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Bioinspired bioartifical polymer hybrid composites for propolis vaginal delivery II: formulation and characterization

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Abstract

In our previous work Box-Behnken experimental design was applied for formulation optimization of the thermoreversible mucoadhesive in situ vaginal hydrogels with propolis and optimized batches were identified. Optimized batches of bioartificial polymer hybrid composites (chitosan, Lutrol® F-127 and Lutrol® F-68 mixture)) (CP1, CP2, CP3) were prepared using so-called cold method. Formulation P3 (chitosan free) was prepared in order to evaluate the effect of chitosan on the physico-chemical and biopharmaceutical properties of the polymer hybrid composites (gels).

The pH values of the gels were 4-4.5. The gelation temperature for all formulations was in a range of 29-33 °C. Total flavonoids content was above 95%. Increase in concentration of Lutrol® F-127 and Lutrol® F-68/Lutrol® F-127 ratio lead to a higher viscosity values and slower gel erosion/dissolution. The presence of chitosan increased gel viscosity and hence slow-down erossion/dissoluiton. Propolis release rate was the highest in P3 which released propolis within 5 h, corresponding to time of complete erosion. The same correlation between erosion process and drug release rate was observed in CP1-CP3, where prolonged propolis release for more than 10 h was achieved. Microbiological quality was in accordance with the requirements of Ph. Eur. 7. All formulations demonstrated adequate stability at 5 ± 3 °C during 6 months. Based on overall results it can be anticipated that bioartificial blended bioinspired polymer hybrid composites for propolis vaginal delivery could represent intelligent delivery systems with physicochemical and biopharmaceutical properties in favor or efficacious and safe therapy of vaginal infections.

Key words: thermoreversible, mucoadhesive, vaginal gels, propolis, physicochemical and biopharmaceutical characterization

Introduction

Propolis (bee glue) exhibits well-known and documented biological and pharmacological properties as antimicrobial and antifungal and most of them antiviral agent. In this context, it is believed that most of the pharmacological effects of this composite biocomplex are due to its flavonoid content, even though the other components also contribute to its biological activity (Casaroto and Lara, 2010; Ramos and Miranda, 2007). A large number of investigations has confirmed the antifungal properties of propolis, especially its prominent activity against Can-

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dida albicans (Fearnley, 2001; Khalil, 2006; Kujumgiev et al., 1999; Marcucci, 1995; Sawaya et al., 2002), as well as its clinical efficacy in the treatment of vaginal infections. It is thought that propolis has local anesthetic and immunostimulatory effects which are also important in the treatment of vaginal infections (Pochinkova, 1995).

The gels as vaginal drug delivery systems are most commonly used dosage forms amongst the patients, due to their advantages such as easy administration and uniform spreading over the surface, allowing more intimate contact with the vaginal mucosa, precise dosing and the absence of discomfort after their administration (Das Neves and Bahia, 2006; Neves et al., 2009). However, despite these advantages, conventional gels demonstrate relatively fast drug release due to their dilution with vaginal fluids and dissolution. Another disadvantage is the possibility of liquefaction at room temperature; therefore they can leak out of the vagina. To avoid these problems, there is a need for formulation of vaginal gels with local antimicrobial activity which can provide uniform distribution over the surface of the vaginal mucosa, along with prolonged residence time and controlled drug release.

In the recent years, thermosensitive in situ-forming gel systems have been receiving a great deal of interest as vaginal delivery systems for efficient local treatment of various diseases. These environmentally sensitive systems can be easily administered due to their liquid state at room temperature, whereas their gelification in response to environmental changes offers significant advantages such as precise dosing, *in vitro* and *in vivo* stability and slow clearance from local sites due to mucoadhesive properties of the polymers used, therefore allowing prolonged drug release.

Poloxamers are poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) copolymers (PEO-PPO-PEO), known under the trade name of Lutrol®, which form micelles at low concentration and clear thermoreversible gels at high concentrations (Alexandridis and Hatton, 1995). Poloxamers differ in their physical and surface properties due to the differences in the chemical structure. Lutrol® F-127 (LF-127) is a hydrophilic PEO-PPO-PEO tri-block copolymer (Escobar-Chávez et al., 2006) which has low toxicity, good dissolving capacity and it considered as a good vehicle for numerous active substances (Kolsure and Rajkapoor, 2012). Because of its thermoreversible gelification properties and low toxicity, LF-127 can be applied in various drug delivery systems. Lutrol® F-68 (LF-68) contains 81% of PEO units in its composition, which makes this poloxamer easily soluble in water (Fussneger, 1999). At higher concentrations, the water solutions of LF-68 exhibit non-Newtonian behavior and thermosensitivity when the concentration exceeds 20%. However, LF-68 is not used alone as gel-forming agent. Usually, it is combined with LF-127 in order to modify the thermorheological properties of the gels. The addition of LF-68 leads to an increase in the gelaton temperature (*Tg*), most probably due to the formation of mixed micelles and offers the advantage of adjusting the *Tg* value to the desired one, by varying the amount of LF-68 up to 20%. (Fussneger, 1999). Notwithstanding the wide application of thermosensitive poloxamer gels in formulation of different drug delivery systems, their main drawback is the fast erosion due to the low mechanical strength. Therefore, prolonged retention time and controlled drug release from poloxamer gels can be achieved by the combination with other polymers, such as chitosan (CTS). The addition of CTS causes the formation of more dense poloxamer network, therefore resulting in increased mechanical strength of the gel matrix, as well as influencing the drug diffusion and slowing its release (Gratieri et al., 2010; Varshosaz et al., 2008).

As it was already rationalized (Simonoska Crcarevska et al., 2013b) by bioartificial blending bioinspired polymer hybrid composites for propolis vaginal delivery could represent intelligent delivery systems with physico-chemical and biopharmaceutical properties in favor or efficacious and safe therapy of vaginal infections. In our previous work, response surface, Box-Behnken, experimental design was applied for formulation optimization of the thermoreversible mucoadhesive in situ vaginal hydrogels with propolis and optimized batches were identified (Simonoska Crcarevska et al., 2013b). The main objective of this work was to characterize and evaluate physicochemical and biopharmaceutical properties of previously identified formulations.

Materials and methods

Materials

Chitosan (CTS, low viscous, 95% deacetylation) was supplied from Sigma-Aldrich, USA. Poloxamers (Lutrol® F-127 and Lutrol® F-68) were obtained from BASF, Ludwigshaften, Germany. 20% propylene-glycolic extract of propolis (PGEP) was kindly donated from Galafarm, Macedonia. All other reagents and solvents were of analytical grade and used as received.

Methods

Preparation of polymer hybrid composites

Optimized batches of bioartificial polymer hybrid composites (CTS, LF-127 and LF-68 mixture)) (thermo reversible mucoadhesive in situ gels) (CP1, CP2, CP3) were prepared according to the previously described procedure using so-called cold method with minor modification (Simonoska Crcarevska et al., 2013b). Detailed composition of the formulations is presented in Table 1.

Formulation P3 was prepared in order to evaluate the effect of CTS on the physicochemical and biopharmaceutical properties of the polymer hybrid composites (gels).

Table 1. Composition of designed polymer hybrid composites (thermo reversible mucoadhesive in situ gels)

Formulation code	CTS (%)	LF-127 (%)	LF-68/LF-127 mass ratio	PGEP (%)
CP1	1.5	16.39	0.06	3.0
CP2	1.5	17.36	0.09	3.0
CP3	1.5	18.24	0.12	3.0
P3	/	18.24	0.12	3.0

Preparation of simulated vaginal fluid (SVF)

Human vaginal fluid comes from several sources such as uterus, cervix and sometimes menstrual secretions and sperm. Due to the limited quantity of human vaginal fluid (approximately 0.5–0.75 mL) and its rapid degradation after collection from its source, a simulated vaginal fluid (SVF) was developed (Aka-Any-Grah et al., 2010). SVF was prepared as follows: NaCl (3.51 g), KOH (1.4 g), $Ca(OH)_2$ (0.22 g), bovine serum albumin (0.018 g), lactic acid (2.00 g), acetic acid (1.00 g), glycerol (0.16 g), urea (0.4 g) and glucose (5.00 g) were added to 900 ml distilled water and stirred mechanically until complete dissolution. The pH of the SVF was then adjusted to 4.5 using 0.1M HCl, and the final volume was adjusted to 1 L.

Characterization of polymer hybrid composites (thermo-reversible mucoadhesive in situ gels)

Visual characterization

Visual characterization of color, appearance, odor, texture and phase separation on designed and prepared formulations were carried out.

pH determination

Adequacy of designed polymer hybrid composites (thermo reversible mucoadhesive in situ gels) for vaginal use were evaluated by determination of their pH value (pH meter, Metler Toledo 340, Germany). pH evaluation was carried out in triplicate.

Determination of of the sol-gel transition temperature

The sol-gel transition temperature (*Tg*) of the designed formulations was measured by tube inversion method (Ur-Rehman et al., 2010) with minor modifications (Simonoska Crcarevska et al., 2013b). Briefly, 2 ml of prepared formulations were transferred into a glass test tube sealed with a parafilm and put in horizontal shaker water bath (50 strokes/ min). The temperature of the water bath was gradually increased (1 °C/ min) and the temperature at which the solution in the sample containing tube stopped flowing upon inverting the tube was recorded. Similarly, the temperature was decreased and the temperature, at which the gel started flowing, was recorded. The average of both temperatures was calculated as the critical *Tg*.

Considering the possibility of change in the *Tg* of the formulations after their dilution with vaginal fluid (Aka-Any-Grah et al., 2010), the *Tg* of each formulation was also investigated after the addition of 0.75 µl of SVF.

Determination of propolis content

Propolis content in the prepared formulations was determined by validated spectrophotometric method (395 nm, Lambda 16, Perkin Elmer, USA) where quantification of the total flavonoid content was done using chrysin as external reference standard. Briefly, 1g of prepared formulations was dissolved in 100 ml methanolic solution of acetic acid (0.5% v/v). 2 ml of prepared solution and exactly 1 ml of aluminum chloride water solution (2% w/w) were transferred in glass volumetric flask and methanolic solution of acetic acid (0.5%, v/v) was added to a total volume of 10ml. In parallel a compensatory solution was prepared on the same way without aluminum chloride. In the same manner external reference standard of chrysin was prepared (0.02% w/v). Three replicates were carried out to estimate the inherent variability of the determination and the total flavonoids content was expressed in mg of chrysin equivalents per gram of prepared formulations.

Viscosity determination

The viscosity (mPa*s) of the prepared polymer hybrid composites (thermo-reversible mucoadhesive in situ gels) was determined using cone/plate viscometer (Myr V2-L, 4.6/MOTv2, Viscotech, Spain). A sample solution (0.5 ml) was applied to the lower plate of the viscometer and viscosity was determined at at 25 ± 0.5 °C and 32 ± 0.5 °C using spindle 52 at a shear rate ranging from 5 to 400 rpm. All samples were analyzed in triplicate.

Erosion/dissolution and in vitro propolis release studies

Thermo-reversible mucoadhesive in situ gels erosion/ dissolution profile and the *in vitro* propolis release from the designed formulations were obtained simultaneously. Briefly, 2 g of each formulation were transferred in glass tubes, weighted and placed in horizontal shaker water bath (40 rpm) previously thermostated at 32 °C. After the gelation, 10 ml of SVF pre-equilibrated at 32 °C were added. At pre-determined time intervals (1, 2, 3, 4, 5, 6, 7, 8 and 12 h) the total volume of liquid medium was removed, and the weight of eroded/dissolved gel was calculated from the change in the weight (glass tube with gel) at the beginning of the experiment and at each time interval.

Afterwards the release medium was completely replaced by 10 ml of fresh medium at predetermined time intervals. The concentration of propolis in the release medium was determined spectrophotometrically as it was previously described. All experiments were performed as triplicates.

In vitro propolis release modeling and release profile comparison were performed with DDSolver 1.0 program (menu-driven add-in program for Microsoft Excel) (Simonoska Crcarevska et al., 2013a; Zhang et al., 2010).

Microbiological quality

The microbiological quality of the prepared formulations was evaluated according to the Ph.Eur.7 method 2.6.12 (Microbiological examination of non-sterile products (total viable aerobic count)) and method 2.6.13 (test for specified microorganisms)). The acceptance criteria for microbiological quality of vaginal preparations stated in the Ph.Eur.7 (5.1.4 - Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use) are based on the total aerobic microbial count (TAMC) and total yeast and mold count (TYMC).

Stability studies

Prepared formulations were sealed in glass vials and stored under controlled conditions (25 ± 2 °C and 5 ± 3 °C). Periodical testing of different parameters (organoleptic properties, pH value, propolis content, microbiological quality) during 6 months was performed.

Results and discussion

In our previous work (Simonoska Crcarevska et al., 2013b) response surface Box-Benken experimental design was used for development and optimization of propolis vaginal delivery system (polymer hybrid composites (thermo-reversible mucoadhesive in situ gels)) with desired gelling properties. Key formulation factors influencing *Tg* were determined and optimization of formulation was carried out. Optimized batches with *Tg* of 32 °C (CP1-CP3) were prepared and characterized accordingly.

Characterization of polymer hybrid composites (thermo-reversible mucoadhesive in situ gels)

The pH values of the prepared propolis vaginal delivery system were in the range of 4 to 4.5 pointing to suitability of formulations for vaginal use (Table 2).

The gelation temperature was determined for all prepared formulations (CP1-CP3, P3) before and after their dilution with 0.75 μL SVF (Table 2).

Obtained results showed an increase of ~ 2 °C in *Tg* of the formulations diluted with SVF. These differences could be explained by the presence of co-solutes, i.e. ions and electrolytes in the SVF which probably interacted with poloxamers. Considering the strong relation between the gelation and micellization processes, the observed increase of *Tg* value is most likely related to the increased critical micelle concentration or critical micelle temperature. The results of our study also showed that the *Tg* of the P3 formulation prepared without chitosan was slightly higher compared to other formulations (CP1-CP3). Similar findings were reported by Gratieri et al. (Gratieri et al., 2010), indicating that CTS has an effect on the crosslinking and the packing of polymer chains, thus resulting in a denser network which gelled at lower temperature.

Total flavonoids content in the prepared formulations (CP1-CP3) was above 95% (Table 2).

The efficacy of gels intended for local treatment of vaginal infections depends to a great extent on the rheological characteristics of the system. Namely, improved therapeutic efficacy could be achieved by prolonged residence time at the site of action (vaginal mucosa) as well as controlled drug release (propolis) from hybrid polymer matrix. Rheological characteristics are influenced by various factors such as the composition of formulations (polymers used as gel-forming agents), temperature, vaginal pH value, amount of vaginal fluid etc. Comparing viscosity values measured at 25 and 32 °C it can be noticed that prepared polymer hybrid composites (thermo-reversible mucoadhesive in situ gels) were characterized by higher viscosity at 32 °C as it was expected due to thermosensitive properties of used poloxamers. Obatined results pointed that by increasing the concentrations of LF-127 and LF-68/LF-127 ratio, higher viscosity values were observed. Formulation P3 was characterized by lower viscosity compared to CP1-CP3 (Table 2).

Erosion/dissolution and in vitro propolis release studies

Fig. 1 presents the effect of formulation variables (LF-127 and LF-68/LF-127 ratio (CP1-CP3)), as well as CTS

Table 2. Gelation temperature (*Tg*) (mean ± SD) of the designed polymer hybrid composites (thermo reversible mucoadhesive in situ gels) (n=3)

Formulation code	pH values ± SD	*Tg* (°C) ± SD		Total flavonoids		Viscosity (mPa*s ± SD)	
		without SVF	with 0.75 μl SVF	mg/g ± SD	% of the declared content ± SD	25 ± 0.5 °C	37 ± 0.5 °C
CP1	4.36 ± 0.03	29 ± 0.03	31 ± 0.05	2.46 ± 0.01	95.17 ± 0.67	1180 ± 0.2	65220 ± 0.5
CP2	4.06 ± 0.02	29 ± 0.02	31 ± 0.01	2.59 ± 0.02	99.87 ± 0.75	1340 ± 0.3	167930 ± 0.3
CP3	4.13 ± 0.04	29 ± 0.03	31 ± 0.05	2.55 ± 0.02	98.55 ± 0.79	1470 ± 0.1	219600 ± 0.3
P3	4.50 ± 0.05	32 ± 0.02	33 ± 0.04	2.58 ± 0.02	99.15 ± 0.74	930 ± 0.1	33700 ± 0.8

(P3 compared to CP3) on the gel erosion/dissolution vs time. Erosion/dissolution of formulation P3 (CTS free) was completed in 4.5 h, while CP1-CP3 showed decrease in the rate of gel erosion, 5.5-8.5 h accordingly. Results obtained for P3 (CTS free) were in accordance with the literature data, which suggest that gels consisted only by poloxamers dissociate rapidly in an aqueous environment (Chung et al., 2009). The observed results for CP1-CP3 pointed that increase in concentration of LF-127 and LF-68/LF-127 ratio lead to a higher viscosity of the system, thereby causing the formation of more dense gel network which erodes at a slower rate. The presence of CTS retarded the gel erosion/dissolution due to its incorporation into the gel skeleton most likely resulting with increased mechanical strength of the gel network (Varshosaz et al., 2008).

The results of *in vitro* propolis release study are presented in Fig. 2. It can be seen that propolis release follows gel erosion/dissolution processes. Propolis release rate was the highest in P3 formulation (CTS free), which released propolis within 5 h, corresponding to time of complete erosion. Furthermore, the same correlation between erosion process and drug release rate was observed in formulations CP1-CP3, where prolonged propolis release for more than 10 h was achieved, due to the effect of CTS.

The continuous swelling of poloxamers accounts for achieving prolonged drug release when used as gelling agents. Additionally, drug diffusion occurs through the extramicellar aqueous channels formed during matrix erosion/dissolution. Hence, the decrease in the rate of propolis release with the increase in LF-127 concentration could be attributed to the increase of number and size of the micelles formed at higher polymer concentration (Radivojša et al., 2013). This could cause a greater extent of polymer chains entanglement in the aqueous phase of the gel structure and slower dissolution rate. Additionally, higher poloxamer concentration could cause shorter intermicellar distance, leading to a larger number of cross-links between neighboring micelles and larger number of micelles per volume, along with subsequently slower rate of dissolution of the incorporated drug (Ibrahim et al., 2012).

Even though the chosen polymers differ in terms of chemical structure, both CTS and poloxamers have hydrophobic regions in their chains (D-glucose residues in chitosan and polypropyloxyethylene blocks in poloxamers). When the temperature is increased, water molecules structured around the hydrophobic regions of polymer chains in a sol state become disarranged. As a result, newly revealed hydrophobic regions attract each other to form bonds, whereas hydrophilic parts reorganize to maximize their contact with the aqueous medium. The resulting structures are micelles, which continue to grow in size and number at higher temperatures, leading to higher viscosity of the gel structure and consequently, slower rate of drug release is achieved (Varshosaz et al., 2008).

Drug release kinetics of propolis from the prepared formulations was determined by analyzing the dissolution data using various mathematical models. The kinetics constants and correlation coefficients for propolis release are shown in Table 3. Taking into account the values of correlation coefficients (R), it can be concluded that Korsmeyer-Peppas model is the best one to describe the propolis release from the prepared formulations. According to the obtained values of release rate constant (K), it can be claimed that the drug dissolution rate decreases with the increase of LF-127 and LF-68 concentrations, as expected. Predominance of diffusion in release mechanisam was observed for CP1-CP3, while P3 (without CTS) demonstrated release mechanism most likely controlled by diffusion and gel erosion/dissolution.

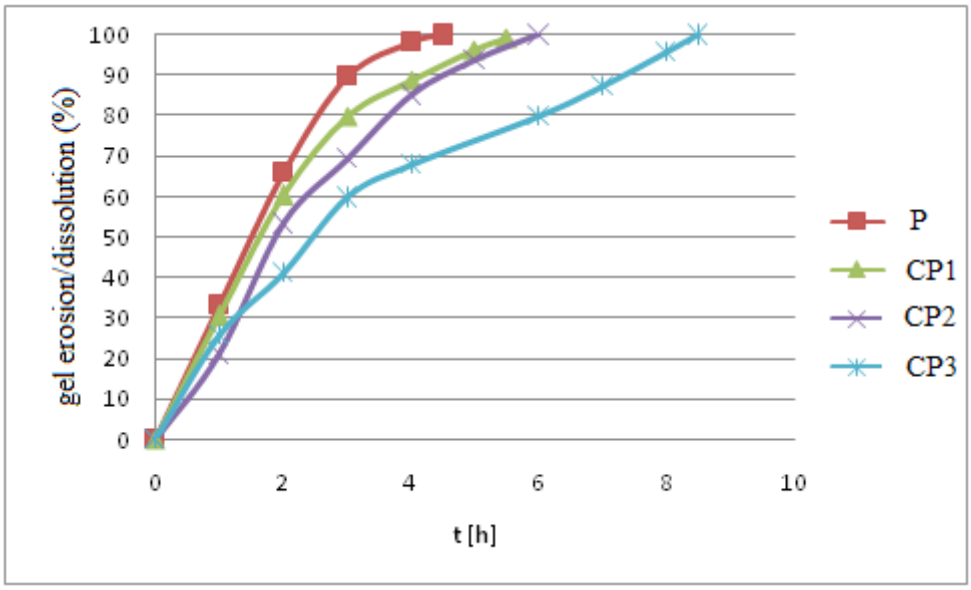

Fig. 1. Gel erosion/dissolution of designed polymer hybrid composites (thermo reversible mucoadhesive in situ gels).

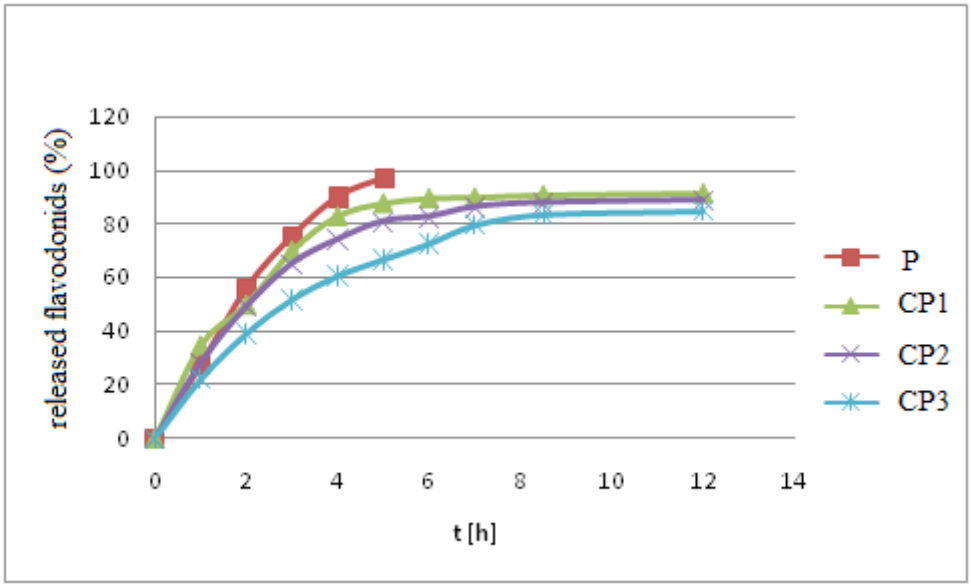

Fig. 2. *In vitro* release profiles of propolis from designed polymer hybrid composites (thermo reversible mucoadhesive in situ gels).

Table 3. Kinetics constants and coefficients for propolis release from the prepared formulations

Formulation code	First order		Higuchi		Korsmeyer-Peppas			Peppas-Sahlin		
	K (1/h)	R	K ($h^{-0.5}$)	R	n	K (h^{-n})	R	k1	k2	R
CP1	0.356	0.9890	33.475	0.9232	0.565	35.165	0.9913	51.756	-6.939	0.9807
CP2	0.367	0.9831	31.638	0.9456	0.613	30.445	0.9888	45.993	-5.449	0.9833
CP3	0.194	0.9811	28.019	0.9800	0.542	27.545	0.9951	33.266	-1.991	0.9856
P3	0.446	0.9869	42.264	0.9809	0.793	29.625	0.9906	25.012	9.175	0.9927

Microbiological quality

The obtained results related to microbiological quality were in accordance with the requirements stated in Ph.Eur. 7.

Stability studies

The results of the stability studies of the prepared formulations stored at 25 ± 2 °C and 5 ± 3 °C during 6 months are presented in Table 4 and 5, respectively.

Formulations CP1 and CP2 demonstrated adequate stability during 6 months of storage at 25 ± 2 °C without any significant changes in their organoleptic properties, pH values, propolis content, viscosity and microbiological quality. CP3 formulation showed adequate stability during 5 months of storage 25 ± 2 °C, but after 6 months changes in its organoleptic properties were observed, i.e. viscous liquid was formed. On the other hand, all formulations demonstrated adequate stability at 5 ± 3 °C during 6 months.

Conclusion

To summarize in this work by bioartifical blending of natural and synthetic polymers thermo reversible mucoadhesive in situ gels were prepared and characterized**.** Addition of bio/mucoadhesive macromolecules (chitosan) to the poloxamers (Lutrol® F-127 and Lutrol® F-68) based vaginal delivery system resulted with increased viscosity, decreased gel erosion/dissolution rate thus improving the sustained release of propolis. Chitosan not only helped to circumvent draw backs of poloxamer gels alone like fast erosion/ dissolution, but also did not adversely affect its thermosensitive behavior. Based on the results obtained it can be concluded that chitosan-poloxamer based systems would enable prolonged residence time allowing prolonged drug release at the desired site of action and hence resulting with better therapeutic efficacy.

Table 4. Results from stability studies of the prepared formulations stored at 25 ± 2 °C during 6 months period

Parameter	Formulation code	Time of evaluation (month) 0	1	2	3	4	5	6
Organoleptic properties	CP1	Dense homogenous liquid with yellow-brownish color and propolis smell						
	CP2							Non-homogeneous liquid with a yellow-brownish color and propolis smell
	CP3	Dense homogenous liquid with yellow-brownish color and propolis smell						
pH value	CP1	4.36 ± 0.2	4.25 ± 0.15	4.31 ± 0.1	4.2 ± 0.13	4.22 ± 0.08	4.18 ± 0.1	4.09 ± 0.11
	CP2	4.06 ± 0.1	3.97 ± 0.12	4.00 ± 0.13	3.95 ± 0.09	3.91 ± 0.1	3.85 ± 0.08	3.79 ± 0.1
	CP3	4.13 ± 0.14	4.00 ± 0.11	4.09 ± 0.1	3.98 ± 0.12	3.94 ± 0.07	3.93 ± 0.1	4.1 ± 0.13
Total flavonoid (% of declared content)	CP1	95.17 ± 1.1	94.83 ± 0.9	98.99 ± 1.07	95.98 ± 1.5	95.64 ± 1.34	95.98 ± 1.46	94.52 ± 0.94
	CP2	99.87 ± 1.5	99.88 ± 1.84	103.63 ± 0.6	101.35 ± 1.2	99.65 ± 1.74	99.77 ± 1.36	102.56 ± 0.8
	CP3	98.55 ± 1.3	98.3 ± 0.5	106.99 ± 2.3	103.78 ± 1.82	97.14 ± 1.12	94.17 ± 1.9	98.19 ± 0.96
Viscosity (mPa*s)	CP1	1180 ± 0.2	/	/	/	/	/	1190 ± 0.4
	CP2	1340 ± 0.3	/	/	/	/	/	1380 ± 0.9
	CP3	1470 ± 0.1	/	/	/	/	/	/
Microbiological quality criteria compliance	CP1	Yes	/	/	/	/	/	Yes
	CP2	Yes	/	/	/	/	/	Yes
	CP3	Yes	/	/	/	/	/	Yes

Table 5. Results from stability studies of the prepared formulations stored at 5 ± 3 °C during 6 months period

Parameter	Formulation code	Time of evaluation (month) 0	1	2	3	4	5	6
Organoleptic properties	CP1	Dense homogenous liquid with yellow-brownish color and propolis smell						
	CP2							
	CP3							
pH value	CP1	4.36 ± 0.2	4.02 ± 0.1	3.99 ± 0.14	3.97 ± 0.1	3.82 ± 0.3	3.90 ± 0.16	3.78 ± 0.2
	CP2	4.06 ± 0.1	4.30 ± 0.9	4.25 ± 0.5	4.20 ± 0.4	4.19 ± 0.1	4.23 ± 0.09	4.09 ± 0.18
	CP3	4.13 ± 0.14	4.09 ± 0.56	4.01 ± 0.28	3.98 ± 0.35	3.95 ± 0.2	3.97 ± 0.25	3.86 ± 0.3
Total flavonoid (% of declared content)	CP1	95.17 ± 1.1	98.84 ± 0.7	96.52 ± 1.22	94.98 ± 2.31	94.21 ± 0.8	96.14 ± 1.98	95.75 ± 2.21
	CP2	99.87 ± 1.5	103.08 ± 1.82	99.61 ± 1.93	99.23 ± 1.85	98.84 ± 1.32	101.54 ± 2.23	102.38 ± 1.3
	CP3	98.55 ± 1.3	107.72 ± 1.31	94.21 ± 2.15	98.84 ± 1.96	92.28 ± 1.64	103.47 ± 1.57	98.07 ± 1.84
Viscosity (mPa*s)	CP1	1180 ± 0.2	1180 ± 0.8	/	/	/	/	1190 ± 0.4
	CP2	1340 ± 0.3	1340 ± 0.7	/	/	/	/	1380 ± 0.9
	CP3	1470 ± 0.1	1470 ± 0.5	/	/	/	/	1560 ± 0.8
Microbiological quality criteria compliance	CP1	Yes	Yes	/	/	/	/	Yes
	CP2	Yes	Yes	/	/	/	/	Yes
	CP3	Yes	Yes	/	/	/	/	Yes

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Резиме

Биоинспирирани биовештачки полимерни хибридни композити со контролирано ослободување на прополис во вагина II: формулација и карактеризација

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Клучни зборови: термореверзибилни, мукоатхезивн, вагинални гели, прополис, физичко-хемиска и биофармацевтска карактеризација

Во нашиот претходен труд со примена на Box-Behnken експериментален дизајн беше направена оптимизација на термореверзибилни мукоатхезивни *in situ* вагинални хидрогели со прополис. Оптимизираните формулации на биовештачки полимерни хибридни композити (смеса на цитозан, Lutrol® F-127 и Lutrol® F-68) (CP1, CP2, CP3) беа подготвени со примена на т.н. ладен метод. Формулацијата P3 која не содржи цитозан, беше подготвена со цел да се евалуира влијанието на цитозанот врз физичко-хемиските и биофармацевтските особини на полимерните хибридни композити (гели). Подготвените гели се карактеризираа со pH од 4-4,5. Температурата на гелирање кај сите формулации беше во опсег од 29-33 °C. Содржината на вкупните флавоноиди беше поголема од 95%. Зголемувањето на концентрацијата на Lutrol® F-127 и односот на Lutrol® F-68/Lutrol® F-127 резултираше со поголеми вредности на вискозитетот и побавна ерозија/дисолуција на гелите. Присуството на цитозанот во формулацијата резултираше со зголемување на вискозитетот на гелот и негова побавна ерозија/дисолуција. Брзината на ослободување на прополисот беше најголема кај формулацијата P3 кај која целата количина на прополис беше ослободена за 5 часа, што кореспондираше со времето на комплетна ерозија на гелот. Слична корелација меѓу процесот на ерозија и брзината на ослободување на прополисот беше забележана и кај формулациите CP1-CP3, кај кои беше постигнато продолжено ослободување на прополисот во период поголем од 10 часа. Микробиолошкиот квалитет на подготвените формулации беше во согласност со барањата на Ph. Eur. 7. Сите формулации беа стабилни на 5 ± 3 °C во тек на 6 месеци. Врз база на севкупните резултати може да се заклучи дека биовештачките блендирани биоинспирарни полимерни хибридни композити со контролирано ослободување на прополис може да преставуваат интелегентни вагинални системи со физичко-хемиски и биофармацевтски особини кои ќе овозможат ефикасен и безбеден третман на вагиналните инфекции.