BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902022e20868

Article

Development and Evaluation of Vaginal Suppository Containing *Althaea officinalis* L. Polysaccharide Extract

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Intrauterine adhesions cause several gynecological problems. *Althaea officinalis* L. roots known as marshmallows contain polysaccharides (M.P.) which possess anti-inflammatory and anti-ulcerogenic activities also can form a bio-adhesive layer on damaged epithelial membranes prompting healing processes. Vaginal formulations of herbal origin are commonly applied to relieve cervico-uterine inflammation. Herein, we aim to develop and evaluate vaginal suppositories containing polysaccharides isolated from the *A. officinalis* root. Six formulations (four P.E.G.-based and two lipid-based suppositories containing 25% and 50% M.P.) met standard requirements, which were then subjected to qualitative and quantitative evaluation. All suppositories exhibited acceptable weights, hardness, content uniformity, melting point, and disintegration time, which fall within the acceptable recommended limits. Higher concentrations of M.P. in PEG-bases moderately increased the hardness ($p<0.05$). PEG-formulations showed content uniformity>90% of the average content while it was 75-83% for suppocire formulations. All formulations disintegrated in<30minutes. In-vitro release test revealed that M.P. release from 25%-MP formulations was higher than that of 50%-M.P. suppositories. Overall, results revealed the feasibility of preparing P.E.G.- or lipid-based suppositories containing M.P., which met the B.P. quality requirement.

Keywords: *Althaea officinalis*. Malvaceae. Polysaccharide. Intrauterine adhesions. Vaginal suppository. Medicinal plant.

INTRODUCTION

Asherman's Syndrome is a condition that is characterized by intrauterine adhesions (I.U.A.), causing several symptoms, including pelvic pain, menstrual anomalies, infertility, miscarriage, and related psychological symptoms (Santamaria, Isaacson, Simón, 2018). The gold standard for I.U.A. treatment would be hysteroscopic lysis of adhesions through surgery. However, recurrence occurs frequently (Salazar, Isaacson, Morris, 2017), and prevention methods including pharmacological treatment and physical barriers have a high recurrence rate (Tu *et al.*, 2013).

Althaea officinalis L. (marshmallow) is a perennial plant with erect and woody stems from Malvaceae (Akbar, 2020). Marshmallow roots have been used in several traditional systems of medicine for more than two millennia (Ibn-Sina,1987).

Marshmallow root contains 5-11% water-soluble mucilage polysaccharides composed of galacturonorhamnans, glucans, arabinans, and arabinogalactans carbohydrates (Dawid-Pać, 2013).

Anti-inflammatory, anti-ulcerogenic, and antimicrobial activities have been reported for different parts of *A. officinalis* (Rezaei *et al.*, 2015; Hage-Sleiman, Mroueh, Daher, 2011; Sleiman, Daher, 2009). Many herbal

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polysaccharides have been shown to enhance intrinsic antioxidant enzymes, induce apoptosis of cervical cancer HeLa cells and possess immunomodulating effects (Li *et al*., 2009; Cao *et al*., 2010; XiaoPing *et al*., 2009). Marshmallow polysaccharides (M.P.) form a bio-adhesive layer on damaged or irritated epithelial membranes prompting healing processes (Schmidgall, Schnetz, Hensel, 2000). Aqueous extract and polysaccharides of marshmallows can effectively stimulate the physiology of epithelial cells through up-regulating genes associated with cell adhesion proteins, growth regulatory factors, cytokine release, extracellular matrix, and apoptosis. Moreover, through passive mucilaginous, barrier-enhancing effects on connective tissue cells, marshmallow roots can accelerate the healing process (Deters *et al*., 2010).

The vaginal route represents a reduced drug degradation, circumvention of the hepatic first-pass effect, ease of use, and high permeability to many drugs (Mahjabeen *et al*., 2018). Vaginal formulations of herbal origin are commonly used in traditional medicine systems to relieve cervico-uterine ulcers, infections, and inflammations. Vaginal suppositories are dosage forms prepared using either fatty bases or water-soluble bases. Suppocire vehicles are complex fat suppository bases containing polyoxylglyceride esters which increase drug solubility and bio-availability. Moreover, Suppocire bases have appropriate solidity, and excellent spreading properties (Regdon *et al*., 1994) Suppocires are widely used as suitable bases for the formulation of vaginal suppositories (Çaliş, Şumnu, Hincal, 1994; Samy *et al*., 2000). Polyethylene glycol (P.E.G.), another widely-used polymer in drug delivery systems, has high structure flexibility, biocompatibility, amphiphilicity, and high hydration capacity. Moreover, P.E.G. suppositories permit better miscibility with the mucus, providing fast drug dissolution. Drug release rate decreases with increasing M.W. of P.E.G.s (D'souza, Shegokar, 2016).

M.P. can provide a bio-adhesive layer with anti-inflammatory and wound healing effects in the uterine cavity (Deters *et al*., 2010). It has been shown that polysaccharides can retard the release of co-administered drugs in suppositories (Uekama *et al*., 1995). This would benefit suppositories to exert local and regional anti-inflammatory and healing effects, including uterine targeting. Therefore, the mucoadhesive properties of M.P. provide promising candidates for vaginal systems (Valenta, 2005).

We aim to develop and evaluate the vaginal suppositories containing polysaccharides isolated from *A. officinalis* root in the present study.

MATERIAL AND METHODS

Ethical approval

This study was approved by the Ethics Committee of Mashhad University of Medical Sciences and supported by a research grant from Mashhad University of Medical Sciences Research Council (number 951701).

Materials

Polyethylene glycol (P.E.G.) 400, P.E.G. 2000, PEG 6000, glycerin, n-hexane, and gelatin were purchased from Merck (Germany). Suppocire AGP® was gifted from Gattefosse (France).

Plant material

The fresh roots of *A. officinalis* L. were obtained in the autumn of 2016 from the research farm of Agriculture Jihad Organization, Mashhad, Iran. The species were identified at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Mashhad University of Medical Sciences, Iran, where a voucher specimen (13236) was deposited. Fresh roots were dried in an oven at 35°C, and then crushed and weighed (Sendker *et al*., 2017; Banaee, Soleimany, Haghi, 2017).

Extraction of marshmallow polysaccharides

Two hundred (200) g of dry-milled marshmallow roots were sieved through a 5 mm sieve, socked in 1 L purified water, and shaken for 15 minutes. The mixture was filtered, and the filtrate was precipitated with ethanol 96%. The residue was mixed with ethanol 70% (v/v), centrifuged, and vacuum-dried. The sediment (M.P.)

was then dissolved in purified water and freeze-dried. The percentage yield of extracted M.P. was calculated based on the gravimetric method (Sendker *et al*., 2017).

Determination of swelling index

The swelling index (S.I.) is the sample volume after it was swollen in an aqueous solution. One gram of dry-milled marshmallow root was added to 25 mL of distilled water, shaken vigorously every ten minutes for an hour in a sealed glass flask, and left to stand for three hours. The final volume was recorded in mL, and the swelling index was calculated using the following equation:

$$SI = [Ws - Wd/Wd] \times 100$$

where Ws and Wd present weights of swollen and dry plant material, respectively.

Displacement factor

Displacement values were calculated using the following equation:

$$f = \left[\frac{100(E-G)}{G.X}\right] + 1$$

where f is the displacement factor, E is the weight of the suppository base without M.P., G is the weight of the suppository containing M.P., and X is the M.P. content in percentage (Hargoli *et al*., 2013).

Preparation of suppositories

Glycerinated gelatin base suppositories

A ratio of 70% glycerin, 20% gelatin, and 10% water was used to prepare the glycerinated gelatin base. Briefly, glycerin and water were mixed in a water bath at 45°C. Then gelatin powder was added slowly while stirring (De Araujo Pereira, Bruschi, 2012).

Accurately weighed quantities of MP (10, 20, and 35% w/w) were mixed with the respective glycerinated gelatin base. After properly dispersing the active ingredient into the base, the mixture was poured into 2-gram molds (lubricated with light mineral oil) and transferred to the refrigerator.

Polyethylene glycol base suppositories

Different formulations of polyethylene glycol (P.E.G.) based suppositories were prepared according to Table I. Different types of P.E.G. were weighed, melted, and mixed homogeneously at 50°C, 25 and 50% (w/w) of M.P. were added and mixed. After completing M.P. in the base, the mixture was poured into 2-gram molds (lubricated with light mineral oil) and placed in the refrigerator for half an hour (Allen, 1997).

TABLE I - Composition of different suppository formulations

Formulation	Suppository Ingredients (%)				
	PEG 2000	**PEG 6000**	**PEG 400**	**Suppocire AGP®**	**M.P.***
F1	15%	45%	15%	-	25%
F2	15%	30%	30%	-	25%
F3	10%	30%	10%	-	50%
F4	10%	20%	20%	-	50%
F5	-	-	-	75%	25%
F6	-	-	-	50%	50%

*M.P., marshmallow polysaccharides

Oleaginous base suppositories

Suppocire AGP® was used to prepare oleaginous base suppositories. The lipid base was melted in a 40°C water bath and mixed homogenously with 25% and 50% (w/w) M.P. to prepare F5 and F6 formulation. The mixture was then molded into a 2-gram mold (lubricated with glycerin) and placed into the refrigerator (Allen, 1997; Mollel, 2006).

Weight uniformity test

The weight uniformity test was carried out according to the British Pharmacopoeia (B.P., 2011). Twenty suppositories of each formulation were randomly selected, and their mean weight and standard deviation were determined (Hargoli *et al.*, 2013).

Hardness test

The mechanical strength of suppositories was determined using the suppository hardness tester (Model S.B.T., Erweka, Germany) at room temperature (25±0.5°C). Ten randomly selected suppositories from each formulation were subjected to different progressive weights (Akl *et al.*, 2019). The weight required for the suppository to collapse was recorded in kg force to measure resistance to crushing. To study the effect of M.P. addition on the mechanical strength of the formulations, base suppositories (without M.P.) were also prepared, and their hardness was tested.

Content uniformity test

Content uniformity test was performed based on a gravimetric method. Ten randomly taken suppositories from each batch were weighed and each placed in a test tube to be melted by heating in a water bath at 50°C. Ten mL of distilled water was then added to each tube, and the mixtures were shaken while adding 4 mL of ethanol 96% to precipitate the M.P. content. The tubes were then centrifuged at 1000 rpm for 2 min. Two mL of hexane were added to each tube and shaken for two minutes while heating at 50°C to exclude the lipophilic phase of the suppositories. The supernatant containing lipophilic base was removed, and the residue containing M.P. was dried at 40-45°C and weighed. Mean weight was determined and used to measure the M.P. content of each suppository (Matsumoto *et al.*, 2016).

Determination of disintegration time

Five randomly chosen suppositories from each formulation were placed in the disintegration test apparatus (TDI2, Electro Farmed®, Iran). Tubes were filled with distilled water (adjusted to pH 4.5 by adding citric acid/ phosphate buffer) and immersed in a water bath at 37±1°C. A plastic disc was placed into each tube to prevent the floating of the suppository (U.S.P., 2018). The time required for complete separation or dissolution of the suppository components was recorded as disintegration time.

Determination of softening and melting points

The melting point of M.P. suppositories was determined according to the U.S.P. 41-NF36 using a melting point apparatus (ElecteroThermal®, England) (U.S.P., 2018). A straight capillary tube with both open ends was dipped into the suppository bases to fill a sufficient base (about 1cm). The capillary tube was then placed in the apparatus attached to a thermometer. The melting point was noted when the contents of the capillary tube started to melt.

In-vitro release test

In-vitro release test was performed using the method described by Hargoli *et al.* (2013) with modifications. Three randomly selected suppositories from each formulation were immersed in falcon tubes containing 10 mL of distilled water as a dissolution medium (adjusted to obtain a pH of 4.5 with phosphate buffer solution to mimic the vaginal pH). Falcon tubes were placed in a shaker water bath (NB-304, N-BIOTEK, INC., Iran) at a temperature of 37°C, and 2 mL samples were withdrawn at different time intervals (0, 5, 15, 25, 35, and 45 min). An equal volume of fresh medium was replaced into the dissolution medium after each sampling to maintain constant volume throughout the study. Four mL of ethanol 96% was added to each tube, which then was centrifuged at 1000 rpm for 2 min. Two mL of hexane was added

to the test tubes, the supernatant containing lipophilic base was removed, and the residue containing M.P. was dried at 40-45°C and weighed. Mean weight and release percentage were calculated for each suppository.

Statistical analysis

Statistical studies of the obtained results were performed using Prism 6 statistical software (GraphPad, Inc. CA, U.S.A.). An unpaired T-test was used to compare each sample group with its base group. To compare the mean in different groups, a one-way ANOVA test and Tukey's multiple comparisons test were used. A P-value less than 0.05 was considered significant.

RESULTS

Evaluation of extract yield and swelling index

The yield percentage of marshmallow polysaccharide extraction was 38.70 ± 1.25. A 36% increase in the volume of marshmallow mucilage was observed after swelling.

Displacement factor

The displacement factors for selected formulations have been presented in Table II.

Qualitative and quantitative evaluation

Appearance

All suppositories (F1-F6) were appropriate for appearance, and there was no cracking, bubble formation, and sediment accumulation.

Hardness

All formulations exhibited a hardness of more than 2.5 Kg. According to the results (Table II), in PEG-based suppositories, the hardness increased by incorporation of M.P. ($p<0.05$). In contrast, in lipid-based formulation, the hardness decreased upon addition of M.P. Formulations with the highest and lowest hardness were F1 (PEG-based with 50% M.P.) and F5 (lipid-based with 25% M.P.), respectively.

Weight uniformity test

The mean weights of P.E.G. formulations ranged from 2.47 to 2.67 g, while those of lipid-base formulations were in the range of 2.18 to 2.42 g (Table II). The results show an increase in weights for all formulations by increasing the percentage of the M.P. ($p <0.05$).

TABLE II - Displacement factor, hardness and weight of selected suppositories

Formulation	Displacement factor	The base amount required for a suppository containing the drug (g)	Mean weight of suppositories containing no drug (g)	Hardness of suppositories (kg) Mean ± SD*	Hardness of suppository bases (kg) Mean±SD	Weight (g) Mean ± SD*	RSD%**
F1	0.626	1.84	2.15	2.22 ± 0.34	2.84 ± 0.556	2.479 ± 0.080	3.2
F2	0.511	1.87	2.12	2.14 ± 0.20	3.54 ± 0.299	2.547 ± 0.061	2.4
F3	0.626	1.53	2.15	2.22 ± 0.34	4.36 ± 0.240	2.658 ± 0.077	2.9
F4	0.511	1.61	2.12	2.14 ± 0.20	4.54 ± 0.250	2.675 ± 0.055	2.1
F5	0.702	1.68	2.03	3.08 ± 0.29	2.44 ± 0.227	2.183 ± 0.102	4.7
F6	0.702	1.32	2.03	3.08 ± 0.29	2.64 ± 0.31	2.420 ± 0.069	2.9

*SD, standard deviation; **RSD, coefficient of variation

Content uniformity

The results of the content uniformity test are shown in Table III.

Disintegration time of suppositories

All suppositories were disintegrated in less than 30 minutes (Table III). It was shown that M.P. incorporation could prolong disintegration time ($p<0.05$). In general, PEG-based formulations were disintegrated in a shorter time.

Melting point of suppositories

The melting points of formulated suppositories and MP-free bases are shown in Table III. The melting points of the fatty bases in 25% and 50% formulations were 37.4°C, which fall in the reported range by the manufacturer (34.5-37.5°C). No significant alteration in melting points of fatty bases was observed after the incorporation of M.P. in different concentrations ($p <0.05$).

The melting points of P.E.G. bases were 40.7-42.8 °C. Except for F4 ($p <0.05$), the addition of M.P. caused no significant alteration in melting points of P.E.G. formulations compared to the plain base ($p <0.05$). The coefficient of variation in all groups was less than 5%.

TABLE III - Disintegration time, melting point and content uniformity of suppositories

Formulations	Disintegration time of MP* suppositories (min) Mean ± SD**	disintegration time of bases (min) Mean ± SD	Melting point of MP suppositories (°C)	RSD%***	Melting point of bases (°C)	RSD%	M.P. content (g) Mean ± SD*	Percentage of mean content	RSD%
F1	25.77± 0.012	12.28 ± 0.121	39.5 ± 1.915	4.9	40.7 ± 0.516	1.3	0.531± 0.058	106.2	10.8
F2	15.27 ± 0.029	10.00 ± 0.099	41.25 ± 1.5	3.6	42.8 ± 0.447	1.05	0.531± 0.051	106.2	9.57
F3	20.80 ± 0.012	12.28 ± 0.121	40.0 ± 1.633	4.1	40.7 ± 0.516	1.3	1.116 ± 0.167	111.6	14.94
F4	22.13 ± 0.022	10.00 ± 0.099	40.25 ± 0.957	2.4	42.8 ± 0.447	1.05	0.899 ± 0.167	89.9	10.64
F5	25.08 ± 0.012	08.13 ± 0.46	37.6 ± 0.547	1.5	37.4 ± 0.527	1.4	0.378 ± 0.059	75.6	15.86
F6	27.25 ± 0.012	08.13 ± 0.046	37.5 ± 0.577	2.6	37.4 ± 0.527	1.4	0.830 ± 0.091	83	11/075

*M.P., marshmallow polysaccharides; **SD, standard deviation; ***RSD, coefficient of variation

In vitro release test

As long as all formulations show a disintegration time of less than 30 min, the in-vitro release test was carried out for 45 min. However, none of the formulations reached 100% release within this period. According to the results (Figure 1), the formulations containing 25% M.P. had better release than those containing 50% M.P. (≥80%) as well as lipid-based formulations that have faster release than PEG-base suppositories.

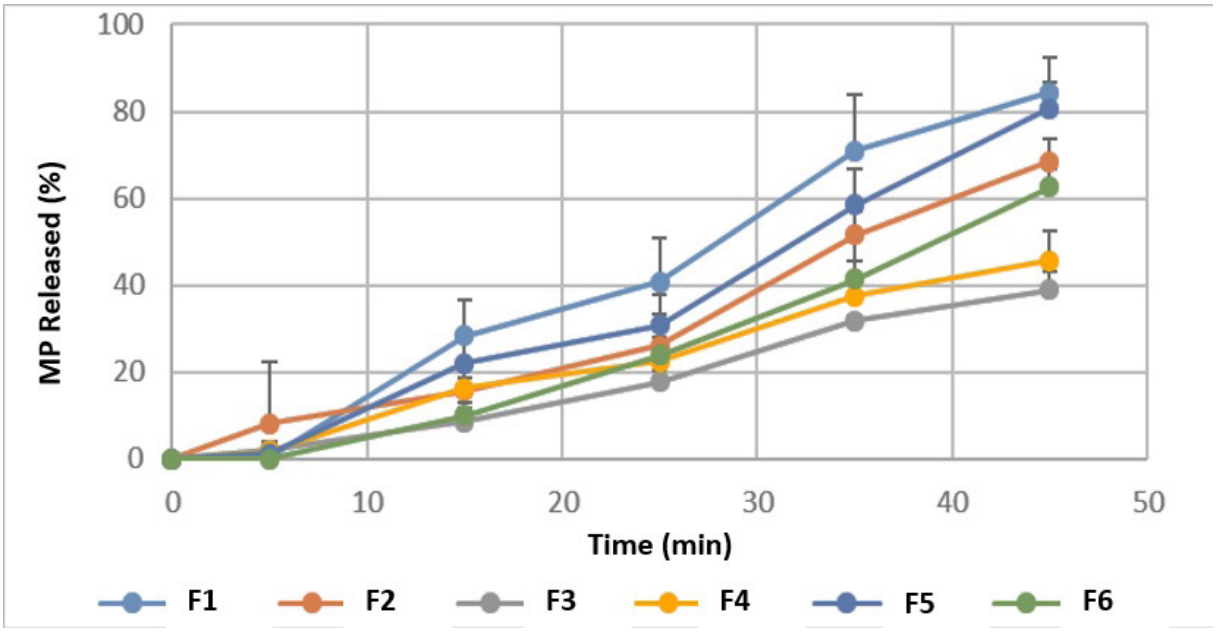

FIGURE 1 - *In vitro* release test. M.P. release (%) = The percentage of marshmallow polysaccharides release. F1 = Formulation 1; F2 = Formulation 2; F3 = Formulation 3; F4 = Formulation 4; F5 = Formulation 5; F6 = Formulation 6.

DISCUSSION

Hage-Sleiman, Mroueh, and Daher (2011) evaluated alleviating effects of aqueous extract of marshmallow on gastric ulcer and inflammation using a rat model. The results revealed significant anti-ulcerogenic and anti-inflammatory activities at all studied doses (50, 100, 250 mg/kg) (Hage-Sleiman *et al.*, 2011). Moreover, marshmallow extract was an effective inhibitor of Gram-positive bacteria. Its topical administration on an excision wound model in rats revealed a significant wound healing activity compared to the control (Rezaei *et al.*, 2015). In another study, anti-inflammatory effects of different fractions and isolated polysaccharides of *Malva sylvestris*, a very close species, were studied on acetic acid-induced ulcerative colitis in rats. Macroscopic and microscopic examinations of colitis revealed that the aqueous fraction and isolated polysaccharides effectively prevented and reduced signs of inflammation (Hamedi *et al.*, 2016).

The vaginal route is capable of the uterine targeting of drugs. After vaginal administration of drugs, the plasma concentrations were reported to be higher in the uterine artery than in the radial artery, indicating a preferential distribution to the uterus. This supports the presence of direct local transport from the vagina to the uterus, namely the "first uterine pass effect" or "direct preferential vagina-to-uterus transport (Shanmugam *et al.*, 2014). This would be important in targeting vaginal active drugs without undergoing hepatic metabolism and causing gastrointestinal side effects (Shaikh *et al.*, 2011).

Accordingly, we conducted a study to develop and evaluate the vaginal suppositories containing M.P.s.

Three glycerinated gelatin suppository formulations containing 10%, 20%, and 35% w/w M.P. were prepared, 20% and 35% suppositories were ruled out due to inappropriate shape, structural instability, and reduced cohesion by increasing M.P. percentage. Only 10% suppositories were physically acceptable; however, 10% suppositories were also ruled out due to the low percentage of the active ingredient (M.P.). After preliminary experiments, four PEG-based (F1-F4) and two lipid-based (F5, F6) suppositories were formulated, showed acceptable physical properties, and were selected for further evaluations. The displacement value was used to determine the amount of M.P. that displaces one part of the suppository basis. This value is dependent on both the active ingredient and suppository base used. The volume of suppositories molded using a particular mold is similar;

however, their weight can be varied because the density of the drug is usually different from the density of the base. Therefore, molds should be calibrated by calculating the displacement factor (Vidras *et al.*, 1982). Accordingly, the displacement factor was used in this study to calibrate the molds before preparing M.P. suppositories.

All suppositories exhibited sufficiently smooth surfaces without any cracks or contraction pores. The average weights of all formulations were within the acceptable range with a coefficient of variation (RSD%) of <5% of the average value (Table II), which indicated homogenous filling and perfect calibration of the molds (BP, 2011; Gomaa *et al.*, 2018).

The suppositories should possess adequate mechanical strength for handling and transportation (Saleem *et al.*, 2008). The hardness test revealed that F4 (PEG-based containing 50% M.P.) achieved the maximum hardness. The hardness of the suppositories was in the following descending order: PEG-based formulations; F4>F3>F2>F1> lipid-based formulations; F6>F5. These results were in harmony with those obtained previously (Gomaa *et al.*, 2018). Moreover, it was revealed that the inclusion of higher concentrations of M.P. in PEG bases moderately increased the hardness ($p<0.05$). This could be attributed to the hydrophilic nature of both the PEG base and the active ingredient (M.P.), which provides a homogeneous distribution of M.P. into the base and forming strong molecular bonds (Brunaugh, Smyth, Williams III, 2019).

In content uniformity testing, PEG formulations showed content uniformity > 90% of the average content while it was 75-83% for Suppocire formulations. This can be the result of a non-homogeneous distribution of hydrophilic M.P. in a lipid base.

Melting point testing revealed that formulations containing P.E.G. showed a higher melting range (39.5-41.25 °C) while the melting point of Suppocire formulations was 37.5 and 37.6 °C. M.P. has a significant effect only on the melting point of F4, which contains 50% of M.P. The melting point of the PEG suppositories is generally higher than lipid-based suppositories. It is important to note that PEG cannot melt at body temperature but instead can dissolve. Therefore, the higher melting point of PEG-based suppositories cannot interfere with the release characteristics of these suppositories. For water-soluble drugs such as M.P., the release rate depends on the molecular weight of the P.E.G. (Brunaugh, Smyth, Williams III, 2019; Rodrigues *et al.*, 2015). Moreover, the use of high melting point suppository bases provides convenient storage of the suppositories without refrigeration and the possibility of excessive softening in warm climates (Hanning *et al.*, 2020).

The disintegration test determines whether suppositories disintegrate or soften within a prescribed time when placed in an immersion fluid using the experimental conditions (Kaewnopparat, Kaewnopparat, 2009). Disintegration time is a crucial parameter, which determines the content release of the suppository. Fast disintegration allows rapid diffusion of drugs in the administered area. The results revealed that all formulations disintegrated in less than 27.5 min. However, plain suppository bases showed significantly shorter disintegration time (12.28-8.13 min) ($p<0.05$). According to B.P., the disintegration time should be shorter than 60 min for hydrophilic-base and 30 min for lipid-based suppositories. The results confirmed their compliance with the B.P. requirements for disintegration (B.P., 2011). However, since the rectal or vaginal administration of a drug in polymer matrices is known to increase the contact time of the drug with the mucus membranes, viscous bases are handy to retain the drug in the area, providing a sustained release delivery (Uekama *et al.*, 1995).

In-vitro release test revealed that M.P. released from 25%-MP formulations was higher than those of 50%-MP suppositories (Figure 1). It was observed that more than 50% of the M.P. was released from all formulations within 60 min. Several factors, including drug-base interactions, the physiochemical nature of the base, and the chemical composition of the additives, have been shown to influence the release of drugs from suppositories (Samy *et al.*, 2000). M.P. is a hydrophilic polymeric carbohydrate; the solubility in P.E.G. bases is relatively higher than lipophilic bases. As a result, M.P. exhibits a higher tendency to P.E.G. bases, and the release rate from P.E.G. bases is relatively low.

On the other hand, M.P. solubility in hydrophobic bases is low. Therefore, MP exhibited a high tendency to diffuse out of hydrophobic bases than PEGs.drug (Gomaa

et al., 2018). Moreover, water-soluble molecules like M.P. can release faster from the hydrophobic bases due to the attraction toward the aqueous fluids (Brunaugh, Smyth, Williams III, 2019). This can adjust our controversial results considering the higher release of lipophilic suppositories.

CONCLUSION

Six formulated suppositories (four PEG- and two lipid-based) met the B.P. quality requirement for weight measurement, hardness, content uniformity, melting point, and disintegration time. The formulations also exhibited acceptable results in the in-vitro release test, which provides reliable evidence supporting the quality and performance of the formulations. Numerous pharmacological studies have demonstrated anti-inflammatory, anti-ulcerogenic and antimicrobial, antioxidant, and immunomodulatory effects of marshmallow and related polysaccharides which support the potential efficacy of the present formulations in alleviating I.U.A. However, future research could focus on the experimental and clinical efficacy and safety of these formulations.

ACKNOWLEDGEMENTS

This study was part of the Pharm. D. student thesis (H.H.) and supported by a research grant from Mashhad University of Medical Sciences Research Council (number 951701).

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Received for publication on 13rd October 2020
Accepted for publication on 08th June 2021