

Development of *in situ* poloxamer-chitosan hydrogels for vaginal drug delivery of benzydamine hydrochloride: Textural, mucoadhesive and *in vitro* release properties

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ABSTRACT

The use of *in situ* hydrogel systems for vaginal drug delivery is an important strategy in the treatment of vaginitis. The aim of this study was to develop *in situ* vaginal hydrogels for benzydamine hydrochloride (BNZ) which were composed of poloxamer and chitosan. The reason for development of these hydrogels was to obtain a vaginal delivery system with improved mechanical and mucoadhesive properties that could provide prolonged retention time for the treatment of vaginitis. The hydrogels were also designed for once a day dosage of the drug and to obtain a controlled release of the BNZ. For this purpose BNZ containing hydrogel formulations using thermosensitive polymer (Poloxamer 407) and mucoadhesive polymer (Chitosan H, Chitosan M and Chitosan L) were developed. The hydrogels are composed of polymers which have promising potential for vaginal delivery systems. These formulations were evaluated by rheology, texture, mucoadhesive profiles and drug

diffusion with a Franz diffusion cell. It was observed that the diffusion of BNZ from the *in situ* poloxamer-chitosan hydrogel was more sustained and controlled than with the poloxamer gel. The hydrogel formulations diffused about 65.3 ± 5.1 , 80.6 ± 3.8 , 88.1 ± 7.3 and $100.4 \pm 4.8\%$ of BNZ at 6h. The formulation containing Poloxamer 407 and Chitosan H has the best controlled release profile and mucoadhesive properties, results which indicate that it could suitable for use once a day on the vaginal route. Moreover, the hydrogels higher mucoadhesion exhibited by this formulation prolongs the drug residence time compared with the poloxamer gel and may increase the BNZ efficacy. These BNZ mucoadhesive vaginal hydrogel formulations may be a promising alternative to conventional dosage forms for vaginal topical therapy.

Key words: Mucoadhesion; poloxamer; chitosan; hydrogel; benzydamine hydrochloride; vaginal drug delivery.

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1. INTRODUCTION

The vagina is the site of various pathologies such as vaginitis caused by bacteria, fungi, protozoa or virus and has been traditionally employed for the delivery of several drugs. Lately, the vagina has been utilized as a possible alternative to the parenteral administration route for the delivery of drugs [1]. Vaginal dosage forms have been developed for many drugs because of the vagina presents several advantages as a site for drug delivery, such as having large surface area, rich blood supply, avoidance of the first-pass effect, relatively high permeability to several drugs, and self-insertion [2]. It should also allow self-administration with minimal interference on body functioning and daily life, and obtain high bioavailability with other medications. The rate and extent of drug absorption after intravaginal administration may vary, depending on formulation factors, vaginal physiology, age of the patient and menstrual cycle. Suppositories, creams, gels, tablets and vaginal rings are commonly used as vaginal

drug delivery systems [3]. Gel formulations are frequently preferred for the vaginal application of various drugs with different effects, such as moisturizing and lubrication effect, physiological pH restoring effect, and also for contraception, labor induction and microbicide activity [4-5]. Different types of polymers are used when preparing mucoadhesive vaginal gels. Many mucoadhesive polymers are suggested in the literature and used in commercial formulations for vaginal use. These mucoadhesive polymers include; polyacrylic acid derivatives such as carbomer and polycarbophil, cellulose derivatives, chitosan and its derivatives, hyaluronic acid, alginate, carrageenan and guar gum [6]. Additionally, an increasing number of *in situ* gel forming systems have been reported in the literature for various pharmaceutical and biomedical applications. Recently, *in situ* gels have also been proved as more appropriate dosage forms for topical applications because they are easy to administer into body cavities. In response to environmental conditions such as pH, temperature or ionic strength of the medium, a polymer solution turns into a gel form [7]. *In situ* gel forming systems based on temperature variation, termed thermogelling systems, present certain advantages; for example they do not need the organic solvents, cross linking agents or external agents for gelling onset. Gelling occurs in response to a temperature increase from ambient to physiological temperature [8]. The sol-gel transition of thermogels occurs at body temperature [9]. Thermogelling systems have been developed to increase distribution in vaginal administration, as they exhibit high spreadability as liquid form at room temperature, and a sol-gel transition when at physiological body temperature. Moreover, once gelation occurs, these systems may exhibit mucoadhesion, and so improve retention time in the vaginal cavity. The specific gelation temperature of each thermogelling system is a crucial parameter for their performance, being expected to lie within the range of 25-37 °C [10, 11]. Poloxamers are synthetic nonionic triblock polymers of polyoxyethylene and polyoxypropylene copolymers which are synthetic polymers with thermoreversible behavior in aqueous solutions and are widely used in pharmaceutical systems [7]. Pharmaceutical grade poloxamers are available in Europe under the brand name Lutrol® and in the USA under the brand name Pluronic® (BASF), Poloxamer 407 and Poloxamer 188 being the most widely used in the literature. Since their mucoadhesiveness is low, poloxamers are usually combined with more bioadhesive polymers. To increase the mucoadhesion properties, thermogel systems based on mixtures of poloxamer and mucoadhesive polymers such as poly (acrylic acid), alginat,

hyaluronic acid, HPMC and chitosan have been developed [12-16].

Chitosan is a hydrolyzed polysaccharide derivative of chitin, a biopolymer widespread in nature, that is non-toxic, biocompatible and biodegradable [17]. It has many additional properties useful for drug delivery systems and, for of these reasons, it is often preferred in the preparation of buccal and vaginal mucoadhesive dosage forms [6]. Some studies aiming to improve the utility of poloxamer gels in drug delivery systems have focused on the mucoadhesive polymer chitosan, because of its mechanical and mucoadhesive properties. The addition of chitosan has been found to improve the mechanical and mucoadhesive properties of thermosensitive (poloxamer) gels [16].

Benzydamine hydrochloride (BNZ) is a topical non-steroidal anti-inflammatory agent that also has local anesthetic activity [18]. Topical application of BNZ can offer some advantages in the treatment of vaginitis in terms of a reduced administered dose, high drug concentrations placed only on the pathological site, and the reduced side effects often associated with systemic administration [19]. BNZ was used as a model drugs in this study because of these properties.

The aim of this study was to develop a vaginal delivery system for BNZ, using poloxamer-chitosan *in situ* hydrogels, taking advantage of hydrogels improved mechanical and mucoadhesive properties, and their retention time, in order to optimize the topical effect of the drug. For this work, poloxamer and different types of chitosan were used to prepare the *in situ* forming hydrogels. Poloxamer was the gelling agent while chitosans were used as a mucoadhesive agent. The rheological and mechanical properties, as well as the mucoadhesive ability of the poloxamer hydrogels as a function of chitosan type, were evaluated.

2. MATERIALS AND METHODS

Materials

The BNZ was a gift from Abdi İbrahim İlaç San. AŞ. (Turkey) and all the other chemicals used were of analytical grade and used as received. Hydrogel formulations containing BNZ were prepared using four polymers: Chitosan-Highly-Viscous (Chitosan H) (MW: 310,000-375,000 Da, degree of deacetylation (DD): >75%, viscosity: 800-2000 cP 1wt. % in 1% acetic acid, 25 °C, Brookfield) (Sigma Aldrich, USA), Chitosan-Middle-Viscous (Chitosan M) (MW: 210,000-275,000 Da, DD: 75-85%, viscosity: 200-800 cP 1wt. % in

1% acetic acid, 25 °C, Brookfield) (Sigma Aldrich, USA), Chitosan-Low-Viscous (Chitosan L) (MW: 50,000-190,000 Da, DD: >75%, viscosity: 20-300 cP 1wt. % in 1% acetic acid, 25 °C, Brookfield) (Sigma Aldrich, USA) and Poloxamer 407 (Pluronic F 127 = Lutrol® F 127) (BASF, Germany).

Preparation of Hydrogel Formulations

When preparing the *in situ* poloxamer-chitosan hydrogel, initially chitosan was initially dissolved in a solution of glacial acetic acid (GAA) 1% v/w. GAA was added to the half of the water to be used and the required amount of chitosan was added to acidic water and mixed slowly. After the chitosan swelling, the rest of the water, containing BNZ, was added and mixed at 500 rpm until a homogenous gel was obtained at +4 °C. After this, poloxamer was added to the chitosan solution at +4 °C and mixed. The compositions of the *in situ* poloxamer-chitosan hydrogel formulations are shown in Table 1.

Thermosensitive BNZ hydrogel was prepared by using the cold method [20]; briefly, poloxamer was added to purified water which contained BNZ at +4 °C and gently mixed. Table 1 shows the contents of the thermosensitive BNZ hydrogel.

Measurement of gelation temperature

This test was performed to determine if the sol-gel transition temperature of the formulations was appropriate for vaginal administration. For the determination of the sol-gel transition temperature a transparent vial containing a magnetic bar and hydrogel was placed in a water bath at 4°C. Each vial was heated 2°C every hour and continuously stirred at 300 rpm. The hydrogels were heated within a range of 4–50°C during the procedure. The temperature at which the rotation of the magnetic bar stopped was measured as the gelation temperature [21].

Drug Content, pH Measurements and Viscosity Studies

One milliliter of *in situ* poloxamer-chitosan hydrogel formulation (0.15 % BNZ) was weighed and dissolved with 30 mL of pH 4.5 citrate phosphate buffer solution. The solution was then left in the ultrasonic bath for 15 minutes, after which it was filtered. The concentration of BNZ in this solution was determined by spectrophotometer at 306 nm. The pH values of the hydrogels were measured with a pH meter (OHAUS, Switzerland) at room temperature 24 h after preparation, and discarding air bubbles. The viscosity of the *in situ* poloxamer-chitosan hydrogel formulations was determined at a temperature of $37.0 \pm 0.5^\circ\text{C}$ with a viscometer (Brookfield DV-III+Rheometer TC-502 Temperature Controller, USA). The spindle speed was set to provide a torque value in the 10–100% range, in accordance with the Brookfield instruction manual. The spindle number and speed used were: Spindle No. 52 and 20 rpm.

Drug Diffusion Studies

The diffusion of BNZ from hydrogel was evaluated by using Franz diffusion cell system. The *in situ* hydrogel was placed to the donor compartment of a Franz diffusion cell system and the temperature was adjusted to 37°C. The diffusional sectional area was 1 cm² and the receptor phase volume was 2.5mL. A dialysis membrane (Sigma®, USA) with a 12,000 Da pore size was used as the diffusion membrane between donor and receptor phase. The receptor phase containing citrate phosphate buffer (pH 4.5) was stirred by magnetic bars at 37°C. Samples were taken periodically from the receptor phase. The received samples were determined spectrophotometrically at 306 nm.

Texture Profile Analysis (TPA)

Hardness, compressibility, adhesiveness, cohesiveness and elasticity of *in situ* hydrogel formulations were determined

Table 1. The composition of hydrogel formulations (BNZ: Benzylamine hydrochloride GAA: glacial acetic acid)

Codes	BNZ %	GAA (%)	Chitosan H (%)	Chitosan M (%)	Chitosan L (%)	Poloxamer 407 (%)
F1	0.15	1	1	-	-	20
F2	0.15	1	-	1	-	20
F3	0.15	1	-	-	1	20
F4	0.15	-	-	-	-	20

using a TA-XT Plus Texture Analyzer (Stable Micro Systems, London, UK) equipped with a 5-kg load cell in TPA mode [22]. The hydrogels were packed into bottles (8 cm) within the ultrasonic water bath to remove air bubbles, at 37 °C. An analytical probe (1 cm diameter) was then twice compressed into each hydrogel formulation to a defined depth (15 mm), and rate (2 mm s⁻¹), with a defined delay period (15 s), between the beginning of the second and the end of the first compression. Using the force–time plot, several mechanical parameters such as hardness (N), compressibility (N mm), adhesiveness (N mm), cohesiveness and elasticity of the hydrogel formulations were determined. These mechanical parameters were calculated according to our previous work [23]. These mechanical parameters were determined and defined as described in the following sentences: Hardness is the force required to attain a given deformation or as the maximum peak force during the first compression cycle and was performed to measure the required force to produce deformation of the gels. Compressibility is the work required to deform the product during the first compression of probe. Adhesiveness is the negative force area for the first compression cycle and represents the work required to overcome the attractive forces between the surface of the gel and the surface of the probe. Cohesiveness is the ratio of the area under the force-time curve produced on the second compression cycle to that produced on the first compression cycle, it is the determination of the reconstruction ability of the gel after application. Elasticity is the ratio of the time required to achieve maximum structural deformation on the second compression cycle to that on the first compression cycle, and also can be defined as the direction of reconstruction of the gel after its deformation by compression over a defined period of time.

Ex-vivo Mucoadhesion studies

Ex-vivo mucoadhesion testing of *in situ* poloxamer-chitosan hydrogel was conducted using a TA-XT Plus Texture Analyzer with a mucoadhesive holder as in a previously work [24]. Freshly obtained cow vaginal mucosa was frozen at –20 °C and then a 2 mm thick section was taken from the inner part of the surface of this mucosa and attached to the lower end of the probe (P 0.5 Perspex: 12.5 mm) of the instrument with cyanoacrylate glue. The mucosa was dipped into the vaginal fluid simulant [25] and kept for 10 min until the start of the experiment. The instrumental parameters specified in a previous study [21] were used to evaluate the mucoadhesive potential of the vaginal gel formulations. The area under the curve was calculated from a force-distance plot as the work

of mucoadhesion. Equation 1 was used to calculate the work of mucoadhesion per cm² (mJ.cm⁻²). (πr^2 : the area of the mucosal surface being in contact with hydrogel)

$$\text{Work of mucoadhesion (mJ/cm}^2\text{)} = \frac{\text{AUC}}{\pi r^2} \quad (\text{Eq. 1})$$

3. RESULTS and DISCUSSION

Measurement of gelation temperature

In this study control of the gelation temperature (T_{gel}) of hydrogels which combined Poloxamer 407 and different types of chitosan was determined. According to the results shown in Table 2, the gelation temperature of the hydrogel formulations varied between 25 and 32°C, and they formed gels at body temperature. The gelation temperature of all the formulations are the suitable for vaginal application. The human vaginal temperature is 37.2°C, so the T_{gel} of the vaginal thermoreversible gels were considered to be suitable in the range of 25–37°C. If the T_{gel} is lower than 25°C, a gel might be formed at room temperature, leading to difficulties in manufacturing, handling, and administering. If the T_{gel} is higher than 37°C, a liquid dosage form still exists at vaginal temperature, resulting in drainage of the formula from the vagina at an early stage [26].

Drug Content, pH Measurements and Viscosity Studies

The prepared *in situ* poloxamer-chitosan hydrogel formulations were transparent and had a homogeneous structure. The drug content, pH and viscosity values of the hydrogel formulations are shown in Table 2. The pH values of the hydrogel formulations ranged from 3.9 to 5.1 and were deemed suitable for vaginal administration. Vaginal pH appears to play an important role in the efficacy of vaginally administered drugs [27].

Combinations of Poloxamer 407 and different types of chitosan were investigated for any differences in hydrogel viscosity. F1 hydrogel formulation which was prepared with the highest molecular weight chitosan exhibited significant increases in viscosity than other formulation. Similar viscosity and gelation temperature results for combinations of with Chitosan MMW (MW: 190,000-310,000 Da; DD: 75–85%) and Poloxamer 407 have previously been reported [16]. In this study, all the prepared hydrogels showed a pseudo-plastic flow behavior, presenting an immediate flow after stress application (Figure 1).

Table 2. Results of drug content, viscosity, and pH values of the hydrogels formulations. The data represent the mean±standard deviation (SD), n=6.

Code	BNZ (%) ± SD	Viscosity (cP) ± SD	pH	Gelation Temperature (°C) ± SD
F1	100±0	949±5	3.9	>31.8±0.6
F2	101±0	491±8	4.1	>30.1±0.3
F3	102±0	462±11	3.9	>28.6±0.4
F4	102±0	423±7	5.1	>25.2±0.0

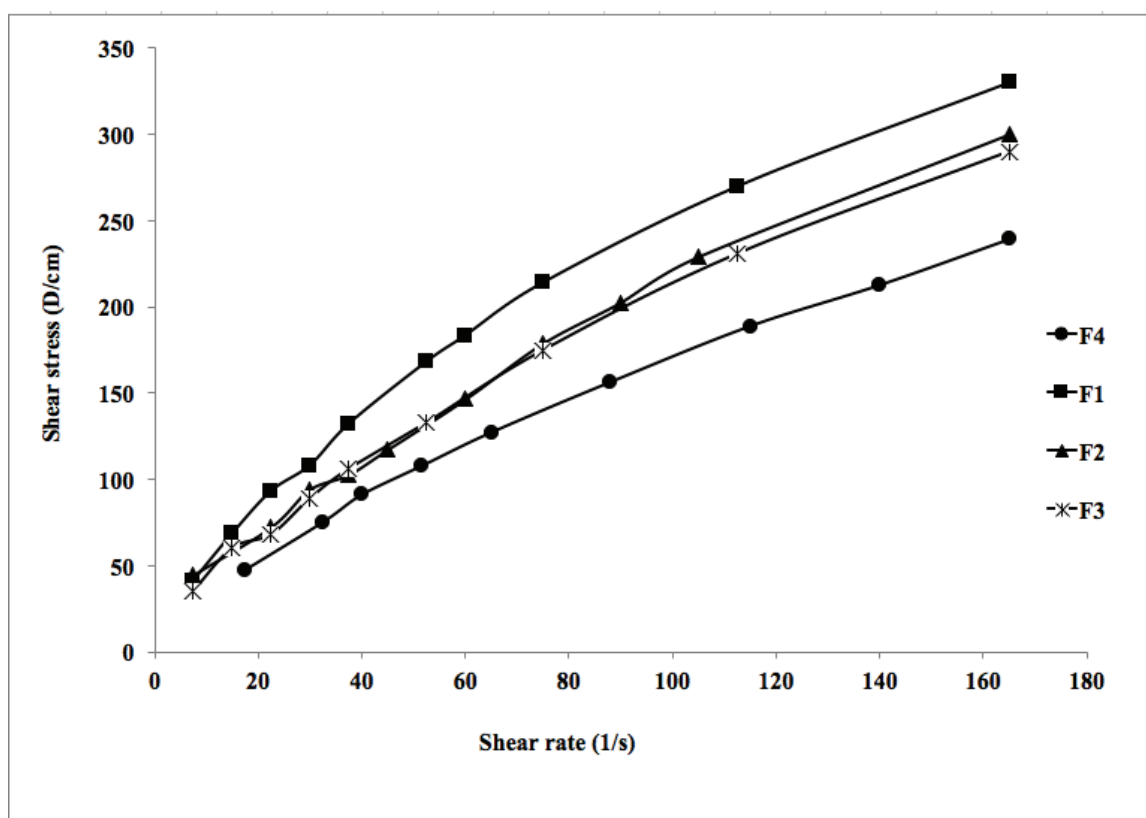


Figure 1. Flow reograms of the hydrogel formulations at 37 °C measured by rheometer (Brookfield DV-III + Rheometer TC-502 Temperature Controller, USA)

Drug Diffusion Studies

This study Franz diffusion cell system was used for all *in vitro* drug diffusion studies of the hydrogel formulations. BNZ diffusion from different hydrogels was examined through cellulose dialysis membrane. The release profiles of BNZ from thermosensitive poloxamer hydrogel (F4) and three hydrogels (F1, F2 and F3) containing a mixture of poloxamer 407 and different types of chitosan are shown in Figure 2. The total amount of drug was diffused from a poloxamer hydrogel formulation (F4) at the end of 6 h. A similar result was seen in the release of metoprolol from the poloxamer

gel [28]. Results for the BNZ-poloxamer hydrogel was not found in the literature. For this reason this study can bring novelty to the literature. It was observed that the release of BNZ from *in situ* poloxamer-chitosan hydrogels (F1, F2 and F3) was more sustained and controlled when compared to the poloxamer gel (F4). Since the viscosity of the F1 formulation is higher than F4, the drug release may be less. Similar results were obtained in our previous study [21, 23]. F1, F2, F3 and when F4 hydrogel formulations were released about 65.3±5.1, 80.6±3.8, 88.1±7.3 and 100.4±4.8 % of BNZ at 6h respectively. The F1 formulation has the

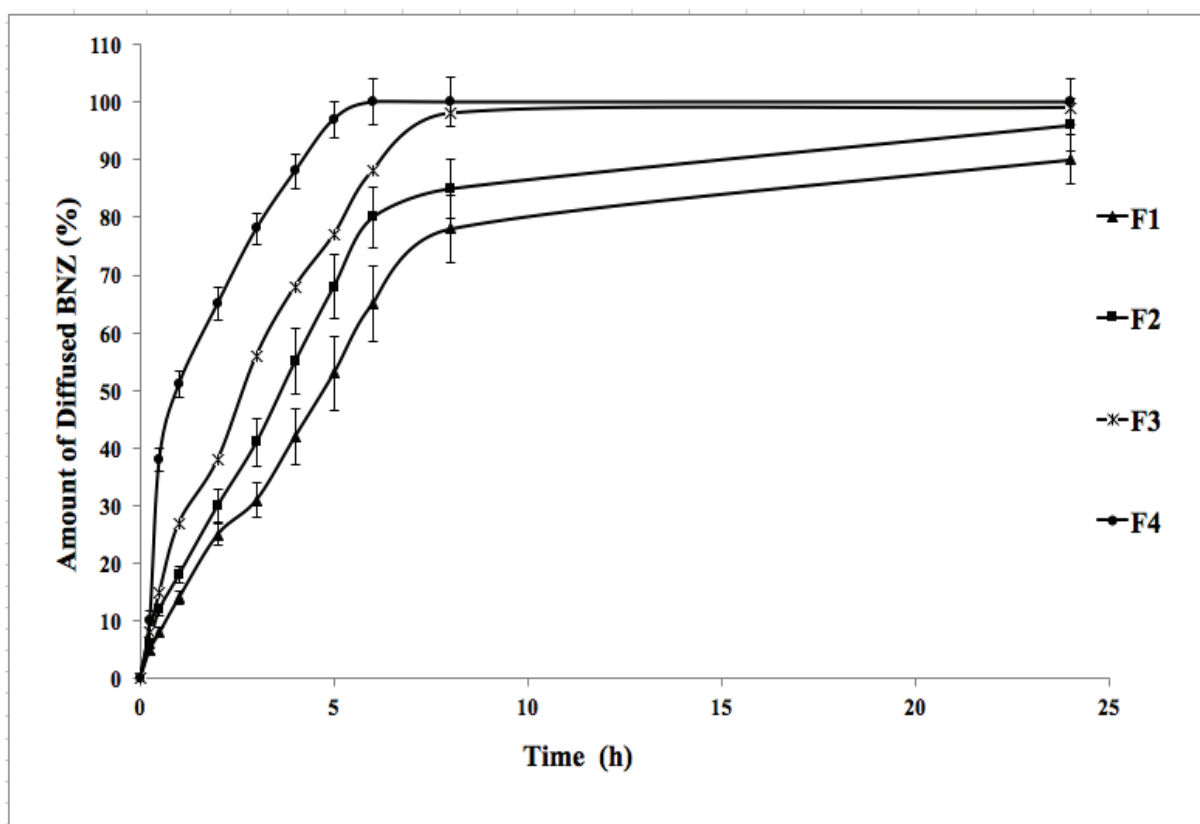


Figure 2. Diffusion profiles of BNZ from *in situ* poloxamer-chitosan hydrogel formulations through a dialysis membrane in pH 4.5 citrate-phosphate buffer using the Franz diffusion cells. Note: The data represent the mean \pm confidence interval, $n = 3$.

best controlled release profile in the study which makes it most suitable for use once a day on the vaginal route. The F1 hydrogel formulation was prepared using Chitosan H. The MW and viscosity value of the Chitosan H polymer are higher than the Chitosan M and Chitosan L polymers. The F1 formulation has a higher viscosity and a controlled BNZ release. Thus, considering the release of hydrogels during vaginal administration, as well as controlled release of BNZ, hydrogels made of Poloxamer 407 and Chitosan H would be the proper choice of vehicle.

Texture Profile Analysis (TPA)

TPA has been used in order to determine the mechanical properties of semi-solids, particularly of vaginal gels [29]. TPA can provide some important parameters such as the hardness, compressibility and adhesiveness of a pharmaceutical preparation [30]. To compare these with the mechanical properties of the hydrogels texture profile analyses were employed at 37°C. The results are given in Table 3.

Table 3. The mechanical properties of the hydrogel formulations measured with texture analyzer. The data represent the mean \pm standard deviation (SD), $n=3$.

Code	Hardness (N) \pm SD	Adhesiveness (N.mm) \pm SD	Cohesiveness \pm SD	Compressibility (N.mm) \pm SD	Elasticity \pm SD
F1	0.310 \pm 0.075	0.431 \pm 0.052	0.711 \pm 0.047	1.174 \pm 0.218	0.739 \pm 0.028
F2	0.247 \pm 0.105	0.292 \pm 0.176	0.572 \pm 0.126	0.849 \pm 0.327	0.615 \pm 0.397
F3	0.183 \pm 0.105	0.269 \pm 0.104	0.459 \pm 0.019	0.783 \pm 0.173	0.582 \pm 0.118
F4	0.168 \pm 0.004	0.238 \pm 0.018	0.411 \pm 0.027	0.648 \pm 0.274	0.429 \pm 0.048

The hardness test was performed to measure the force required to produce deformation of gels. Vaginal gels are required to have a low hardness value for ease of administration to the vagina [31]. The results showed that F1 had the highest hardness value. A low compressibility value is required to enable ease of removal of the gel from the container and ease of the spreadability of the gel on the application site [7]. In this study it was apparent that both concentration and type of polymer affected product compressibility. The results showed that the compressibility of the hydrogels, it increased from 0.648 ± 0.274 to 1.17 ± 0.218 according to the chitosan type. When the chitosan type used in the preparation of hydrogels were compared, the elasticity ranked in the order of Chitosan H > Chitosan M > Chitosan L. Cohesion introduces the measure of the reconstruction of the gel after application. Cohesiveness increases the performance of the product on the application site [32]. A high value of cohesiveness indicates full structural recovery following gel application. F1 formulations showed higher cohesiveness than other formulations. According to the polymer type, the cohesiveness of the *in situ* poloxamer-chitosan hydrogels was arranged in the order of F1 > F2 > F3 > F4. When the adhesiveness of the gel formulations prepared with different types of chitosan was examined, the increase in the chitosans MW and viscosity expanded the adhesiveness of gels. The highest adhesiveness was determined in the formulations including Chitosan H. Ideally, poloxamer-chitosan hydrogels as a vaginal formulation should have high adhesiveness, cohesiveness, and elasticity appropriate for application to the vaginal mucosa. The hydrogel formulations containing Chitosan H (formulation F1) showed maximum mechanical properties and the type of polymer used in the preparation affected the mechanical properties of the resultant hydrogel. The results suggest that the F1 hydrogel formulation may be with the most suitable mechanical properties for vaginal drug delivery systems.

Ex-Vivo Mucoadhesion Studies

Vaginal mucoadhesion relies on the type, molecular weight and architecture of polymer and the polymer concentration in the gel can influence the mucoadhesive performance of the formulation [31]. Chitosan is a linear polycation that readily adheres to negatively charged surfaces [33]. Interactions with mucin appear to be both electrostatic, via positively charged amino groups on the chitosan and negatively charged sialic acid residues of mucus glycoproteins or mucins, and/or hydrophobic, via methyl groups on acetylated chitosan residues with methyl groups on mucin side chains [34]. This

study it was evaluated whether the mucoadhesive properties of chitosan would be maintained even after being prepared with poloxamer. Therefore *ex vivo* mucoadhesion tests were performed. Mucoadhesive force here means the force required to detach the formulation from a fresh sample of cow vaginal mucosa. The results obtained for maximum detachment force (F_{max}), work of adhesion (W_{ad}) and work of mucoadhesion (W_{mucoad}) are shown in Table 4. The highest values were determined in the F1 formulation that included Poloxamer 407 (20%), Chitosan H (1%) and BNZ (0.15%).

Table 4. Mucoadhesion values of the hydrogel formulations using texture analyzer with cow vaginal mucosa (n = 6) (SD: Standart deviation).

Code	F_{max} (N)±SD	W_{ad} (Nmm) ±SD	W_{mucoad} (mj/cm ²) ±SD
F1	0.188±0.021	0.997±0.059	0.316±0.031
F2	0.170±0.019	0.785±0.016	0.250±0.018
F3	0.157±0.027	0.621±0.043	0.198±0.026
F4	0.128±0.047	0.574±0.016	0.183±0.047

The *in vitro* mucoadhesion tests confirmed that the Poloxamer 407 hydrogel (formulation F4) mucoadhesive properties were increased with chitosan. Statistical significant differences were observed for formulations containing Chitosan H, M and L. Thus, chitosan confers mucoadhesive properties to the *in situ* hydrogel.

4. CONCLUSION

In the present study, an *in situ* hydrogel with improved mechanical and mucoadhesive properties as well as improved retention time in the vagina, was obtained by the combination of poloxamer and chitosan. Based on this study's *in vitro* characterization, it was concluded that the *in situ* vaginal hydrogel of BNZ with Poloxamer 407 and Chitosan H may be a promising and innovative therapeutic system. *In situ* hydrogels containing BNZ can offer some advantages in the treatment of vaginitis treatment in terms of suitable controlled release, mucoadhesion, and mechanical properties. No poloxamer-chitosan hydrogel formulation containing BNZ has been found in the literature. For this reason this study can bring novelty to the literature.

Conflict of Interest

The author declares no conflict of interest.

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