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Natural polymers for vaginal mucoadhesive delivery of vinegar, using design of experiment methods

Prirodni polimeri za vaginalnu mukoadhezivnu primenu sirćeta, korišćenjem dizajna eksperimentalnih metoda

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Abstract

Background/Aim. Vinegar is one of the main international traditional nutraceuticals, and it has been used as a vaginal health protectant due to vagina pH balance maintenance and antimicrobial properties. Since the main form of vinegar is liquid, it is difficult to apply vaginally due to its short retention. The aim of this study was to design a vaginal mucoadhesive gel made of vinegar. **Methods.** Xanthan gum and tragacanth were utilized as natural gel-forming polymers. The effects of xanthan gum and tragacanth on mucoadhesion strength and drug release of the gel formulations were optimized using a 3 level (3^2) factorial design. Several physicochemical properties of the gel formulations, including gel viscosity, lubricity, scanning electron microscopy (SEM) images of hydrogel chains, and chain release kinetic, were also investigated. **Results.** It was found that tragacanth possessed a statistically significant effect on release rate control (p-value = 0.0027), while both tragacanth and xanthan gum had a significant effect (p-value = 0.0001 and 0.0017, respectively) on mucoadhesion property. Formulation F7 with 5% xanthan gum and 1% tragacanth (mucoadhesion = 0.4632 N and release rate = 88.8% in 6 hours) considered as the optimum formulation with some modifications. **Conclusion.** Xanthan gum and tragacanth can be considered as appropriate natural polymers for vaginal drug delivery.

Key words:
acetic acid; gels; polymers; tragacanth; vaginal creams, foams, jellies; xanthan gum.

Apstrakt

Uvod/Cilj. Sirće je jedno od glavnih internacionalnih tradicionalnih nutraceutika koje se koristi kao sredstvo za zaštitu vagine, zahvaljujući osobini da održava pH vagine i poseduje antibakterijska svojstva. S obzirom na to da je sirće tečnost, zbog kratkog zadržavanja je teško primenljiv vaginalno. Cilj rada bio je da se od sirćeta dizajnira vaginalni mukoadhezivni gel. **Metode.** U studiji su kao polimeri koji formiraju prirodni gel korišćeni ksantan guma i tragakant. Efekti ksantan gume i tragakanta na jačinu mukoadhezije i oslobađanje lekova u formulacijama gela optimizovani su korišćenjem faktorijalnog dizajna на 3 nivoa (3^2). Takođe, istraženo je nekoliko fizičko-hemijskih svojstava formulacije gela, uključujući viskoznost gela, mazivost, slike *scanning* elektronske mikroskopije lanaca hidrogela i kinetiku oslobađanja lanaca. **Rezultati.** Utvrđeno je da je tragakant imao statistički značajan uticaj na kontrolu brzine oslobađanja ispuštanja (p = 0,0027), dok su i tragakant i ksantan guma imali značajan uticaj (p = 0,0001 i 0,0017, redom) na svojstva mukoadhezije. Formulacija F7, sa 5% ksantan gume i 1% tragakanta (mukoadhezija = 0,4632 N i stopa oslobađanja = 88,8% tokom 6 h) sa nekim modifikacijama, bila je optimalna. **Zaključak.** Ksantan guma i tragakant mogu se smatrati odgovarajućim prirodnim polimerima za vaginalnu „isporuku" lekova.

Ključne reči:
sirćetna kiselina; gelovi; polimeri; tragakant; vaginalne kreme, pene i želei; ksantan guma.

Introduction

The vagina is considered as a drug delivery site to obtain a local as well as systemic pharmacological effect [1, 2]. Thanks to the presence of *Lactobacilli*, the normal pH of the vaginal fluid in healthy women is maintained between 4 and 5.5 [3]. Low pH is one of the natural factors in resistance to the colonization of pyogenic organisms [4]. Lactic acid has been

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shown to deactivate a wide range of reproductive tract pathogens, including HSV-2 [5], *Neisseria gonorrhoeae* [6], and uropathogenic *Escherichia coli* [7]. In addition to direct inactivation of pathogens, vaginal acidity potentiates the slowing and trapping of HIV-1 virions by cervicovaginal mucus [8, 9]. Moreover, studies have confirmed the coincidence between the increase in vaginal pH along with bacterial vaginitis and trichomoniasis [10, 11].

Conventional vaginal drug delivery systems such as creams, tablets, capsules, pessaries, liquid dosage forms, etc., are associated with some disadvantages including leakage, messiness, and poor retention time (due to vagina self-cleaning action), which requires multiple daily doses and decreases patient compliance [12, 13]. In this way, mucoadhesive dosage forms seem to be a promising choice [14]. Among them, gels have received great attention due to their high water content [15] and rheological behavior causing increased vaginal retention time, lubrication, and patient compliance [16, 17]. Gels are also easy to manufacture and scale up and are the preferred vaginal drug delivery system among ladies [18, 19]. Natural polymers are valuable candidates for mucoadhesive gel formulations due to their proven safety, high biocompatibility [20, 21], ability to conjugate with other polymers [22], and eco-friendliness [23].

Xanthan gum and tragacanth are two examples of natural anionic polysaccharide gums that have been widely used in various industries including food, oil-recovery, cosmetics, water-based paints, petroleum, tissue engineering, biomedical, and drug delivery [24, 25]. Among several drug delivery systems incorporating these two polymers, mucoadhesive dosage forms are of interest due to their proper mucoadhesion property [26–28].

Vinegar called "*serkeh*" in Persian medicine [29] is produced from fermentable glucose in carbohydrate-rich foods such as grape, apple, fig, rice, etc., through fermentation processes (alcoholic and subsequently acetous fermentation) [30]. Vinegar has a long history and was widely prescribed by physicians of Persian medicine for several therapeutic purposes including oral consumption as an appetizer, digestive, thirst relief, treatment of warm headache, decreasing the bile flow [29, 31], and also in hypertension [32]. External dosage forms of vinegar separately or in combination with other medicines have been utilized for treating several illnesses including hemorrhoid, tinnitus, hearing loss, halitosis, gingivitis, mouth ulcers, toothache, intestinal worm infections, fire burning, removal of an attached leech from the pharynx [29, 31], and female genital infections [33]. Historically, the use of vinegar against infections dates back to Hippocrates (460-377 BC), who prescribed vinegar for treating sores and ulcers and its oral products in combination with honey named "oxymel" for improvement of persistent cough [34]. Furthermore, there are a few studies about the formulation of dosage forms using vinegar against vaginal infections [35, 36]. This liquid nutraceutical has also been studied in current medicine. Different studies show several health benefits of the vinegar such as improving lipid profile and suppressing fat accumulation, reduction of hyperglycemia and improvement of insulin secretion, inhibition of proliferation and induction of apoptosis in human cancer cells, antioxidant properties, exhaustion recovery effects, regulation of blood pressure, and natural disinfectant [30, 37].

Nowadays, in many analytical methods, experimental design is used for optimization instead of the traditional one variable-at-a-time (OVAT) approach [38, 39]. There are several reasons for the superiority of the design of experiment (DOE) approach over the OVAT approach. DOE yields the best possible formulation, while OVAT may find only suboptimal formulation. In addition, DOE is armed to estimate any synergistic or antagonistic interaction among constituents, whereas OVAT is inept to reveal any possible interaction. In the DOE approach, all response variables are quantitatively governed by a set of input factors (variables); therefore, any change in the optimized formulation for scale-up can be easily incorporated. On the other hand, the OVAT approach is restricted to the suboptimal point, and it is very difficult to modify the optimal points for any possible scale-up. Besides, the DOE approach is highly economistic in terms of resources and time. Factorial design is one of the most popular techniques of DOE. In situations when there are several factors (e.g., 2 or 3 factors), a three-level factorial design is certainly a possible choice by an experimenter who is concerned about curvature in the response function (Montgomery: design and analysis of experiments). The addition of a third-factor level allows the relationship between the response and the design factor to be modeled as a quadratic.

To the best of our knowledge, although there are vinegar douche products in the market, vinegar has not been formulated as a mucoadhesive gel yet. Therefore, in the present study, considering antimicrobial potential and pH modification of vinegar as a natural remedy, a 3-level factorial design was incorporated for the formulation of vinegar vaginal mucoadhesive gel, using tragacanth and xanthan gum natural polymers.

Methods

Materials

Tragacanth and xanthan gum were purchased from Gol Darou Co, Tehran, Iran, and vinegar was obtained from Bidestan co, Qazvin, Iran. Sodium hydroxide of analytical grade was obtained from Merck (Darmstadt, Germany). Dialysis bags with a molecular weight cut-off of 14 kDa were purchased from Sigma (Steinheim, Germany). All gel formulations were prepared with deionized water.

Experimental design

A 2 factor, 3-level (3^2) full factorial design was utilized for the optimization of vinegar gel using Design Expert 10 software (Stat-Ease Inc., Minneapolis, USA). Amounts (w/w %) of tragacanth and xanthan gum as independent variables were categorized in three (low, intermediate, and high) levels. Mucoadhesion strength and 6 hrs drug release were considered as dependent variables. Each design point was

tested three times, while the center point was assessed four times to consider the pure experimental error of the model. Furthermore, two formulations with zero percent of tragacanth or xanthan gum were also investigated. The design matrix and corresponding response values are presented in S1 (Supplementary Information) [40]. The statistical validation was confirmed by ANOVA at a significance level of $p < 0.05$ [41].

Gel preparation

According to the design matrix, a proper amount of each polymer was mixed with deionized water in a 250 mL beaker. The solution was stirred with a magnetic stirrer at 500 rpm, and vinegar was slowly added to reach the pH of 4 (around the vagina's natural pH). The final volume of each formulation was adjusted to 150 mL.

Viscosity studies

The viscosity of samples was measured using a DV-3 con viscometer (Brookfield, USA). A total of 50 mL of samples were applied to the viscometer container at 37 °C (n = 3).

pH measurement

To confirm the compatibility of the prepared formulations for vaginal mucosa, their pH values were measured by a pH meter (Mettler-Toledo GmbH, Switzerland) at room temperature (n = 3).

Gel spreadability

Of each gel formulation, 0.5 g was placed on a circular glass plate with 1 cm diameter, and another glass plate was placed on the gel. A 50 g weight was put on the upper glass for 5 min. The spreading area was calculated using the measurement of the increase in gel diameter (n = 3) [42, 43].

Mucoadhesion studies

The mucoadhesion study was performed according to Tasdighi's method [44] with some modifications. As is presented in Figure 1, 0.5 g of each gel formulation (A) was placed between two circle glasses (B) covered with sheep intestinal mucus (C). The bottom glass was fixed in a crystallizer, and the top glass was linked to a balance measuring the required force for detachment of the gel from the mucosal membrane. The test was performed in phosphate buffer medium pH = 4.5 and 37 °C.

In vitro release profile

The *in vitro* release was performed using a 14 kDa Dialysis tubing Cellulose Membrane. The membrane was soaked in distilled water for 24 hrs before the experiment. Five grams of each formulation was packed in a dialysis tube and placed in 200 mL distilled water (receptor medium) at 37 °C. Medium was stirred at 100 rpm during the release test, and samples were withdrawn at certain time intervals of 0.5, 1, 2, 4, and 6 hrs. Acetic acid was considered as the

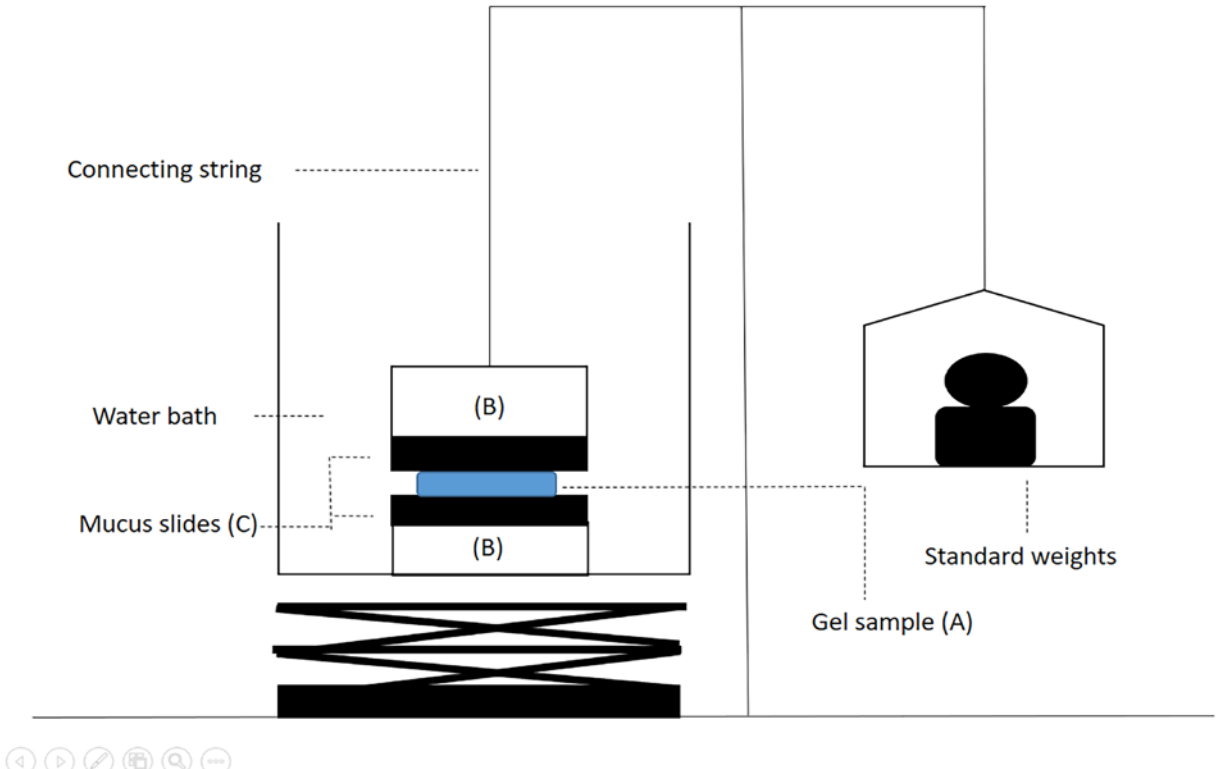

Fig. 1 – Schematic figure of apparatus used for assessment of gel's mucoadhesion.

main index in vinegar for quantitative analysis. The content of acetic acid in each sample was analyzed with pH metric titration with NaOH 0.005 M to equivalence point pH = 8.2 (Proline B210 pH meter Netherland). The amount of released acetic acid from each sample was calculated.

Release mechanism determination

The release kinetic of formulations F2, F3, F7, F9, and F10 were investigated by fitting the *in vitro* release data to various mathematical kinetic models. Models included zero order, first order, Higuchi, and Korsmeyer-Peppas [45].

Drug content

One gram of gel was vigorously stirred with 10 mL distilled water, using a sonicator and vortex, resulting in a transparent solution. Then the volume was adjusted to 100 mL, and the acetic acid content was measured using pH metric titration with NaOH as described in the release study (n = 3).

Scanning electron microscopy analysis

Scanning electron microscopy (SEM) evaluations were performed for the characterization of hydrogel polymeric chain micro-morphology.

Polymer chain morphology was investigated by FESEM Sigma VP (Zeiss, Germany) with an accelerating voltage of 10 kV under vacuum conditions. For sample preparation, gels were freeze-dried at -15 °C, and dried gels were gold sputter-coated before FESEM.

The study protocol was approved by the research committee of Guilan University of Medical Sciences by the ethics code of IR.GUMS.REC.1396.283.

Results

According to Table 1, gelling agents (polymers) were slowly dispersed in water with a range of 0–5% for both tragacanth and xanthan gum, and the pH was adjusted to 4, using vinegar.

The pH of formulations is one of the important parameters for vaginal compatibility. The vaginal pH of healthy women of reproductive age is around 4–5.5, changing during the menstrual cycle. Lactic acid produced by *Lactobacilli* present in the healthy vagina plays an important role in the control of infection by common pathogens. In our formulations, acetic acid as the index component of vinegar plays such a role.

The results showed that all the formulations were suitable for vaginal application as they could maintain the acidic pH value of the vagina (Table 1).

Viscosity influences other mechanical properties of formulations, such as spreadability and mucoadhesion, which are in direct correlation with patient compliance. The viscosity of each formulation was measured and stated in Table 1. The spreading area of each gel formulation was evaluated by measuring the diameter of the circle, which is formed under the pressure of glass. Spreading values are mentioned in Table 1.

Acceptable content of active pharmaceutical ingredient (API) or its indexes ensures the producer and consumer about receiving the adjusted or necessary dose. Since there is no defined monograph for vaginal vinegar gel in pharmacopeias, the prevalent range of 90–105% was considered acceptable in this study. The drug content of each formulation is presented in Table 1.

Four samples were subjected to SEM, including F9 and F10, which were simply made of xanthan gum and tragacanth, respectively, and F2 with the lowest amount of xanthan gum (2%) and the highest amount of tragacanth (5%), and F7 with the lowest amount of tragacanth (1%) and the highest amount of xanthan gum (5%). Figure 2 shows a homogenous wavy morphology for F9 (Figure 2a) and a relatively smoother morphology for F10 (Figure 2b), while in F2 and F7, a kind of polymeric chain integration is observed (Figures 2c, 2d). It seems that formulations containing both polymers show special polymeric interactions due to probable hydrogen or van der Waals bonds.

The acetic acid release profile was evaluated for all formulations in distilled water at 37 °C. Release profiles of F2, F7, F9, F10, and F3 (which is the center point of

Table1

Polymer composition and physical properties of formulations F1-F11

Formulation	XG (%)	TG (%)	pH	Viscosity (cp) mean ± SD	Diameter (cm) mean ± SD	Mucoadhesion (N x10^{-2})	Drug content (%) mean ± SD	Release rate (%)
F_1	2	1	4.01	21,100 ± 1,462.2	3.8 ± 1.3	41.9	95.5 ± 6.2	86.4
F_2	2	5	4.09	54,000 ± 2,581.5	3.0 ± 0.5	51.5	92.4 ± 4.4	73.3
F_3	3.5	3	4.21	34,000 ± 1,652.4	3.1 ± 0.9	45.2	95.1 ± 8.2	78.3
F_4	5	5	4.16	*	2.8 ± 0.4	56.5	89.3 ± 5.7	78.0
F_5	5	3	4.19	95,900 ± 4,235.9	3.7 ± 2.0	49.5	94.3 ± 7.0	84.7
F_6	3.5	5	4.08	*	2.9 ± 1.3	52.2	88.7 ± 9.3	76.4
F_7	5	1	4.14	29,000 ± 1,874.2	3.7 ± 2.8	46.3	91.3 ± 4.6	88.8
F_8	3.5	1	4.11	24,700 ± 1,423.5	3.6 ± 1.7	43.3	92.2 ± 11.6	88.2
F_9	5	0	4.23	28,600 ± 1,685.6	3.5 ± 1.4	46.0	97.6 ± 9.4	87.3
F_{10}	0	5	4.17	25,100 ± 1,584.5	3.0 ± 2.8	53.8	93.8 ± 6.3	86.4
F_{11}	2	3	4.16	26,400 ± 1,376.1	3.3 ± 1.7	44.3	84.0 ± 8.3	81.4

XG – xanthan; TG – tragacanth; SD – standard deviation.

***Due to very high viscosity, it was not measurable by the apparatus.**

experiment design) are presented in Figure 3. Release data for other formulations is accessible in a supplementary file.

According to the release results, F7 showed the highest (88.8%) while F2 showed the lowest (73.3%) release rate among other formulations in 6 hours. Considering F7 and F2 gums composition, they are a combination of both xanthan gum and tragacanth, in which F7 had the highest amount of xanthan gum (5%) with the lowest amount of tragacanth (1%) and F2 had the lowest amount of xanthan gum (2%) with the highest amount of tragacanth (5%).

Data obtained from *in vitro* release F2, F3, F7, F9, and F10 formulations were fitted to zero order, first order, Higuchi and Korsmeyer-Peppas models [44]. The drug release kinetic data are listed in Table 2, the highest regression coefficient (R^2) was considered the best fitted kinetic model for each vinegar gel formulation.

All chosen formulations showed the highest regression coefficient in the first-order model, which confirms that the drug release rate depends on its concentration.

Analysis of variances was used to assess the suitable response surface model and its significance. Tables 3 and 4

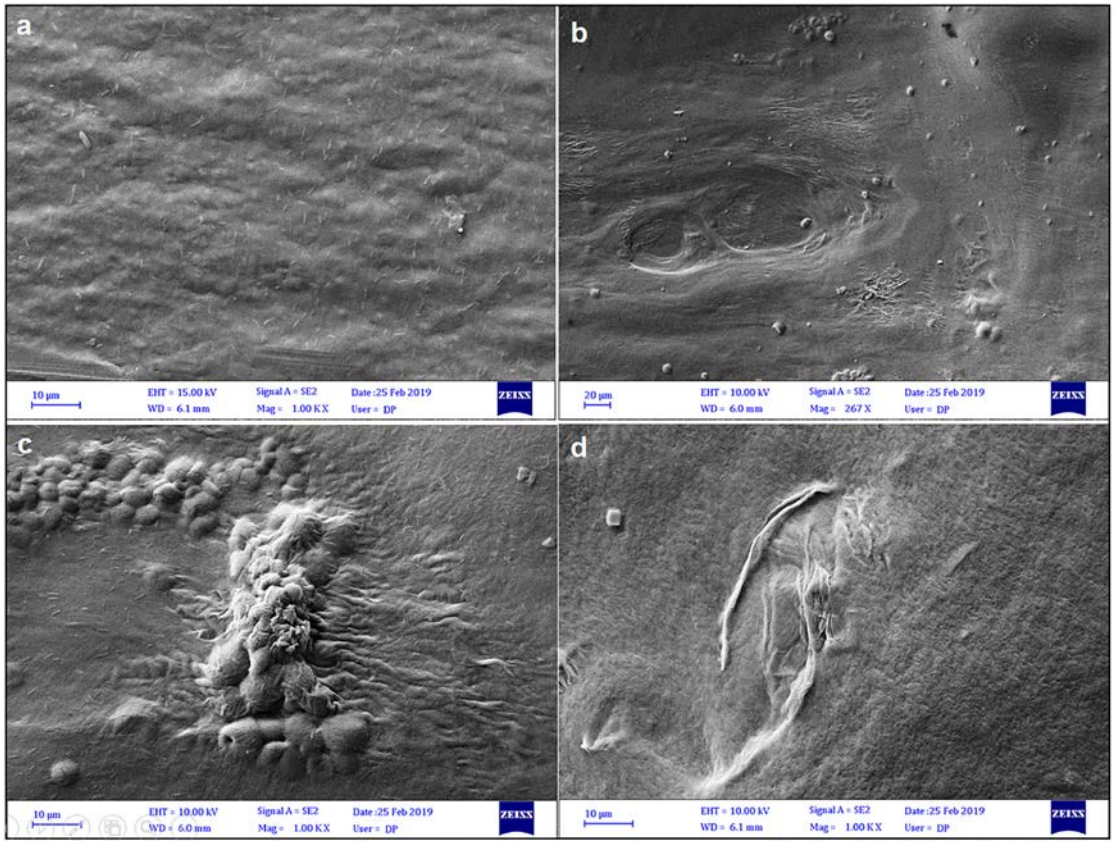

Fig. 2 – Scanning electron microscopy (SEM) images of hydrogel polymeric chain arrangement in: a) xanthan gum (5%), b) trgacanth (5%), c) xanthan gum (2%) and tragacanth (5%), d) tragacanth (1%) and xanthan gum (5%).

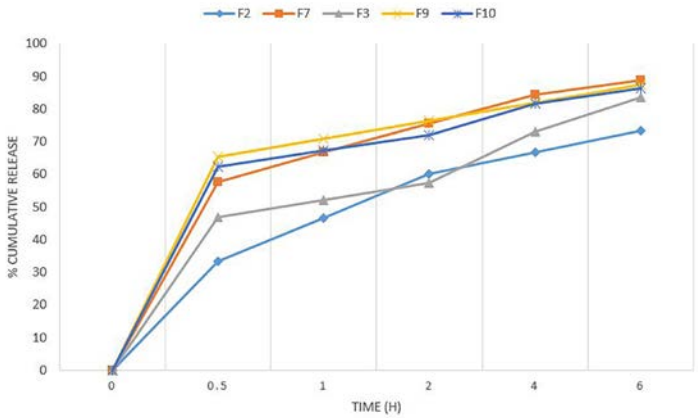

Fig. 3 – Release profile of formulations F2, F3, F7, F9, and F10 indicates the cumulative drug release vs. time.
Data on formulations are given in Table 1.

show ANOVA tables for mucoadhesion strength and release rate, respectively. A *p*-value lower than 0.05 was used to select significant parameters. As seems in Tables 3 and 4, the models are significant, and the *p*-value of the lack of fit implies that it is not significant relative to the pure experimental error and confirms the validity of the models.

Besides, the R-squares value and other statistical parameters of the models are all acceptable. Table S2 (supplementary materials) shows the statistical parameters of the models.

A dimensional graph of the effect of tragacanth and xanthan gum on A) vinegar *in vitro* release rate and B) mucoadhesion of gels is shown in Figure 4.

Table 2

Release kinetic parameters for F2, F3, F7, F9, and F10 formulations based on different mathematical models

Formulation	Zero order		First order		Higuchi		Korsmeyer-Peppas	
	K_0	R^2	K_1	R^2	K_H	R^2	K_K	R^2
F_2	13.709	0.9033	-0.1113	0.9843	13.709	0.9033	0.2954	0.5931
F_3	14.309	0.8549	-0.1383	0.9399	14.309	0.8549	0.2921	0.5526
F_7	15.229	0.7678	-0.1768	0.9627	15.229	0.7678	0.2941	0.5165
F_9	14.027	0.6676	-0.1544	0.8844	14.027	0.6676	0.2865	0.4858
F_{10}	14.126	0.7055	-0.1523	0.9074	14.126	0.7055	0.2875	0.4963

Data on formulations are given in Table 1.

Table 3

Analysis of variance (ANOVA) table for mucoadhesion strength of the formulations

Source	Sum of squares	df	Mean square	F-value	*p*-value	
Model	191.9	3	64.0	38.6	4.2 E-05	significant
A: Xanthan gum	35.5	1	35.5	21.4	1.7 E-03	
B: Tragacanth	137.0	1	137.0	82.6	1.72 E-05	
B^2	19.4	1	19.4	11.7	9.1 E-03	
Residual	13.3	8	1.6			
Lack of fit	4.8	5	0.9	0.4	0.9	not significant
Pure error	8.5	3	2.8			
Cor total	205.2	11				

Table 4

Analysis of variance (ANOVA) table for release rate of the formulations

Source	Sum of squares	df	Mean square	F-value	*p*-value	
Model	230.9	2	115.46	9.1	7.0 E-03	significant
A: Xanthan gum	18.0	1	18.0	1.4	0.3	
B: Tragacanth	212.9	1	212.9	16.7	2.7 E-03	
Residual	114.7	9	12.8			
Lack of fit	8.6	6	1.4	0.04	1.0	not significant
Pure error	106.1	3	35.4			
Cor total	345.6	11				

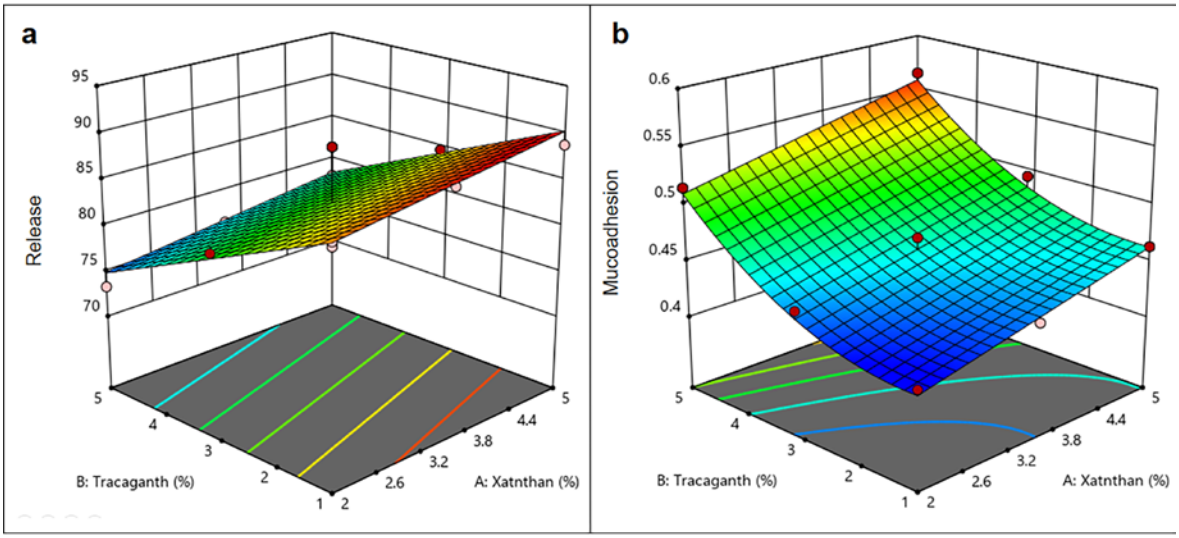

Fig. 4 – 3-Dimensional graph of effect of tragacanth and xanthan gum on a) vinegar *in vitro* release rate, b) mucoadhesion of gels.

Discussion

Paying attention to formulations F9 (5% xanthan gum) and F10 (5% tragacanth), it seems that an increase in tragacanth percent leads to decreases in vinegar release rate whilst xanthan gum increases the release rate of vinegar. Although tragacanth's behavior on release control has been observed in other studies [46], xanthan gum's influence on increasing the vinegar release rate is in contrast with former studies [47]. However, most studies have investigated the release of lipophilic API from tragacanth and xanthan gum matrices, while vinegar is a completely hydrophilic API. On the other hand, it's hypothesized that tragacanth can be adsorbed to the oil droplets and therefore possesses a more hydrophobic nature, while xanthan gum just acts through the viscosity increase [47, 48]. Therefore, it seems that in this study, tragacanth plays the main role in lowering the release rate, and xanthan gum modulates the rate by decreasing the tragacanth percent in the formulation because in this formulation maximum release of vinegar in 6 hours is desirable.

The release kinetic data is somehow in agreement with results of Salamanca et al. [47] that used xanthan and tragacanth gum in the formulation of a sustained-release tablet and drug release kinetic best fitted with first order and Higuchi models.

According to the models, tragacanth has a significant effect (p-value = 0.0027) on release rate, while xanthan gum has no statistically significant effect (p-value = 0.3). Figure 4a demonstrates the response surface graphically to help visualize the shape of the response surface of release rate. An increase in tragacanth weight (%) leads to a decrease in the release rate. As can be seen, there is no curvature effect of any parameter on the release rate. On the other hand, both tragacanth and xanthan gum, together with the curvature of tragacanth, have a significant effect on the mucoadhesive properties of the formulation. Figure 4b shows the response surface of mucoadhesive strength. Both tragacanth and xanthan gum have a positive effect on mucoadhesive strength.

Conclusion

The main objective of the optimization is to determine the optimum values of the parameters. After rigorous analysis of the selected models and their corresponding parameters, F7 has been selected as the best formulation of the study.

This study demonstrates that xanthan gum and tragacanth can be considered appropriate natural polymers for vaginal drug delivery. A certain combination of these two polymers with specific ratios (F7: 5% xanthan gum and 1% tragacanth) presented high mucoadhesion properties (0.4632 N) and good release characteristics (88.8%) in 6 hrs as a vinegar vaginal natural mucoadhesive gel.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. *das Neves J, Bahia MF.* Gels as vaginal drug delivery systems. Int J Pharm 2006; 318(1–2): 1–14.
2. *Sarwal A, Singh G, Singh S, Singh K, Sinha V.* Novel and effectual delivery of an antifungal agent for the treatment of persistent vulvovaginal candidiasis. J Pharm Investig 2018; 49(4): 135–47.
3. *Andrade AO, Parente ME, Ares G.* Screening of mucoadhesive vaginal gel formulations. Braz Jf Pharm Sci 2014; 50(4): 931–41.
4. *Boris S, Barbés C.* Role played by lactobacilli in controlling the population of vaginal pathogens. Microbes Infect 2000; 2(5): 543–6.
5. *Conti C, Malacrino C, Mastromarino P.* Inhibition of herpes simplex virus type 2 by vaginal lactobacilli. J Physiol Pharmacol 2009; 60(Suppl 6): 19–26.
6. *Graver MA, Wade JJ.* The role of acidification in the inhibition of Neisseria gonorrhoeae by vaginal lactobacilli during anaerobic growth. Ann Clin Microbiol Antimicrob 2011; 10: 8.
7. *Juárez Tomás MS, Ocaña VS, Wiese B, Nader-Macías ME.* Growth and lactic acid production by vaginal Lactobacillus acidophilus CRL 1259, and inhibition of uropathogenic Escherichia coli. J Med Microbiol 2003; 52(Pt 12): 1117–24.
8. *Shukair SA, Allen SA, Cianci GC, Stieh DJ, Anderson MR, Baig* SM, et al. Human cervicovaginal mucus contains an activity that hinders HIV-1 movement. Mucosal Immunol 2013; 6(2): 427–34.
9. *Lai SK, Hida K, Shukair S, Wang YY, Figueiredo A, Cone R,* et al. Human immunodeficiency virus type 1 is trapped by acidic but not by neutralized human cervicovaginal mucus. J Virol 2009; 83(21): 11196–200.
10. *Das P, Swain T, Mohanty JR, Sinha S, Padhi B, Torondel B, et al.* Higher vaginal pH in Trichomonas vaginalis infection with intermediate Nugent score in reproductive-age women-a hospital-based cross-sectional study in Odisha, India. Parasitol Res 2018; 117(9): 2735–42.
11. *Brotman RM, Bradford LL, Conrad M, Gajer P, Ault K, Peralta L,* et al. Association between Trichomonas vaginalis and vaginal bacterial community composition among reproductive-age women. Sex Transm Dis 2012; 39(10): 807–12.
12. *Patel P, Patel P.* Formulation and evaluation of clindamycin HCL in situ gel for vaginal application. Int J Pharm Investig 2015; 5(1): 50–6.
13. *Rencber S, Karavana SY, Şenyiğit ZA, Erac B, Limoncu MH, Baloğlu E.* Mucoadhesive in situ gel formulation for vaginal delivery of clotrimazole: formulation, preparation, and in vitro/in vivo evaluation. Pharm Dev Technol 2017; 22(4): 551–61.
14. *de Araujo Pereira RR, Bruschi ML.* Vaginal mucoadhesive drug delivery systems. Drug Dev Ind Pharm 2012; 38(6): 643–52.
15. *Khan M, Shah LA, Rehman T, Khan A, Iqbal A, Ullah M,* et al. Synthesis of physically cross-linked gum Arabic-based polymer hydrogels with enhanced mechanical, load bearing and shape memory behavior. Iran Polym J 2020; 29(4): 1–10.

16. *Bonferoni MC, Giunchedi P, Scalia S, Rossi S, Sandri G, Caramella C.* Chitosan gels for the vaginal delivery of lactic acid: relevance of formulation parameters to mucoadhesion and release mechanisms. AAPS PharmSciTech 2006; 7(4): 104.
17. *Ramadan AA, Elbakry AM, Esmaeil AH, Khaleel SA.* Pharmaceutical and pharmacokinetic evaluation of novel rectal mucoadhesive hydrogels containing tolmetin sodium. J Pharm Investig 2018; 48(6): 673–83.
18. *Braunstein S, van de Wijgert J.* Preferences and practices related to vaginal lubrication: implications for microbicide acceptability and clinical testing. J Womens Health (Larchmt) 2005; 14(5): 424–33.
19. *Vandebosch A, Goetghebeur E, Ramjee G, Alary M, Ettiègne-Traoré V, Chandeying V,* et al. COL-1492 Study Group. Acceptability of COL-1492, a vaginal gel, among sex workers in one Asian and three African cities. Sex Transm Infect 2004; 80(3): 241–3.
20. *Wadhera P, Jindal R, Dogra R.* Insight into adsorption kinetics and isotherms for adsorption of methylene blue using gum rosin alcohol/psyllium-based green adsorbent. Iran Polym J 2020; 29: 501–14.
21. *Şenyiğit ZA, Karavana SY, Eraç B, Gürsel Ö, Limoncu MH, Baloğlu E.* Evaluation of chitosan based vaginal bioadhesive gel formulations for antifungal drugs. Acta Pharmaceutica 2014; 64(2): 139–56.
22. *Malik NS, Ahmad M, Minhas MU, Tulain R, Barkat K, Khalid I* et al. Chitosan/Xanthan Gum Based Hydrogels as Potential Carrier for an Antiviral Drug: Fabrication, Characterization, and Safety Evaluation. Front Chem 2020; 8: 50.
23. *Apoorva A, Rameshbabu AP, Dasgupta S, Dhara S, Padmavati M.* Novel pH-sensitive alginate hydrogel delivery system reinforced with gum tragacanth for intestinal targeting of nutraceuticals. Int J Biol Macromol 2020; 147: 675–87.
24. *Selig MJ, Gamaleldin S, Celli GB, Marchuk MA, Smilgies DM, Abbaspourrad A.* The stabilization of food grade copper-chlorophyllin in low pH solutions through association with anionic polysaccharides. Food Hydrocoll 2020; 98: 105255.
25. *Farzi M, Emam-Djomeh Z, Mohammadifar MA.* A comparative study on the emulsifying properties of various species of gum tragacanth. Int J Biol Macromol 2013; 57: 76–82.
26. *Cazorla-Luna R, Notario-Pérez F, Martín-Illana A, Bedoya Lm, Tamayo A, Rubio J*, et al. Development and in vitro/ex vivo characterization of vaginal mucoadhesive bilayer films based on ethylcellulose and biopolymers for vaginal sustained release of Tenofovir. Biomacromolecules 2020; 21(6): 2309–19.
27. *Ghosal K, Adak S, Agatemor C, Praveen G, Kalarikkal N, Thomas S.* Novel interpenetrating polymeric network based microbeads for delivery of poorly water soluble drug. J Polym Res 2020; 27(4): 1–11.
28. *Ugoeze KC.* Bioadhesive Polymers for Drug Delivery Applications. In: *Mittal KL, Bakshi IS, Narang JK,* editors. Bioadhesives in Drug Delivery. Hoboken, New Jersey; Wiley; 2020. p. 29–56.
29. *Aghili Khorasani M.* Makhzan al-Advie. Teheran: Iran University of Medical Science, Research Institute for Islamic and Complementary Medicine; 2009.
30. *Samad A, Azlan A, Ismail A.* Therapeutic effects of vinegar: A review. Food Sci 2016; 8: 56–61.
31. *Tonekaboni M.* Tohfat al Momenin. Tehran: Nashre Shahr; 2008.
32. *Heydari M, Dalfardi B, Golzari SE, Habibi H, Zarshenas MM.* The medieval origins of the concept of hypertension. Heart Views 2014; 15(3): 96–8.
33. *Tansaz M, Meyari A, Naeemi SA, Meybodi RN, Memarzade H, Shirooye P*, et al. Recommended treatment methods of Iranian traditional medicine for female genital tract infections. Avicenna J Phytomed 2015; 5: 88–9.
34. *Johnston CS, Gaas CA.* Vinegar: medicinal uses and antiglycemic effect. MedGenMed 2006; 8(2): 61.
35. *Pavlova SI, Tao L.* In vitro inhibition of commercial douche products against vaginal microflora. Infect Dis Obstet Gynecol 2000; 8(2): 99–104.
36. *Martino JL, Vermund SH.* Vaginal douching: evidence for risks or benefits to women's health. Epidemiol Rev 2002; 24(2): 109–24.
37. *Ho CW, Lazim AM, Fazry S, Zaki UKHH, Lim SJ.* Varieties, production, composition and health benefits of vinegars: A review. Food Chem 2017; 221: 1621–30.
38. *Ebrahimi-Najafabadi H, Leardi R, Jalali-Heravi M.* Experimental design in analytical chemistry - part I: theory. J AOAC Int 2014; 97(1): 3–11.
39. *Ebrahimi-Najafabadi H, Pasdaran A, Rezaei Bezenjani R, Bozorgzadeh E.* Determination of toxic heavy metals in rice samples using ultrasound assisted emulsification microextraction combined with inductively coupled plasma optical emission spectroscopy. Food Chem 2019; 289: 26–32.
40. *Montgomery DC.* Design and Analysis of Experiments. 5th ed. Chichester, UK: John Wiley & Sons; 2001. p. 363–73.
41. *Soares PA, de Seixas JR, Albuquerque PB, Santos GR, Mourão PA, Barros W Jr,* et al. Development and characterization of a new hydrogel based on galactomannan and κ-carrageenan. Carbohydr Polym 2015; 134: 673–9.
42. *Dantas MG, Reis SA, Damasceno CM, Rolim LA, Rolim-Neto PJ, Carvalho FO,* et al. Development and Evaluation of Stability of a Gel Formulation Containing the Monoterpene Borneol. ScientificWorldJournal 2016; 2016: 7394685.
43. *Deuschle VCKN, Deuschle RAN, Bortoluzzi MR, Athayde ML.* Physical chemistry evaluation of stability, spreadability, in vitro antioxidant, and photo-protective capacities of topical formulations containing Calendula officinalis L. leaf extract. Braz J Pharm Sci 2015; 51(1): 63–75.
44. *Tasdighi E, Jafari Azar Z, Mortazavi SA.* Development and In-vitro Evaluation of a Contraceptive Vagino-Adhesive Propranolol Hydrochloride Gel. Iran J Pharm Res 2012; 11(1): 13–26.
45. *Nie S, Hsiao WL, Pan W, Yang Z.* Thermoreversible Pluronic F127-based hydrogel containing liposomes for the controlled delivery of paclitaxel: in vitro drug release, cell cytotoxicity, and uptake studies. Int J Nanomedicine 2011; 6: 151–66.
46. *Rasul A, Iqbal M, Murtaza G, Waqas MK, Hanif M, Khan SA*, et al. Design, development and in-vitro evaluation of metoprolol tartrate tablets containing xanthan-tragacanth. Acta Pol Pharm 2010; 67(5): 517–22.
47. *Salamanca CH, Yarce CJ, Moreno RA, Prieto V, Recalde J.* Natural gum-type biopolymers as potential modified nonpolar drug release systems. Carbohydr Polym 2018; 189: 31–8.
48. *Pocan P, Ilhan E, Oztop MH.* Characterization of Emulsion Stabilization Properties of Gum Tragacanth, Xanthan Gum and Sucrose Monopalmitate: A Comparative Study. J Food Sci 2019; 84(5): 1087–93.

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