optimized bio-adhesive spray formulation was quantified using a UV-Visible spectrophotometer (i7 UV-VIS Spectrophotometer, China). Initially, a stock solution of TXA (5 mg/ml) was prepared in distilled water. The absorption spectrum of a suitably diluted standard solution was scanned over 190–400 nm against distilled water as the blank to determine the analytical wavelength. The wavelength of maximum absorbance was then selected for quantitative analysis.

A series of standard TXA solutions was prepared by serial dilution of the stock solution to obtain concentrations of 50, 5, 2.5, 1.25, 0.625, 0.50, and 0.3125 $\mu\text{g/ml}$, corresponding to dilution ratios of 1:100, 1:1000, 1:2000, 1:4000, 1:8000, 1:10000, and 1:16000, respectively. The absorbance of each standard solution was measured at 273 nm using a 1-cm quartz cuvette, and a calibration curve was constructed by plotting absorbance against concentration. TXA concentrations in the bio-adhesive spray samples were calculated from the corresponding linear regression equation.

For sample analysis, an accurately measured aliquot of the optimized formulation was appropriately diluted with distilled water to obtain a concentration within the linear range of the calibration curve before absorbance measurement. All analyses were performed in triplicate, and the results were expressed as mean $\pm$ SD.

To evaluate the method's specificity, a placebo formulation containing all excipients except TXA was prepared and analyzed under identical experimental conditions. The placebo showed negligible absorbance at the selected analytical wavelength, indicating no significant interference from formulation excipients.

Accelerated stability study

The formulation was filled into amber spray containers and stored at $25 \pm 2^\circ\text{C}$ for 30 days. Samples were evaluated on days 0, 15, and 30 for changes in appearance, pH, and viscosity. Physical stability was assessed by monitoring precipitation, phase separation, color changes, and loss of homogeneity.

Hemostatic evaluation

The *in vivo* hemostatic efficacy of the developed bio-

adhesive chitosan-TXA spray was evaluated using a standardized rat tail bleeding model. Healthy adult male Wistar rats (8–10 weeks old, 220–250 g) were randomly allocated into three experimental groups ($n=6$ per group): Group I received purified water as the negative control, Group II received topical TXA solution as the positive control, and Group III received the bio-adhesive chitosan-TXA spray. Animals were housed under controlled environmental conditions ($22 \pm 2^\circ\text{C}$, relative humidity 50–60%, 12 hr light/12 hr dark cycle) with ad libitum access to standard laboratory chow and water. They were acclimatized for at least one week before experimentation. All experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and complied with the ARRIVE 2.0 guidelines (21). The experimental protocol was approved by the Institutional Animal Ethics Committee (Approval No. IR.BMSU.BLC.1404.042).

General anesthesia was induced by intraperitoneal administration of ketamine (80 mg/kg) and xylazine (10 mg/kg). After anesthesia and surgical site preparation, a standardized incision injury was created on the ventral aspect of the tail. A longitudinal incision measuring 3 cm in length and approximately 3 mm in depth was created under identical experimental conditions for all animals. Immediately after injury induction, the assigned formulation was applied directly to the wound surface. For the intervention group, exactly 2 actuations of the bio-adhesive chitosan-TXA spray were applied, delivering a total mass of approximately 237.4 mg (equivalent to 11.87 mg of TXA, based on the 5% w/w concentration and the mean delivered dose of 118.7 mg/actuation). For the positive-control group, 0.24 ml of topical TXA solution (5% w/v) was applied directly to the wound surface, delivering 12.0 mg of TXA to ensure strict dose comparability between groups. This 5% w/v solution was prepared by diluting the commercial TXA injection (500 mg/5 ml, equivalent to 10% w/v) with an equal volume of purified water. After a one-minute application period, bleeding assessment began.

Blood loss was quantified using a gravimetric filter paper method. Pre-weighed filter papers were carefully applied to the bleeding site at 30-second intervals until active bleeding ceased. After blood absorption, the filter papers were reweighed, and the mass increase was recorded as blood loss (mg).

Bleeding time was assessed based on serial blood spot formation on the filter papers. The number of blood spots generated during the observation period was recorded, and bleeding time was calculated by converting the number of spots according to the 30-sec sampling interval and adding the initial one-minute post-treatment period. Bleeding time was expressed in seconds.

Sample size and statistical analysis

The animal experiment was designed as a pilot proof-of-concept study to provide an initial estimate of the hemostatic efficacy of the optimized bio-adhesive TXA spray. Sample size estimation was informed by a previous study by Li *et al.* (2012), which evaluated a chitosan-containing TXA formulation in a rabbit liver bleeding model and reported bleeding times of 3.07 ± 0.84 min in the control group and 1.90 ± 0.75 min in the chitosan-TXA formulation group. Based on these preliminary effect estimates, an a priori power analysis was performed using G*Power version 3.1.9.7 (22). Assuming a one-way ANOVA design with

three experimental groups, an effect size of $f=0.58$, a two-sided significance level of $\alpha=0.05$, and 70% statistical power, the estimated minimum sample size was six animals per group. Accordingly, 18 animals were included and equally allocated to three groups: distilled water (negative control), 5% TXA spray (positive control), and the optimized chitosan-based bio-adhesive TXA spray (intervention). Given the exploratory proof-of-concept nature of the study, this sample size was considered appropriate for detecting preliminary between-group differences while minimizing animal use.

All quantitative data are presented as mean $\pm$ SD. The assumptions required for parametric analysis were assessed before statistical testing. Data distribution was evaluated using the Shapiro–Wilk normality test accompanied by visual examination of Q–Q plots, while equality of variances was assessed using Levene's test.

Differences among treatment groups were analyzed using one-way analysis of variance (ANOVA). When a significant overall treatment effect was observed, Tukey's honestly significant difference (HSD) test was applied for adjusted pairwise comparisons. Effect sizes were calculated using eta squared (η^2) to quantify the magnitude of between-group differences. Statistical significance was defined as a two-sided adjusted P -value < 0.05 . Statistical analyses were performed using IBM SPSS Statistics.

Results

All experimental results presented in this section pertain to the optimized formulation (F3), selected for its integration of therapeutic activity with pharmaceutical performance characteristics. The reference formulations (F1 and F2) were used only for design rationale and were not evaluated in the subsequent characterization or biological experiments.

Appearance

The optimized bio-adhesive TXA spray exhibited a homogeneous milky-white appearance with no visible sedimentation, particulate matter, or phase separation immediately after preparation. The formulation remained physically uniform, with no evidence of creaming or precipitation, indicating satisfactory dispersion of all formulation components within the polymeric matrix. A faint characteristic odor of acetic acid was perceptible, which was expected due to the use of dilute acetic acid as the chitosan solvent. No discoloration or visible changes in appearance were observed during refrigerated storage, confirming acceptable preliminary physical stability.

pH measurement

The pH of the optimized formulation was 6.36 ± 0.01 ($n=3$) at room temperature. The formulation showed excellent reproducibility across triplicate measurements, with minimal variation. The measured pH was within the near-physiological range, indicating suitability for topical application and compatibility with chitosan-based formulations.

Rheological behavior

Rheological evaluation showed characteristic shear-thinning behavior in the optimized bio-adhesive TXA spray. As rotational speed increased from 10 to 100 rpm, apparent viscosity decreased from 40.5 ± 0.3 to 21.8 ± 0.2

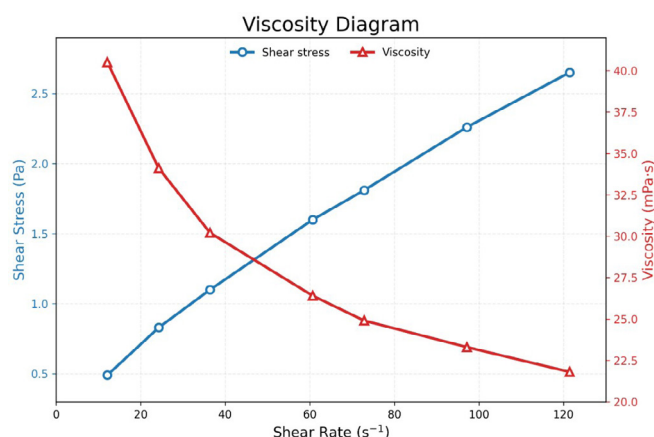


Figure 1. Shear-thinning rheological profile of the optimized chitosan-based tranexamic acid (TXA) spray

Apparent viscosity (mPa·s) was measured at rotational speeds ranging from 10 to 100 rpm at $25 \pm 1^\circ\text{C}$ using a rotational viscometer equipped with a coaxial cylinder spindle. Data are presented as mean $\pm$ standard deviation (SD) of triplicate measurements ($n=3$). The progressive decrease in viscosity with increasing shear rate demonstrates characteristic shear-thinning (pseudoplastic) behavior, which facilitates atomization during spraying while maintaining adequate viscosity for tissue retention.

mPa·s (Figure 1), indicating reduced flow resistance under increasing shear. This rheological profile is consistent with chitosan-based polymeric systems. It supports efficient atomization during spraying while maintaining relatively higher viscosity under low-shear conditions, which may favor prolonged residence at the application site.

Spray pattern and dose delivery uniformity

The optimized formulation achieved consistent spray characteristics and uniform dose delivery. The formulation generated a homogeneous circular spray pattern with a mean diameter of 3.2 cm and a central deposition zone of approximately 2.6 cm. No evidence of nozzle blockage or spray pattern distortion was observed during repeated actuations. Gravimetric assessment revealed a mean delivered dose of 118.7 ± 0.4 mg per actuation, equivalent to 5.94 ± 0.02 mg of TXA per actuation for the 5% (w/w) formulation (Table 2). The delivered dose exhibited an RSD

Table 2. Delivered dose uniformity and tranexamic acid (TXA) content per actuation

Actuation No.	Delivered dose (mg)	TXA (mg/actuation)
1	118.6	5.93
2	119.3	5.97
3	117.9	5.90
4	118.8	5.94
5	119.1	5.96
6	118.2	5.91
7	119.0	5.95
8	118.4	5.92
9	118.7	5.94
10	118.5	5.93
Mean $\pm$ SD	118.7 ± 0.4	5.94 ± 0.02
RSD (%)	0.34	0.34

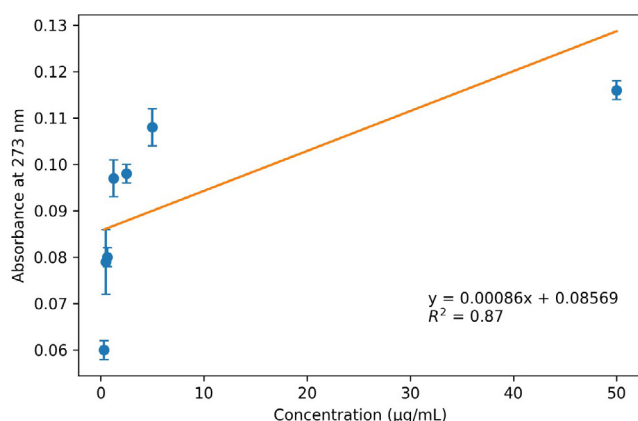


Figure 2. UV-Visible spectrophotometric calibration curve of tranexamic acid (TXA) based on the Beer-Lambert relationship

Absorbance was measured at the maximum absorption wavelength (λ_{max}) of 273 nm using a 1-cm quartz cuvette against distilled water as the blank. TXA standard solutions were prepared over the concentration range of 0.3125–50 $\mu\text{g/mL}$. Each data point represents the mean absorbance $\pm$ standard deviation (SD) of triplicate measurements ($n=3$). Linear regression analysis yielded the equation $y=0.00086x+0.08569$, with a coefficient of determination (R^2) of 0.87. This calibration curve was used to determine TXA content in the optimized spray formulation quantitatively.

of 0.34%, demonstrating excellent dose uniformity.

Quantitative determination of TXA and accelerated stability

The UV-Visible spectrophotometric method was used to quantify TXA in the optimized bio-adhesive spray formulation. Spectral scanning of TXA over the wavelength range of 190–400 nm identified a maximum absorption wavelength (λ_{max}) at 273 nm, which was subsequently selected for quantitative analysis.

A calibration curve was established using TXA standard solutions over the concentration range of 0.3125–50 $\mu\text{g/mL}$. Mean absorbance increased with TXA concentration, ranging from 0.060 ± 0.002 at 0.3125 $\mu\text{g/mL}$ to 0.116 ± 0.002 at 50 $\mu\text{g/mL}$. Linear regression analysis yielded the equation ($y=0.00086x+0.08569$), with a coefficient of determination of ($R^2=0.87$) (Figure 2). The calibration data demonstrated a concentration-dependent increase in absorbance over the investigated concentration range.

The optimized bio-adhesive spray formulation was subsequently evaluated during storage at $25 \pm 2^\circ\text{C}$ for 30 days. The formulation was diluted appropriately before spectrophotometric analysis, and absorbance was measured at 273 nm. On day 1, the mean absorbance was 0.09000 ± 0.00120 , corresponding to a calculated TXA concentration of 5.01 $\mu\text{g/mL}$. Absorbance decreased slightly during storage, reaching 0.08995 ± 0.00130 on day 15 and 0.08992 ± 0.00140 on day 30, corresponding to calculated TXA concentrations of 4.95 and 4.92 $\mu\text{g/mL}$, respectively.

Relative to the initial measured concentration, the formulation retained approximately 98.8% and 98.2% of the initial TXA content on days 15 and 30, respectively. Thus, the spectrophotometrically determined TXA content showed only a modest reduction over the 30-day storage period, indicating relatively good short-term chemical stability under the tested storage conditions.

In parallel with drug-content determination, the study monitored the formulation's physical stability throughout. The study evaluated appearance, color, homogeneity, precipitation, phase separation, pH, and viscosity at predetermined sampling intervals. These assessments identified potential physicochemical changes that could compromise the bio-adhesive spray's stability or performance.

Overall, the optimized formulation showed limited variation in TXA content over 30 days of storage at $25 \pm 2^\circ\text{C}$, retaining more than 98% of the initial measured TXA content at the end of the study. These findings support the short-term stability of the developed bio-adhesive spray formulation and warrant further evaluation under longer-term and accelerated stability conditions.

In vivo hemostatic efficacy

The hemostatic effects of the three spray formulations were evaluated by measuring blood loss and bleeding time following standardized tail incision injury. Figure 3 presents individual animal-level measurements, and Tables 3 and 4 summarize group-level statistical comparisons. Figure 4 provides a schematic overview of the experimental setup, including the standardized rat tail-incision model, topical formulation application, and gravimetric blood loss assessment.

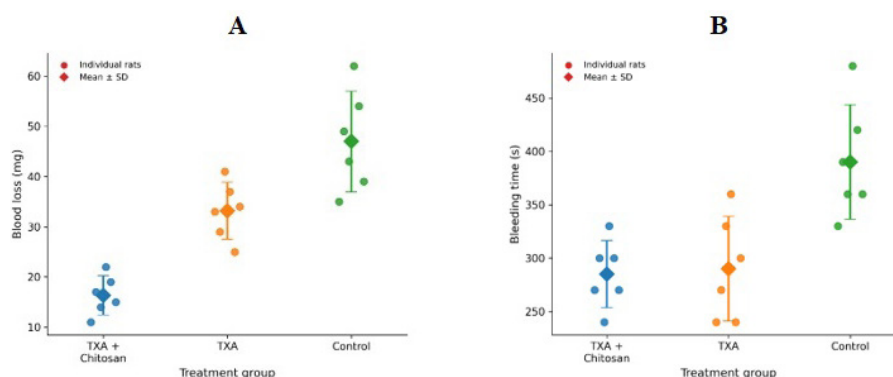


Figure 3. Hemostatic effects of tranexamic acid (TXA)-chitosan spray compared with TXA alone and untreated control in a standardized rat tail-incision bleeding model

Healthy adult male Wistar rats (8–10 weeks old, 220–250 g) were randomly allocated into three experimental groups ($n=6$ per group): TXA-chitosan spray, topical TXA solution (5% w/v), and untreated control (purified water). A standardized longitudinal incision (3 cm length, ~ 3 mm depth) was created on the ventral aspect of the tail, and the assigned treatment was applied immediately after injury. (A) Blood loss (mg) was quantified gravimetrically using pre-weighed filter papers applied at 30-second intervals until hemostasis. (B) Bleeding time (seconds) was calculated based on the number of blood spots and the sampling interval. Each dot represents an individual animal. Diamonds indicate group means, and error bars represent standard deviations (SD). Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's honestly

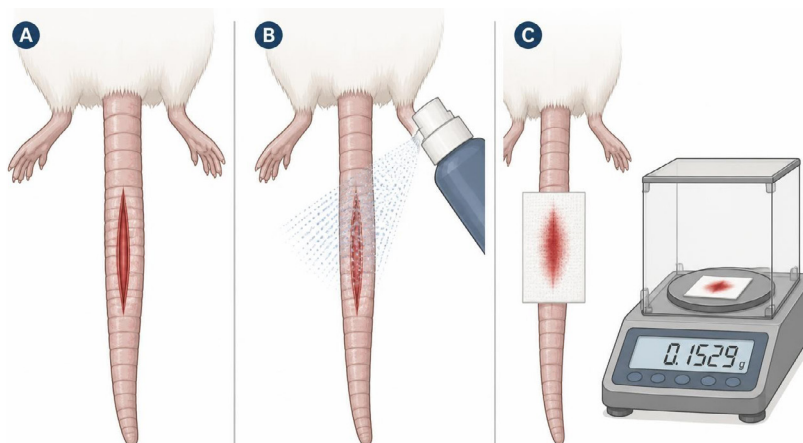


Figure 4. Schematic illustration of the *in vivo* rat hemostatic evaluation protocol

(A) Standardized longitudinal incision (3 cm length, ~3 mm depth) created on the ventral aspect of the rat tail. (B) Topical application of the assigned formulations (control, TXA, or TXA-chitosan spray) directly onto the wound bed using the spray device. (C) Gravimetric assessment of blood loss using pre-weighed filter papers applied at 30-second intervals to quantify hemostatic efficacy.

Time to hemostasis

Bleeding time differed significantly among the experimental groups (one-way ANOVA, $F(2,15)=10.07$, $P=0.002$, $\eta^2=0.57$).

The mean bleeding time was 285 ± 31 sec in the TXA+Chitosan group, 290 ± 49 sec in the TXA group, and 390 ± 54 sec in the untreated control group (Table 3). Pairwise comparison demonstrated no significant difference between the TXA+Chitosan and TXA groups (mean difference, -5.0 sec; 95% CI, -73.6 to 63.6 ; adjusted $P=0.980$).

However, both active formulations significantly reduced bleeding time compared with untreated control animals. TXA+Chitosan treatment decreased bleeding time by 105.0 sec compared with control (95% CI, -173.6 to -36.4 ; adjusted $P=0.03$), while TXA alone reduced bleeding time by 100.0 sec (95% CI, -168.6 to -31.4 ; adjusted $P=0.05$).

Taken together, topical administration of TXA-containing spray formulations significantly improved bleeding control in this rat tail incision model. Incorporating chitosan into the TXA formulation was associated with a further

reduction in blood loss, although the effect on bleeding time was comparable to TXA alone.

Bleeding volume

Blood loss differed significantly among the three experimental groups (one-way ANOVA, $F(2,15)=28.76$, $P<0.001$, $\eta^2=0.79$). The TXA+Chitosan group showed the lowest blood loss (16.3 ± 3.9 mg), followed by the TXA group (33.2 ± 5.7 mg), while the untreated control group had the highest blood loss (47.0 ± 10.0 mg).

Post hoc Tukey's HSD analysis confirmed significant differences across all pairwise comparisons. Compared with TXA alone, the TXA + Chitosan formulation reduced blood loss by 16.8 mg (95% CI, -27.4 to -6.3 ; adjusted $P=0.02$). Similarly, TXA+Chitosan treatment reduced blood loss by 30.7 mg compared with the untreated control group (95% CI, -41.2 to -20.1 ; adjusted $P<0.001$). The TXA group also showed significantly lower blood loss compared with control animals (mean difference, -13.8 mg; 95% CI, -24.4 to -3.3 ; adjusted $P=0.10$) (Table 4).

Table 3. Effects of tranexamic acid (TXA) and TXA -chitosan spray formulations on bleeding outcomes in rats

Outcome measure	TXA+Chitosan (n=6)	TXA (n=6)	Control (n=6)	F statistic (df=2,15)	P-value	η^2
Blood loss (mg)	16.3 ± 3.9	33.2 ± 5.7	47.0 ± 10.0	28.76	<0.001	0.79
Bleeding time (s)	285 ± 31	290 ± 49	390 ± 54	10.07	0.002	0.57

†Values are presented as mean $\pm$ standard deviation (SD). Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) *post hoc* test

Table 4. *Post hoc* comparisons of bleeding outcomes between rat experimental groups

Outcome measure	Comparison	Mean difference	95% CI	Adjusted P-value
Blood loss (mg)	TXA+Chitosan vs TXA	16.8	27.4 to -6.3	0.002
	TXA+Chitosan vs Control	30.7	41.2 to -20.1	<0.001
	TXA vs Control	13.8	24.4 to -3.3	0.010
Bleeding time (s)	TXA+Chitosan vs TXA	5.0	73.6 to 63.6	0.980
	TXA+Chitosan vs Control	105.0	173.6 to -36.4	0.003
	TXA vs Control	100.0	168.6 to -31.4	0.005

†Tukey HSD-adjusted pairwise comparisons are presented as mean differences with 95% confidence intervals
TXA: Tranexamic acid

Discussion

This study developed a bio-adhesive chitosan-based TXA spray that combines the antifibrinolytic activity of TXA with the intrinsic hemostatic and adhesive properties of chitosan. The optimized formulation demonstrated favorable physicochemical characteristics, including shear-thinning behavior, reproducible spray performance, high dose-delivery uniformity, and short-term stability. Importantly, both TXA-containing formulations significantly reduced bleeding time compared with the untreated control, whereas adding chitosan further reduced blood loss by 51% compared with topical TXA alone. These findings support the formulation's potential as a reproducible topical hemostatic dosage form that integrates therapeutic activity with pharmaceutical performance, rather than merely a chitosan-TXA hemostatic composition.

Our findings build upon and extend previous evidence supporting the hemostatic potential of chitosan-TXA systems. While the synergistic combination of TXA's antifibrinolytic activity and chitosan's intrinsic hemostatic properties is a recognized strategy, the choice of delivery platform critically influences clinical utility. For instance, Sasmal and Datta (2019) developed TXA-loaded chitosan electrospun nanofibers that showed excellent hemostatic properties and controlled drug release; however, solid nanofiber mats suit accessible, flat wound beds and may lack the rapid deployability needed for irregular or deep traumatic wounds (15). Similarly, Gholizadeh *et al.* (2019) reported a thermosensitive *in situ* gel of a chitosan-based nasal spray loaded with TXA, which effectively optimized spray pattern, droplet deposition, and local biological effects for mucosal surfaces (23). While highly effective for specific anatomical sites, thermosensitive gels may present viscosity and sprayability challenges for general topical trauma applications compared with optimized liquid formulations.

Furthermore, it is important to acknowledge prior art, such as the patent WO2019053579A1, which discloses the general concept of topical spray compositions comprising TXA and chitosan for hemorrhage control (20). Therefore, the primary contribution of the present study is not introducing the TXA-chitosan spray concept itself, but systematically optimizing its formulation and comprehensively characterizing it, which are prerequisites for clinical translation.

Specifically, this work advances beyond previously disclosed concepts by providing rigorous, quantifiable data on critical performance parameters not fully detailed in the prior art. These include: (1) the demonstration of favorable shear-thinning rheological behavior that facilitates atomization while promoting tissue retention; (2) precise spray pattern metrics (mean diameter of 3.2 cm); (3) exceptional dose-delivery uniformity (RSD of 0.34%), ensuring reproducible drug administration; and (4) verified short-term physicochemical stability. Most importantly, our *in vivo* evaluation provides direct, quantitative evidence that this systematically optimized spray formulation reduces blood loss by 51% compared to topical TXA alone, moving the TXA-chitosan spray from a theoretical disclosure to a robust, evidence-based topical hemostatic platform.

A key contribution of this work is integrating therapeutic activity with pharmaceutical performance. Beyond demonstrating hemostatic efficacy, we characterized rheological behavior, spray pattern, delivered dose, dose uniformity, drug content, and short-term stability, addressing several formulation attributes required for a

practical topical delivery system. The low variability in delivered dose (RSD, 0.34%) further supports reproducible administration. This distinction is important because successful translation of a hemostatic material requires not only biological activity but also reliable delivery to the bleeding surface.

The differential effects on bleeding time and blood loss provide an additional clinically relevant observation. Although TXA and TXA-chitosan produced comparable reductions in bleeding time, the chitosan-containing formulation reduced blood loss by approximately 51% relative to TXA alone. This finding suggests that chitosan's main contribution may not be faster initial clot formation, but rather improved persistence or stabilization of the local hemostatic interface. TXA can inhibit fibrinolysis and preserve the developing fibrin network, whereas chitosan may provide complementary local hemostatic and adhesive effects. The combination may therefore better maintain the developing clot after initial hemostasis. However, this interpretation remains mechanistic and should be confirmed by direct assessment of tissue retention, clot architecture, and local TXA exposure.

From a pharmaceutical perspective, the observed shear-thinning behavior further strengthens the rationale for the spray platform. Decreased viscosity under increasing shear may facilitate atomization during actuation, whereas higher viscosity under low-shear conditions may favor retention after deposition. Coupled with the highly reproducible delivered dose, these characteristics suggest that the formulation can combine ease of topical application with controlled drug deposition. Thus, compared with previously reported chitosan-TXA solutions, adducts, and particulate systems, this work advances the concept toward a more readily deployable topical dosage form.

Several limitations should be considered. The *in vivo* evaluation was conducted in a small-animal tail-incision model, which cannot fully reproduce the complexity of traumatic or surgical hemorrhage in humans. The stability assessment was limited to 30 days, and the authors did not directly measure bio-adhesion, local TXA retention, release kinetics, or clot architecture. Accordingly, the proposed contribution of chitosan to blood-loss reduction should be regarded as a biologically plausible interpretation rather than a demonstrated mechanism. Future studies should evaluate long-term stability, tissue adhesion, local drug retention, clot structure, safety, and efficacy in clinically relevant bleeding models.

Conclusion

This study provides proof of concept for a reproduced and optimized chitosan-based bio-adhesive TXA spray as a localized topical hemostatic platform. By integrating the antifibrinolytic activity of TXA with the intrinsic hemostatic properties of chitosan, the formulation offers a rational strategy for sustained drug retention at the bleeding site. The optimized spray demonstrated favorable physicochemical characteristics, excellent dose uniformity, and short-term stability. Most importantly, it substantially enhanced blood-loss control compared to TXA alone in an *in vivo* model. These findings support the further translational development of this system as a practical, locally applied hemostatic intervention for trauma and surgical bleeding.

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Limitations

Several limitations warrant acknowledgment. First, the *in vivo* hemostatic evaluation was conducted in a small-animal tail-incision model, which cannot fully reproduce the complexity of traumatic or surgical hemorrhage in humans, including hemodynamic variables, coagulopathy, and wound geometry. Therefore, the translational relevance of these findings should be confirmed in clinically relevant large-animal bleeding models. Second, the stability assessment was limited to 30 days under controlled storage conditions, and long-term stability evaluation remains necessary. Third, although chitosan is widely recognized for its mucoadhesive properties, this study did not directly quantify bioadhesion (e.g., detachment force, work of adhesion, or *ex vivo* tissue-retention assays). Accordingly, the contribution of bioadhesion to the observed reduction in blood loss should be regarded as a biologically plausible mechanistic hypothesis rather than a demonstrated mechanism. Fourth, direct measurements of local TXA retention, release kinetics, and clot architecture were not conducted, and these parameters require dedicated experimental validation in future studies. Finally, while quantitative gravimetric and temporal endpoints were rigorously recorded in accordance with ARRIVE 2.0 guidelines, this pilot phase did not include systematic macroscopic photographic documentation of wound beds across different bleeding severities. Future comprehensive studies should incorporate standardized visual documentation (e.g., macroscopic or microscopic imaging) to complement and further validate these quantitative hemostatic assessments.

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Authors' Contributions

E M contributed to conceptualization, study design, formulation development, experimental work, data collection, data analysis, and writing the original draft. G K contributed to study design, methodology, supervision of experimental procedures, data interpretation, and critical revision of the manuscript. M MAB contributed to conceptualization, methodology, statistical analysis, interpretation of results, and critical review and editing of the manuscript. M I contributed to study design, project supervision, validation of analytical methods, interpretation of findings, and critical revision of the manuscript. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Conflicts of Interest

The authors declare that they have no competing interests.

Declaration

The authors used OpenAI solely to improve the English language, grammar, and readability of the manuscript. No AI tools were used to generate scientific content, analyze data, interpret results, or draw conclusions. The authors reviewed and revised the final manuscript and take full responsibility for its content.

References

1. Bernhardt IM, Moore EE, Sauaia A, Cotton BA, Cannon JW, Schreiber MA, *et al.* Timing of trauma deaths due to uncontrolled

bleeding have not changed in three decades: A multicenter study of patients in hemorrhagic shock. *Am J Surg* 2025;250:116510.

2. Ter Avest E, Carenzo L, Lendrum RA, Christian MD, Lyon RM, Coniglio C, *et al.* Advanced interventions in the pre-hospital resuscitation of patients with non-compressible haemorrhage after penetrating injuries. *Crit Care* 2022;26:184.

3. Jamal L, Saini A, Quencer K, Altun I, Albadawi H, Khurana A, *et al.* Emerging approaches to pre-hospital hemorrhage control: A narrative review. *Ann Transl Med* 2021;9:1192.

4. Barrett WJ, Kaucher KA, Orpet RE, Campion EM, Goodloe JM, Fischer PE, *et al.* Tranexamic acid in trauma: A joint position statement and resource document of NAEMSP, ACEP, and ACS-COT. *J Trauma Acute Care Surg* 2025;99:357-363.

5. Roberts I, Shakur H, Coats T, Hunt B, Balogun E, Barnetson L, *et al.* The CRASH-2 trial: A randomised controlled trial and economic evaluation of the effects of tranexamic acid on death, vascular occlusive events and transfusion requirement in bleeding trauma patients. *Health Technol Assess* 2013;17:1.

6. Peng HT, Kastrup CJ, Tenn C, Beckett A. Local delivery of tranexamic acid for hemorrhage control in trauma: A narrative review. *Front Drug Deliv* 2026;6:1771095.

7. Barrett WJ, Kaucher KA, Orpet RE, Campion EM, Goodloe JM, Fischer PE, *et al.* Prehospital trauma compendium: Tranexamic acid in trauma - A joint position statement and resource document of NAEMSP, ACEP, and ACS-COT. *Prehosp Emerg Care* 2025;1-8. doi: 10.1080/10903127.2025.2497056. Online ahead of print

8. Cai J, Ribkoff J, Olson S, Raghunathan V, Al-Samkari H, DeLoughery TG, Shatzel JJ. The many roles of tranexamic acid: An overview of the clinical indications for TXA in medical and surgical patients. *Eur J Haematol* 2020;104:79-87.

9. Artykbay S, Susantitaphong P, Tantavisut S. Efficacy and safety of topical tranexamic acid in elderly hip fractures undergoing surgical treatment: Meta-analysis of randomized controlled trials. *Clin Orthop Surg* 2025;17:16-28.

10. Aoki M, Goto Y, Ogawa S, Baba H, Okawa Y. Can local administration of tranexamic acid reduce aspirin-induced bleeding in off-pump coronary artery bypass grafting? *Kyobu Geka* 2013;66:1119-1125.

11. Boccio E, Hultz K, Wong AH. Topical tranexamic acid for hemostasis of an oral bleed in a patient on a direct oral anticoagulant. *Clin Pract Cases Emerg Med* 2020;4:146-149.

12. Ausen K, Fossmark R, Spigset O, Pleym H. Safety and efficacy of local tranexamic acid for the prevention of surgical bleeding in soft-tissue surgery: A review of the literature and recommendations for plastic surgery. *Plast Reconstr Surg* 2022;149:774-787.

13. Ker K, Beecher D, Roberts I. Topical application of tranexamic acid for the reduction of bleeding. *Cochrane Database Syst Rev* 2013;2013:CD010562.

14. Baylis JR, Lee MM, St John AE, Wang X, Simonson E, Cau M, *et al.* Topical tranexamic acid inhibits fibrinolysis more effectively when formulated with self-propelling particles. *J Thromb Haemost* 2019;17:1645-1654.

15. Sasmal P, Datta P. Tranexamic acid-loaded chitosan electrospun nanofibers as drug delivery system for hemorrhage control applications. *J Drug Deliv Sci Technol* 2019;52:559-567.

16. Yu L, Luo Z, Chen T, Ouyang Y, Xiao L, Liang S, *et al.* Bioadhesive nanoparticles for local drug delivery. *Int J Mol Sci* 2022;23:2370.

17. Cassano R, Perri P, Scarcello E, Piro P, Sole R, Curcio F, Trombino S. Chitosan hemostatic dressings: Properties and surgical applications. *Polymers* 2024;16:1770.

18. Aranaz I, Alcántara AR, Civera MC, Arias C, Elorza B, Heras Caballero A, Acosta N. Chitosan: An overview of its properties and applications. *Polymers* 2021;13:3256.

19. Antonyuk V, Manko N, Panchak L, Khomyak S, Stoika R. Chitosan adduct with tranexamic acid and its haemostatic effect. *Prog Chem Appl Chitin Deriv* 2022;27:35-42.

20. Rangabhatla RPAPRGLA, inventor. Tranexamic acid spray for knee arthroplasty. World Patent WO2019053579A1. 2019.

21. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT,

Baker M, *et al.* The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *J Cereb Blood Flow Metab* 2020;40:1769-1777.

22. Li D, Li P, Zang J, Liu J. Enhanced hemostatic performance of tranexamic acid-loaded chitosan/alginate composite microparticles. *J Biomed Biotechnol* 2012;2012:981321.

23. Gholizadeh H, Messerotti E, Pozzoli M, Cheng S, Traini D, Young P, Kourmatzis A, *et al.* Application of a thermosensitive in situ gel of chitosan-based nasal spray loaded with tranexamic acid for localised treatment of nasal wounds. *AAPS PharmSciTech* 2019;20:299.