ORIGINAL ARTICLE

Formulation and technology development of vaginal pessaries with probiotic activity

Formulace a technologický vývoj vaginálních globulí s probiotickou aktivitou

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Received December 19, 2019 / Accepted March 3, 2020

Summary

Lactobacilli use in treatment and prevention of the vaginal microflora disorders, such as bacterial vaginosis and vulvovaginal candidiasis, is highly promising. The objective of this study was is to develop formulation and technology of the extemporal *Lactobacillus casei* (*L. casei*) IMB B-7280-containing medicinal product in the form of vaginal pessaries**.**

The quality control parameters were defined in accordance with the State Pharmacopeia of Ukraine (2nd edition) and included appearance, uniformity of texture, uniformity of mass and disintegration test. *Lactobacilli* assay was determined after preparation and within the storage period. Thus, feasible formulation and technology were selected for vaginal pessaries with an expected 6-month shelf life. The results of the hereby described research will be used for technological instruction development for extemporaneous vaginal pessaries with defined probiotic activity.

Key words: vaginal pessaries • *Lactobacilli* • vaginal dysbiosis

Souhrn

Použití laktobacilů při léčbě a prevenci poruch vaginální mikroflóry, jako je bakteriální vaginóza a vulvovaginální kandidóza, je velmi slibné. Cílem této studie bylo optimalizovat složení a technologii v současné době vyvíjeného léčivého přípravku IMB 72-7280 ve formě vaginálních globulí obsahujícího *Lactobacillus casei* (*L. casei*).

Parametry kontroly kvality byly definovány v souladu se Státním lékopisem Ukrajiny (2. vydání) a zahrnovaly posouzení vzhledu, stejnoměrnost textury, hmotnostní stejnoměrnost a zkoušku rozpadavosti. Obsah laktobacilů byl hodnocen po přípravě a během doby skladování. V rámci studie bylo vybráno vhodné složení a technologie pro přípravu vaginálních globulí s očekávanou skladovací dobou 6 měsíců. Výsledky popsané studie budou použity pro vývoj technologických instrukcí pro extemporózní vaginální pesary s definovanou probiotickou aktivitou.

Klíčová slova: vaginální globule • *Lactobacilli* • vaginální dysbióza

Introduction

Bacterial vaginosis (BV) is the most common disease of the female genital tract affecting premenopausal, postmenopausal and pregnant women (prevalence from 5% to 50%)[1)]. It is characterized by a complete change of vaginal microbiota, reduction or loss of *Lactobacilli* and increased numbers of anaerobes or facultative anaerobic organism (mostly *Gardnerella vaginalis* and *Atopobium vaginae*, *Prevotella species, Mobiluncus species*), followed by alternation of normal pH[2–6)]. *Lactobacilli* are the predominant microorganisms in the healthy human vagina microenvironment[4, 7, 8)], among which *L. crispatus, L. gasseri, L. jenesenii, L. iners, L. vaginalis* та *L. rhamnosus*[1, 9–13)] strains are the most common ones.

According to the scientific knowledge, more than 200 *Lactobacilli* strains are defined[14)], however only 1–3 of them are dominant in the individual vaginal microenviroment[12)].

Lactobacilli are capable of vaginal microbiota homeostasis maintenance by means of auto-aggregation, production of lactic and acetic acids, H_2O_2, bacteriocins and biologically active substances, co-aggregation with pathogenic microorganisms, adhesion to epithelial cells and immunomodulatory effect[1, 5, 11, 15)].

The healthy vaginal epithelium demonstrates a resistance to the invasion of the pathogenic microorganisms. The amount of glycogen in the surface

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area cells is one of the key parameters reflecting the resistance ability of the vaginal epithelium. In the process of constant renovation, old cells, being destroyed in the cycle of constant renovation, release glycogen that is the substrate for normal microbiota. The amount of glycogen in the vaginal epithelium cells may differ within the time of the year and depends on the menstrual cycle phases (the highest levels are accumulated during ovulation)[16].

Women of reproductive age demonstrate vaginal pH in the rage of 3.8–4.4. However, deviations are possible from 6.6 (± 0.3) to 4.2 (± 0.2) between day 2 and day 14 of the menstrual cycle.

Vaginal glycogen serves as the nutrient source for *Lactobacilli* and is transformed into lactic acid by fermentation. Lactic acid enables the acidic pH of vaginal flora to keep to 3.8–4.4, causing unfavourable conditions for other microorganisms' development. Exceptions resulting in a pH increase are observed during menstruation and within 24–48 hours after the intercourse, due to the presence of nitrogen-containing bases in sperm that are potent to neutralize the vaginal natural acidity level[12, 17–19].

Adhesive capacity of *Lactobacilli* empowers them to compete with pathogenic and opportunistic pathogenic bacteria for nutrient sources. Adherence of *Lactobacillus* is mediated by lipoteichoic acid. Due to physicochemical interaction between *Lactobacilli* and the vaginal epithelium, allowing *Lactobacilli* colonization, a specific biofilm is generated to cover the mucosa and vaginal epithelium. Being attached to epitheliocytes, *Lactobacilli* create a solid cover on the vagina walls preventing other microorganism from adhesion to epitheliocytes receptors[20, 21].

Most of *Lactobacilli* (70–96%) can produce hydrogen peroxide (H_2O_2), which interacts with cervical mucus. Due to this interaction, growth suppression and abortion of obligate anaerobes and multiplication of opportunistic pathogenic microorganisms are possible[1, 12, 18, 19].

Data available show that *Lactobacilli* can produce antimicrobial substances such as bacteriocins (nisin, diplocin, lactospretcin, helveticin, calycin, microcin, pesticin, pyocin, etc.). These are low-molecular-weight peptides capable of adjusting to specific microbial cell receptors in order to cause membrane destabilization

Table 1. Several studies with different dosage forms for vaginal use containing Lactobacillus

Author of study	Microorganism	Vaginal dosage form	Reference
Anukam KC, et al.	*L. rhamnosus*, *L. reuteri*	capsules	1, 24
Mastromarino P, et al.	*L. brevis, L. salivarius*, *L. plantarum*	vaginal tablets	1, 25
Parent D, et al.	*L. acidophilus*	vaginal tablets	1, 26, 38
Hallén A, et al.	*L. acidophilus*	suppositories	1, 27, 38
Ehrström S, et al.	*L. gasseri, L. fermentum, L. casei subsp. rhamnosus*, *P. acidi lactici*	vaginal capsules	17, 28
Maggi L, et al.	*L. brevis, L. salivarius, L. crispaius, L. gasseri*	tablets	17, 29
Burton JP, et al.	*L. fermentum*, *L. rhamnosus*	vaginal capsules	17, 30
Eriksson K, et al.	*L. fermentum, L. casei var. rhamnosus*, *L. gasseri*	tampons	17, 31, 38
Czaja CA, et al.	*L. crispatus*	suppositories	17, 32
Uehara S, et al.	*L. crispatus*	suppositories	40
Drago L, et al.	*L. acidophilus*	vaginal douche	17, 33
Kale V, et al.	*Lactobacilli lyophilizate*	vaginal tablets	17, 34
Kale V, et al.	*L. sporogenes*	suppositories	41
Larsson PG, et al.	*L. gasserii*, *L. rhamnosus*	vaginal gelatin capsules	17, 35
Kaewnopparat S, Kaewnopparat N.	*L. paracasei*	vaginal suppositories	17, 36
Rodrigues F, et al.	*L. acidophilus*	vaginal suppositories	37
Ozkinay E, et al.	*L. acidophilus*	vaginal tablets	38, 39
Ya W, et al.	*L. rhamnosus, L. acidophilus*, *St. thermophilus*	vaginal capsules	42
Kaewsrichan J, et al.	*L. jensenii*, *L. crispatus*	vaginal tablets	43
Kaewsrichan J, et al.	*L. jensenii*, *L. crispatus*	suppositories	43
Fazeli MR, et al.	*L. acidophilus*	vaginal tablets	44

and endoplasma leaks resulting in the destruction of pathogenic, opportunistic pathogenic microorganisms and fungi[16]. Lactocidin, acidolin and lactacin B effects are significant for maintaining healthy vaginal microbiome. Lactocins B, F, J, M, acidolin and lactocidin, bulgaricin, lactobrevin, helveticin, lactolin, and reuterin demonstrate an ability to inhibit growth and multiplying of a vast number of bacilli, *Clostridia, Saccharomycetes, Streptococcus, Staphylococcus, Enterobacteriaceae, Pseudomonas, Listeria,* and *Candida*[22, 23].

Lactobacilli protective function is realized by means of various biologically active substances such as glycolipids, lipopeptides, polysaccharide-peptide complexes, phospholipids, fatty acids and neutral lipids that contribute to pathogens' growth suppression and prevent microorganisms' adhesion to epithelial cells[19].

The *Lactobacilli* immunostimulatory effect is revealed through activation of macrophages, accumulation of phagocytes, cytokine synthesis and an Ig level increase[11, 15].

Data obtained from literature review have shown numerous studies on efficacy and wide prospective of various *Lactobacilli* strains use in various vaginal dosage forms prophylaxis and treatment of vaginal flora disorders (Table 1).

Thus, vaginal dosage forms available around the world include creams, gels, tablets, capsules, pessaries, foams, ointments, films, tampons, rings, and douches[17]. Numerous researches demonstrate profound use of *Lactobacilli*-containing dosage forms for vaginal delivery.

The effect of vaginal application of medicinal products is achieved by uniform distribution throughout the vaginal cavity. The choice of dosage form depends on the expected therapeutic effect. Semi-solid and solid dosage forms are required for local effect, achieved mostly by pessaries and vaginal tablets widely used in gynaecological practice[45, 46].

Extemporaneous production is an integral part of the pharmaceutical market in highly developed European countries. In Europe two types of pharmaceutical preparations are prepared in drugstores: extemporaneous preparations and stock preparations[47].

Pharmaceutical compounding in the European Union is carried out in accordance with the Good Pharmacy Practice standards. In Ukraine, this pharmacy production activity is also regulated by appropriate orders, instructions and other legal acts.

The objective of this study is the formulation and technology development of vaginal pessaries with the substance *L. casei* for the treatment and prevention of vaginal flora disorders (extemporaneous production), as well as the quality control of the dosage form according to the requirements of the State Pharmacopoeia of Ukraine (2nd edition).

Experimental part

Materials

Freeze-dried *L. casei* IMB B-7280 (10^{13} CFU/g) extracted from biological material and synthesized in the laboratory at Danylo Zabolotny Institute of Microbiology and Virology NASU were used as the active substance of vaginal pessaries[48, 49]. Samples were prepared with an approximate count of *Lactobacilli* 10^9 CFU per 1 pessary.

As the base for vaginal pessaries, the following components were used: hard fat Novata®B PH with the melting point of 35.2 °C – *Ph. Eur.* (BASF, Germany), polyoxyethylene glycols (PEGs) with the molecular weight of 1500 and/or 4000 (JSC Fine Organic Synthesis Plant Barva, Ukraine), PEG Kollisolv® 400 (BASF, Germany). Polysorbate 80 (ERCA, Italy) was used as the emulsifier and purified water as the solvent.

Preparation of vaginal pessaries

Pessaries were compounded at the scientific laboratory of O. O. Bogomolets National Medical University Pharmacy and Industrial Technology of Drugs Department. For pessaries preparation, diphilic base was chosen in order to avoid main disadvantages of both hydrophilic and lipophilic bases. Moulding was used as the pessaries preparation method. The composition of the pessaries samples is presented in Table 2.

Technology of preparation

Hydrophobic and hydrophilic bases were melted separately in porcelain dishes on a VB-8 stainless steel water bath (Medika, Ukraine) at the temperature of 50–55 °C. The freeze-dried substance with probiotic activity diluted with a small (as small as possible) volume

Table 2. Composition of the experimental samples

Components	Components concentration in % to 100 g of base				
	Sample №				
	1	2	3	4	5
PEG-400	16.0	17.0	15.0	24.0	15.0
PEG-1500	32.0	–	25.0	40.0	65.0
PEG-4000	32.0	73.5	10.0	16.0	–
Hard fat	17.5	6.5	46.5	16.5	17.0
Polysorbate 80	1.5	2.0	2.5	2.5	3.0
Purified water	up to 100.0				

of purified water was incorporated into a base cooled down to the temperature of 40–45 °C being thoroughly stirred till uniform (homogeneous). Hydrophobic and hydrophilic fractions were mixed with the emulsifier polysorbate 80 and poured into a fluoroplastic (teflon) suppository mould F-4 (TC 6-05-810-88) (TM Promvit, Ukraine) (claimed mass of 1 pessary made in the mould matrix was 3.9 g), firstly cooled down for 1 hour at room temperature, then refrigerated at the temperature of 2–8 °C until the pessaries were fully congealed.

Methods

Microbiological study

The number of *Lactobacilli* was determined in the prepared sample by a microbiological method using pessaries prior melted in a thermostat (within 1 hour at the temperature of 37 °C). A fixed volume of the melted pessaries was applied onto a Petri dish covered with solid selective growth media. The nutrient medium MRSA (Merck, Germany) was used to identify *L. casei* IMB B-7280 strain in pessaries.

The following growth media were used for opportunistic pathogenic microorganisms' microbiological tests; BAIRD-PARKER-Agar (Merck, Germany) was utilized as the selective medium for *Staphylococcus*; KF--Streptococcusagar (Merck, Germany) was used as the selective medium for *Streptococcus*; ENDO (Farmactive Ltd., Ukraine) was chosen as the selective medium for coliform bacteria; Saburo (Farmactive Ltd., Ukraine) served as the selective medium for microscopic fungi. The bacteria count was counted up in a Petri dish after cultivation within 24 hours at 37 °C, presuming that one colony equals one bacterium.

Samples of pessaries as a particular dosage form were tested for quality parameters (appearance, uniformity of texture, uniformity of mass, disintegration) defined in accordance with the State Pharmacopeia of Ukraine (2nd edition).

Appearance and uniformity of texture tests

Pessaries must be of the same size and shape. Uniformity of texture is defined by splitting the pessary longitudinally. No inclusions on the split are acceptable, while axial air cavities inside or funnel-shaped indentation are allowed.

Uniformity of mass: 20 sample units must be taken at random, each weighed separately with a following average mass calculation. The individual deviation must be not more than ± 5%.

Disintegration (method 2.9.2): hydrophilic-based pessaries are examined in 60 minutes, while the hydrophobic-based ones undergo examination in 30 minutes. The aim of disintegration test is to determine whether a pessary sample softens or disintegrates within the required time period. Disintegration tests were performed at a suppository and pessaries disintegration tester PTS 3E (PHARMA TEST, Austria). Three pessaries of each prepared samples were placed into a perforated basket, then dumped into a water bath and heated up to 37 °C, being turned every 10 minutes through 180 °. The procedure identified the time needed for samples disintegration, leading to the following effect: melted fatty components piled on the surface, while soluble components were completely dissolved.

Stability test

All five samples of vaginal pessaries were kept in glass containers at room temperature (25 ± 2 °C) and 2–8 °C for 6 months. At appropriate time intervals 0, 1 month, 3 months and 6 months, the assay of *Lactobacilli* was determined by a microbiological method using MRSA (Merck, Germany) as the medium.

Results and discussion

Diphilic base was chosen for vaginal pessaries formulation based on the peculiarity of its properties.

The main advantages of lipophilic bases include an optimal melting point range (30–36 °C), good release of water-soluble drugs, and no irritability on the mucous membranes. Compared to theobroma oil, the positive properties of semisynthetic hard fat bases can be seen in the next points: solidifying points unaffected by overheating, good resistance to oxidation because of a lower content of unsaturated fatty acids and good water-absorbing capacities. Moreover, no mould lubricant is necessary to use because of excellent volume contractility on cooling. On the other hand, hard fat also shows some disadvantages: brittleness if cooled rapidly, impossibility to use a refrigerator during preparation, and low viscosity in melted state. The latter factor could be responsible for drug particles sedimentation during preparation and non-uniform drug distribution causing local irritability[50–52].

Hydrophilic pessaries need water for the medicine to be successfully dissolved and absorbed. Unlike hydrophobic-based pessaries, hydrophilic ones can dissolve in physiological fluids[50] In accordance with the accepted general assumption, PEGs have long been claimed to be favourable compounds of the hydrophilic base due to their chemical stability, inertia, and wide availability. Basic quality control parameters of pessaries such as mechanical hardness, solubility, and appropriate softening temperature can be easily achieved by using PEGs with different molecular weights[51]. However, PEGs have significant disadvantages compared to fats. Firstly, they tend to be more chemically reactive, therefore the preparation technology must be carefully followed to avoid organoleptic parameters of pessaries being non-compliant with the norms set. Secondly, the rate of water-soluble substances release decreases noticeably with the PEG molecular weight increase. In addition, PEGs appear to be more irritating to mucous membranes[37, 51].

Thus, the diphilic base was considered to be a highly promising option to avoid both hydrophobic and hydrophilic bases demerits and was selected for *Lactobacilli* incorporation. *Lactobacilli* substance is in

fact a water-soluble lyophilizate, consequently, a better release rate is expected from hydrophilic bases, since they allow direct diffusion on substances into physiological fluids. Still, the use of a PEGs mixture only is regarded to be non-feasible, since these ingredients, being highly hydrophilic and hygroscopic, may cause mucous dehydration and thus, lead to a substances absorption rate decrease[52].

The first phase of the research was devoted to a microbiological study of pessaries to identify an assay of *Lactobacilli* (Table 3) in the experimental samples that also were tested for microbiological purity (Table 4).

Efficacy of probiotic microorganisms is determined by appropriate delivery of active substances in reasonable concentrations. From the technological perspective, a probiotic medicine must be composed of particular *Lactobacilli* strains capable of passing all the technological stages in high living concentrations along with maintaining relevant probiotic activity within the shelf life. Consequently, it is important to consider ranges of probiotic microorganisms in the terms of minimal therapeutic doses[53].

According to scientific data, viable *Lactobacilli* concentrations of 10^9 CFU for vaginal administration claim their potential to restore and maintain the urogenital tract microflora[53].

Our samples № 3 and № 5 complied with the microbiological purity criteria set out and demonstrated satisfactory results with an identified count of *Lactobacilli* not less than 10^9 CFU. *Lactobacilli* assay in samples № 1, № 2 and № 4 did not correspond to the claimed value. Moreover, in sample № 4 the growth of *Streptococcus* and microscopic fungi was detected.

The obtained results might have been induced either by main physical and chemical properties of the base used or by difference in base components and excipients proportion.

Table 3. Lactobacilli-viability in the experimental samples

Sample №	Batch №	Amount of *Lactobacilli*, CFU/ml	Mean value
1	1	$7.22 \pm 0.20*10^8$	$7.21 \pm 0.20*10^8$
	2	$7.12 \pm 0.18*10^8$	
	3	$7.29 \pm 0.23*10^8$	
2	1	$5.23 \pm 0.15*10^8$	$5.26 \pm 0.17*10^8$
	2	$5.36 \pm 0.17*10^8$	
	3	$5.21 \pm 0.20*10^8$	
3	1	$2.1 \pm 0.17*10^{10}$	$2.14 \pm 0.17*10^{10}$
	2	$2.17 \pm 0.19*10^{10}$	
	3	$2.14 \pm 0.16*10^{10}$	
4	1	$9.11 \pm 0.11*10^7$	$9.14 \pm 0.14*10^7$
	2	$9.08 \pm 0.15*10^7$	
	3	$9.23 \pm 0.17*10^7$	
5	1	$7.54 \pm 0.16*10^9$	$7.54 \pm 0.17*10^9$
	2	$7.58 \pm 0.18*10^9$	
	3	$7.49 \pm 0.17*10^9$	

P ± 95%, n = 5

Table 4. Microbiological purity test results of the experimental samples

Sample №	Amount of pathogenic microorganisms, CFU/ml			
	Streptococcus	***Staphylococcus***	***Coliform bacteria***	***Microscopic fungi***
1	–	–	–	–
2	–	–	–	–
3	–	–	–	–
4	$2.25 \pm 0.18*10^2$	–	–	$1.0 \pm 0.08*10^{-1}$
5	–	–	–	–

P ± 95%, n = 5

Polysorbate 80 – a well-known non-ionic surfactant and emulsifier – is widely used in food, pharmaceutical and cosmetic (beauty) industry. Moreover, it is utilized as a nutrient medium for *Lactobacilli*, capable of boosting *Lactobacilli* growth and contribute to their protection from unfavourable outer factors, including acids, lyophilization, nutrients deficiency, and bile salts influence[54]. Being included into formulations, polysorbate 80 stimulates production of biologically active substances, namely bacteriocins[55] and lactic acid[56] that maintain healthy vaginal microbiome.

In addition, due to surfactants hydrophobic bases are much better absorbed by mucous, which is realized by greater interaction of particles surface with mucous[57].

What is more, non-ionic surfactant polysorbate 80 use in medicine formulation causes less damage on mucous compared to anionic.

A large number of existing studies in the broader literature have examined the role of polysorbates on substances release. For example, Hanaee et al. research has shown that the release rate of salbutamol in Witepsol® H15-based suppositories altered linearly with the amount of polysorbate 80 in suppository formulations[58].

Thus, the results obtained in our research might be interpreted from the perspective of polysorbate presence and concentration in the formulations. Basically, sample № 3 and sample № 5 with polysorbate 80 concentrations in the pessary base up to 2.5–3.0%, demonstrated the highest *Lactobacilli* count. Sample № 4, with polysorbate concentration up to 2.5%, presented a 10^7 CFU *Lactobacili* count. It must be noted that sample № 4 was contaminated with *Streptococcus* and fungi, being the reason of *Lactobacilli* growth suppression.

Moreover, diphilic bases have never been used by the authors before for *Lactobacilli*-containing pessaries formulation. Nonetheless, a number of researches have illuminated the data on probiotic bacteria viability both in hydrophilic and lipophilic based suppositories.

Kaewnopparat et al. investigated *L. paracasei HL32* containing solid body and hollow-type suppositories based on Witepsol H-15 or PEGs mixture. The research has shown the identical viability of *Lactobacilli* from both hydrophilic and lipophilic bases, however the hollow-type suppositories 10^8 CFU viability was higher than that of 10^5 CFU solid body ones[36].

Rodrigues et al. examined solid body and hollow-type *L. acidophilus* containing vaginal pessaries. Formulation included Witepsol H-15 or PEGs. All the samples obtained showed a *Lactobacilli* count of not less than 10^8 CFU[37].

Kale et al. studied three types of pessaries containing lyophilized *Lactobacillus* spp. based on a PEG mixture. Plain pessaries survival was 10^5 CFU, while 10^7 CFU viability was shown by multifunctional bilayer ones and 10^8 CFU by hollow-type units[59].

Pashayan research was devoted to double layer vaginal pessaries with lyophilized *L. delbrueckii* MH-10 and *Achillea millefolium* extract powder. All four samples were lipophilic based and indicated 10^8 CFU viability[60].

The results of our study demonstrated the viability of *Lactobacilli* ranged between 10^7 and 10^{10} CFU in all samples. The best value of *Lactobacilli* assay was identified in sample № 3 which consists of hydrophilic and lipophilic phases in the ratio of almost 1 : 1 and 2.5% of polysorbate 80. Thus, we can confirm that our results show some better viability of *Lactobacilli* than the subsequences of other researchers.

It is also current to compare the assay results of pessaries and suppositories with other dosage forms for vaginal use. For example, Zárate et al. analyzed gelatin capsules with *Lactobacilli*. The values of survival are between 10^7 and 10^9 CFU[37, 53]. Fazeli et al. investigated vaginal tablets with *Lactobacillus acidophilus* and demonstrated viability ranging from 10^8 to 10^{10} CFU in different types of vaginal tablets[44]. These results are in accordance with those of Maggi et al. and Mastromarino et al. whose viability was about 10^8–10^9 CFU/ tablet[5, 29, 37, 44].

So, the subsequences of other researchers do not object to our results.

Table 5. Experimental samples quality control according to the requirements of the State Pharmacopeia of Ukraine (2nd edition)

Parameter	Sample №				
	1	2	3	4	5
Appearance	White-yellowish pessaries with no specific odour.	White-yellowish pessaries with no specific odour.	White-yellowish pessaries with no specific odour.	White-yellowish pessaries with no specific odour.	White-yellowish pessaries with no specific odour.
Uniformity of texture	No inclusions presented on the split. No axial air cavity or indentation found inside.	Inclusions presented on the split. Axial air cavity was detected.	No inclusions presented on the split. No axial air cavity or indentation found inside.	No inclusions presented on the split. No axial air cavity or indentation found inside.	Inclusions presented on the split. Axial air cavity was detected.
Uniformity of mass (g)	3.83 ± 0.03	3.85 ± 0.07	3.88 ± 0.05	3.92 ± 0.06	3.81 ± 0.07
Disintegration (min)	43 ± 0.9	45 ± 1.0	41 ± 1.2	44 ± 1.1	45 ± 1.2

P ± 95%, n = 5

The next phase of the research was devoted to experimental samples quality control according to the requirements of the State Pharmacopeia of Ukraine (2nd edition) with results reflected in the Table 5.

According to the obtained results, only sample № 2 and sample № 5 did not adequate the requirements of the Uniformity of Texture parameter, since inclusions were detected on the split of the pessaries. The described non-conformity is presumed to be a result of preparation process disruptions, presumably at the moulding phase. Samples № 1, № 3 and № 4 fully complied with the above-mentioned parameter. All samples demonstrated compliance with the requirements of the State Pharmacopeia of Ukraine (2nd edition) on appearance, uniformity of mass, and disintegration.

Solid form disintegration is the first step of substance release[61]. It should be considered that the disintegration time of sample № 3 was the lowest (41 ± 1.2 min). It might be dependent on the base characteristics, since there was a nearly equal proportion of both hydrophobic and hydrophilic phases (1 : 1) in the sample № 3 formulation, while the hydrophilic phase prevailed in the rest of the samples.

Release of active substance in hydrophilic PEG based pessaries is realized through dissolution, while hydrophobic based pessaries melt[62]. Thus, melting time of pessaries is shorter than dissolution time.

The experimental samples were also tested for viability of *Lactobacillus* in prepared samples within the storage period of 6 months at 2–8 °C and at room temperature (25 ± 2 °C) (Table 6).

According to the obtained results, only sample № 3 demonstrated *Lactobacilli* assay of not less than 10^9 CFU/ml being stored within 6 months at 2–8 °C.

As it was already stated, samples № 1, № 2 and № 4 *Lactobacilli* assay did not correspond to the claimed value. In sample № 4 a growth of *Streptococcus* and microscopic fungi was detected, while samples № 2 failed to comply with the requirements of the Uniformity of Texture parameter.

Nevertheless, according to the data obtained, sample № 3 being stored within 6 months at 2–8 °C showed an appropriate *Lactobacilli* assay of not less than 10^9 CFU, while at room temperature it appeared to be $7.29 \pm 0.13*10^8$ CFU.

So, it was demonstrated that in all samples the values of *Lactobacilli* viability decrease during the storage period at both temperature regimes. The more significant reduction of *Lactobacilli* was observed at

Table 6. Viability of Lactobacillus in prepared samples after stability tests

Sample №	Storage period	Total amount of *Lactobacilli*, CFU/ml	
		+2+8 °C	Room temperature
1	day 0	$7.21 \pm 0.20*10^8$	$7.21 \pm 0.20*10^8$
	1 month	$4.98 \pm 0.18*10^6$	$5.94 \pm 0.19*10^5$
	3 months	$8.12 \pm 0.18*10^5$	$7.13 \pm 0.20*10^3$
	6 months	$7.24 \pm 0.18*10^4$	$3.45 \pm 0.17*10^2$
2	day 0	$5.26 \pm 0.17*10^8$	$5.26 \pm 0.17*10^8$
	1 month	$3.54 \pm 0.14*10^7$	$1.12 \pm 0.15*10^7$
	3 months	$1.25 \pm 0.18*10^7$	$5.45 \pm 0.18*10^6$
	6 months	$7.32 \pm 0.13*10^6$	$7.48 \pm 0.17*10^5$
3	day 0	$2.14 \pm 0.17*10^{10}$	$2.14 \pm 0.17*10^{10}$
	1 month	$8.91 \pm 0.18*10^9$	$5.23 \pm 0.18*10^9$
	3 months	$5.07 \pm 0.16*10^9$	$1.12 \pm 0.20*10^9$
	6 months	$3.29 \pm 0.17*10^9$	$7.29 \pm 0.13*10^8$
4	day 0	$9.14 \pm 0.14*10^7$	$9.14 \pm 0.14*10^7$
	1 month	$3.54 \pm 0.12*10^7$	$5.15 \pm 0.17*10^6$
	3 months	$6.48 \pm 0.15*10^6$	$4.45 \pm 0.19*10^5$
	6 months	$6.47 \pm 0.18*10^5$	$5.15 \pm 0.18*10^4$
5	day 0	$7.54 \pm 0.17*10^9$	$7.54 \pm 0.17*10^9$
	1 month	$3.42 \pm 0.20*10^9$	$1.13 \pm 0.20*10^9$
	3 months	$5.45 \pm 0.19*10^8$	$2.54 \pm 0.18*10^8$
	6 months	$1.05 \pm 0.21*10^8$	$5.15 \pm 0.14*10^7$

P ± 95%, n = 5

the room temperature mode because of the temperature effect.

The similar are the results of research by Pashayan on *Lactobacilli* viability in suppositories at the temperature of 2–8 °C which demonstrated a decrease in *Lactobacilli* count in all four samples: within a 6-month storage time the *Lactobacilli* count decreased from 4.3–5.4*10^8 CFU to 6.0–9.1*10^7 CFU[60]. Kale et al. studies described a decrease in the *Lactobacilli* count in plain, hollow-type and multifunctional bilayer pessaries during a 1-month storage time. Those results showed a more significant *Lactobacilli* reduction in plain pessaries at the temperature of 2–8 °C and in all types of pessaries at ambient temperature[59].

The study of Kaewnopparat et al. described also a demonstrative decrease in *Lactobacilli* survival in conventional and hollow-type suppositories on Witepsol H-15 or mixed PEGs during 3 months at ambient temperature than at the temperature of 2–8 °C [36].

But some our samples (№ 3 and № 5) demonstrated a not very high reduction of the *Lactobacilli* count and showed better results than those in the scientific works of other researchers.

Thus, a significant decrease in *Lactobacilli* viability is considered to be dependent on the temperature within the storage time[36]. However, we presume that the decrease in the *Lactobacilli* count within the shelf life might be related to the nature of the base used, quality of the substance with probiotic activity, and preparation technology.

Sample № 5 also displayed lower *Lactobacilli* viability not only within shelf life, but also right after preparation. Moreover, sample № 5 did not comply with the requirements of the Uniformity of Texture parameter.

Sample № 3 appeared to be the most promising for further study and development, as it demonstrated strict compliance with the quality parameters set (appearance, uniformity of texture, uniformity of mass, disintegration), along with a satisfactory *Lactobacilli* count both after preparation and within the 6-months shelf life.

Conclusions

This study was carried out to develop the formulation and technology of vaginal pessaries with probiotic activity for vaginal flora disorders treatment and prophylaxis**.**

The following formulation was determined as feasible for the investigated dosage form (for 100 g of the base): *L. casei* IMB B-7280 substance for bacteria assay 10^9 CFU per 1 pessary, PEG-400 – 15.0, PEG-1500 – 25.0, PEG-4000 – 10.0, hard fat – 46.5, polysorbate 80 – 2.5, purified water – up to 100.0. Feasible extemporaneous technology was elaborated for diphilic-based suppositories. Experimental samples proved complete compliance with the requirements for the following parameters: appearance, uniformity of texture, uniformity of mass, and disintegration.

The experimental samples being tested for viability of *Lactobacillus* right after preparation and within the storage period demonstrated satisfactory stability results for a 6-month shelf life at 2–8 °C. Thus, the results of this study will be used for further development of technological instruction for extemporaneous preparation of vaginal pessaries with probiotic activity.

Acknowledgement

This research was carried out as a part of grand: «Probiotics development for the treatment and prophylaxis infection-inflammatory diseases» (State registration number in Ukraine 0118U005399) and was financed by the Ministry of Education and Science of Ukraine. Research, analysis and paper development were not sponsored by commercial organizations.

Conflicts of interest: none.

References

1. **Mastromarino P., Vitali B., Mosca L.** Bacterial vaginosis, a review on clinical trials with probiotics. New Microbiol. 2013; 36(3), 229–238.
2. **Kusuma Naik, Avinash, H. Nusrat, L. Krishna, Ravi Kumar.** An RCT for efficacy of oral probiotics in treatment of cases with symptomatic white discharge per vagina in rural population. Journal of SAFOG 2012; 4(3), 126–129. doi:10.5005/jp-journals-10006-1193
3. **Marcotte H., Krogh Andersen K., Lin Y., Zuo F., Zeng Z., Larsson P. G., Brandsborg E., Brønstad G., Hammarström L.** Characterization and complete genome sequences of L. rhamnosus DSM 14870 and L. gasseri DSM 14869 contained in the EcoVag® probiotic vaginal capsules. Microbiol. Res. 2017; 205, 88–98. doi:10.1016/j.micres.2017.08.003
4. **Tomusiak A., Strus M., Heczko P., Adamski P., Stefański G., Mikołajczyk-Cichońska A., Suda-Szczurek M.** Efficacy and safety of a vaginal medicinal product containing three strains of probiotic bacteria, a multicenter, randomized, double-blind, and placebo-controlled trial. Drug Des. Devel. Ther. 2015; 9, 5345–5354. doi:10.2147/DDDT.S89214
5. **Mastromarino P., Brigidi P., Macchia S., Maggi L., Pirovano F., Trinchieri V., Conte U., Matteuzzi D.** Characterization and selection of vaginal Lactobacillus strains for the preparation of vaginal tablets. J. Appl. Microbiol. 2002; 93(5), 884–893.
6. **Powell A. M., Nyirjesy P.** Recurrent vulvovaginitis. Best Pract. Res. Clin. Obstet. Gynaecol. 2014; 28(7), 967–976. doi:10.1016/j.bpobgyn.2014.07.006
7. **Bertuccini L., Russo R., Iosi F., Superti F.** Effects of Lactobacillus rhamnosus and Lactobacillus acidophilus on bacterial vaginal pathogens. Int. J. Immunopathol. Pharmacol. 2017; 30(2), 163–167. doi:10.1177/0394632017697987
8. **Fuochi V., Cardile V., Petronio G., Furneri P. M.** Biological properties and production of bacteriocins-like-inhibitory substances by Lactobacillus sp. strains from human vagina. J. Appl. Microbiol. 2019; 126(5), 1541–1550. doi:10.1111/jam.14164
9. **Pendharkar S., Brandsborg E., Hammarström L., Marcotte H., Larsson P. G.** Vaginal colonisation by probiotic lactobacilli and clinical outcome in women conventionally treated for bacterial vaginosis and yeast infection. BMC Infect. Dis. 2015; 3(15), 255. doi:10.1186/s12879-015-0971-3

10. **Gaspar C., Donders G. G., Palmeira-de-Oliveira R., Queiroz J. A., Tomaz C., Martinez-de-Oliveira J., Palmeira-de-Oliveira A.** Bacteriocin production of the probiotic Lactobacillus acidophilus KS400. AMB Express 2018; 8(1), 153. doi:10.1186/s13568-018-0679-z
11. **Petricevic L., Witt A.** The role of Lactobacillus casei rhamnosus Lcr35 in restoring the normal vaginal flora after antibiotic treatment of bacterial vaginosis. BJOG 2008; 115(11), 1369–1374. doi:10.1111/j.1471-0528.2008.01882.x
12. **Neut C., Verrière F., Nelis H. J., Coenye T.** Topical treatment of infectious vaginitis, effects of antibiotic, antifungal and antiseptic drugs on the growth of normal vaginal Lactobacillus strains. Open Journal of Obstetrics and Gynecology 2015; 5(3), 173–180 doi:10.4236/ojog.2015.53024
13. **Marcotte H., Krogh Andersen K., Lin Y., Zuo F., Zeng Z., Larsson P. G., Brandsborg E., Brønstad G., Hammarström L.** Characterization and complete genome sequences of L. rhamnosus DSM 14870 and L. gasseri DSM 14869 contained in the EcoVag® probiotic vaginal capsules. Microbiol. Res. 2017; 205, 88–98. doi:10.1016/j.micres.2017.08.003
14. **Hill D., Sugrue I., Tobin C., Hill C., Stanton C., Ross R. P.** The Lactobacillus casei Group, History and Health Related Applications. Front. Microbiol. 2018; 9, 2107. doi:10.3389/fmicb.2018.02107
15. **Melgaço A. C., Blohem Pessoa W. F., Freire H. P., Evangelista de Almeida M., Santos Barbosa M., Passos Rezende R., Timenetsky J., Miranda Marques L., Romano C.** Potential of maintaining a healthy vaginal environment by two Lactobacillus strains isolated from cocoa fermentation. Biomed. Res. Int. 2018; 30, 7571954. doi:10.1155/2018/7571954
16. **Sobel J. D., Schneider J., Kaye D., Levison M. E.** Adherence of bacteria to vaginal epithelial cells at various times in the menstrual cycle. Infect Immun. 1981; 32(1), 194–197.
17. **Borges S., Silva J., Teixeira P.** The role of lactobacilli and probiotics in maintaining vaginal health. Arch. Gynecol. Obstet. 2014; 289(3), 479–489. doi:10.1007/s00404-013-3064-9
18. **Laue C., Papazova E., Liesegang A., Pannenbeckers A., Arendarski P., Linnerth B., Domig K. J., Kneifel W., Petricevic L., Schrezenmeir J.** Effect of a yoghurt drink containing Lactobacillus strains on bacterial vaginosis in women – a double-blind, randomised, controlled clinical pilot trial. Benef. Microbes 2018; 9(1), 35–50. doi:10.3920/BM2017.0018
19. **Homayouni A., Bastani P., Ziyadi S., Mohammad-Alizadeh-Charandabi S., Ghalibaf M., Mortazavian A. M., Mehrabany E. V.** Effects of probiotics on the recurrence of bacterial vaginosis, a review. J. Low. Genit. Tract. Dis. 2014; 18(1), 79–86. doi:10.1097/LGT.0b013e31829156ec
20. **Kumar N., Behera B., Sagiri S. S., Pal K., Ray S. S., Roy S.** Bacterial vaginosis, Etiology and modalities of treatment – A brief note. J. Pharm. Bioallied. Sci. 2011; 3(4), 496–503. doi:10.4103/0975–7406.90102
21. **Leyva-Gómez G., Prado-Audelo M. L. D., Ortega-Peña S., Mendoza-Muñoz N., Urbán-Morlán Z., González-Torres M., González-Del Carmen M., Figueroa-González G., Reyes-Hernández O. D., Cortés H.** Modifications in Vaginal microbiota and their influence on drug release, challenges and opportunities. Pharmaceutics 2019; 11(5). pii:E217. doi:10.3390/pharmaceutics11050217
22. **Balish E., Wagner R. D.** Probiotic bacteria for prophylaxis and therapy of candidiasis. Rev. Iberoam. Micol. 1998; 15(4), 261–264.
23. **Cadieux P., Burton J., Gardiner G., Braunstein I., Bruce A. W., Kang C. Y., Reid G.** Lactobacillus strains and vaginal ecology. JAMA 2002; 287(15), 1940–1941.
24. **Anukam K. C., Osazuwa E., Osemene G. I., Ehigiagbe F., Bruce A. W., Reid G.** Clinical study comparing probiotic Lactobacillus GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. Microbes Infect. 2006; 8(12–13), 2772–2776.
25. **Mastromarino P., Macchia S., Meggiorini L., Trinchieri V., Mosca L., Perluigi M., Midulla C.** Effectiveness of Lactobacillus-containing vaginal tablets in the treatment of symptomatic bacterial vaginosis. Clin. Microbiol. Infect. 2009; 15(1), 67–74. doi:10.1111/j.1469-0691.2008.02112.x
26. **Parent D., Bossens M., Bayot D., Kirkpatrick C., Graf F., Wilkinson F. E., Kaiser R. R.** Therapy of bacterial vaginosis using exogenously-applied Lactobacilli acidophili and a low dose of estriol, a placebo-controlled multicentric clinical trial. Arzneimittelforschung 1996; 46(1), 68–73.
27. **Hallen A., Jarstrand C., Pahlson C.** Treatment of bacterial vaginosis with lactobacilli. Sex Transm. Dis. 1992; 19(3), 146–148.
28. **Ehrström S., Daroczy K., Rylander E., Samuelsson C., Johannesson U., Anzén B., Pahlson C.** Lactic acid bacteria colonization and clinical outcome after probiotic supplementation in conventionally treated bacterial vaginosis and vulvovaginal candidiasis. Microbes Infect. 2010; 12(10), 691–699. doi:10.1016/j.micinf.2010.04.010
29. **Maggi L., Mastromarino P., Macchia S., Brigidi P., Pirovano F., Matteuzzi D., Conte U.** Technological and biological evaluation of tablets containing different strains of lactobacilli for vaginal administration. Eur. J. Pharm. Biopharm. 2000; 50(3), 389–395.
30. **Burton J. P., Cadieux P. A., Reid G.** Improved understanding of the bacterial vaginal microbiota of women before and after probiotic instillation. Appl. Environ Microbiol. 2003; 69(1), 97–101.
31. **Eriksson K., Carlsson B., Forsum U., Larsson P. G.** A double-blind treatment study of bacterial vaginosis with normal vaginal lactobacilli after an open treatment with vaginal clindamycin ovules. Acta Derm. Venereol. 2005; 85(1), 42–46.
32. **Czaja C. A., Stapleton A. E., Yarova-Yarovaya Y., Stamm W. E.** Phase I trial of a Lactobacillus crispatus vaginal suppository for prevention of recurrent urinary tract infection in women. Infect. Dis. Obstet. Gynecol. 2007; 2007, 35387. doi:10.1155/2007/35387
33. **Drago L., de Vecchi E., Nicola L., Zucchetti E., Gismondo M. R., Vicariotto F.** Activity of a Lactobacillus acidophilus-based douche for the treatment of bacterial vaginosis. J. Altern. Complement Med. 2007; 13(4), 435–438.
34. **Kale V., Trivedi R., Muley P.** Proposed design of a dissolution apparatus for vaginal formulations containing probiotics. Dissolution Technologies 2008; 15(2). doi:10.14227/DT150208P27
35. **Larsson P. G., Stray-Pedersen B., Ryttig K. R., Larsen S.** Human lactobacilli as supplementation of clindamycin to patients with bacterial vaginosis reduce the recurrence rate; a 6-month, double-blind, randomized, placebo-controlled study. BMC Womens Health 2008; 8, 3. doi:10.1186/1472-6874-8-3
36. **Kaewnopparat S., Kaewnopparat N.** Formulation and evaluation of vaginal suppositories containing Lactobacillus. International Scholarly and Scientific Research & Innovation 2009; 3(7), 117–120.
37. **Rodrigues F., Maia M. J., Neves J., Sarmento B., Amaral M. H., Oliveira M. B.** Vaginal suppositories containing Lactobacillus

acidophilus, development and characterization. Drug Dev. Ind. Pharm. 2015; 41(9), 1518–1525. doi:10.3109/03639045.2014.963864

38. **Falagas M., Betsi G. I., Athanasiou S.** Probiotics for the treatment of women with bacterial vaginosis. Clin. Microbiol. Infect. 2007; 13(7), 657–664.
39. **Ozkinay E., Terek M. C., Yayci M., Kaiser R., Grob P., Tuncay G.** The effectiveness of live lactobacilli in combination with low dose oestriol (Gynoflor) to restore the vaginal flora after treatment of vaginal infections. BJOG 2005; 112(2), 234–240.
40. **Uehara S., Monden K., Nomoto K., Seno Y., Kariyama R., Kumon H.** A pilot study evaluating the safety and effectiveness of Lactobacillus vaginal suppositories in patients with recurrent urinary tract infection. Int. J. Antimicrob. Agents 2006; 28(1), S30–34.
41. **Kale V. V., Trivedi R. V., Wate S. P., Bhusari K. P.** Development and evaluation of a suppository formulation containing Lactobacillus and its application in vaginal diseases. Ann NY Acad. Sci. 2005; 1056, 359–365.
42. **Ya W., Reifer C., Miller L. E.** Efficacy of vaginal probiotic capsules for recurrent bacterial vaginosis, a double-blind, randomized, placebo-controlled study. Am. J. Obstet. Gynecol. 2010; 203(2), 120.e1–6. doi:10.1016/j.ajog.2010.05.023
43. **Kaewsrichan J., Chandarajoti K., Kaewnopparat S., Kaewnopparat N..** Evaluation of Lactobacilli containing suppository formulation for probiotic use. Mahidol University Journal of Pharm. Sci. 2007; 34(1–4), 1–8.
44. **Fazeli M. R., Toliyat T., Samadi N., Hajjaran S., Jamalifar H.** Viability of lactobacillus acidophilus in various vaginal tablet formulations. DARU Journal of Pharmaceutical Sciences 2006; 14(4), 172–178.
45. **Ashok V. R. Manoj Kumar, D. Murali, Arkendu Chatterjee**. A review on vaginal routeas a systemic drug delivery. https://www.researchgate.net/publication/224318371_A_review_on_vaginal_route_as_a_systemic_drug_delivery (18. 10. 2019).
46. **Ham A. S., Buckheit R. W. Jr.** Designing and developing suppository formulations for anti-HIV drug delivery. Ther. Deliv. 2017; 8(9), 805–817. doi:10.4155/tde–2017–0056
47. **Zujkina S. V.** Stan ekstemporal`noyi receptury` Ukrayiny` ta problemy` s`ogodennya. Zb. nauk. pracz` spivrobit. NMAPO imeni P. L. Shupy`ka 2018; 32, 294–307.
48. Patent Ukrainy na korysnu model UA № 98881, C12N 1/00 vid 12.05.2015, biul. № 9.
49. Patent Ukrainy na vynakhid UA № 93133, C12N 1/20 vid 10.01.2011, biul. № 1.
50. **Ham A. S., Buckheit R. W. Jr.** Designing and developing suppository formulations for anti-HIV drug delivery. Ther. Deliv. 2017; 8(9), 805–817. doi:10.4155/tde-2017-0056
51. **Rowe R. C., Sheskey P. J., Quinn M. E.** Handbook of pharmaceutical excipients. London: Pharmaceutical Press 2009; 517–521.
52. **Jankowski A., Dyja R., Sarecka-Hujar B.** Dermal and transdermal delivery of active substances from semisolid bases. Indian. J. Pharm. Sci. 2017; 79(4), 488–500. doi:10.4172/pharmaceutical-sciences.1000255
53. **Zárate G., Juárez Tomás M. S., Nader-Macias M. E.** Effect of some pharmaceutical excipients on the survival of probiotic vaginal lactobacilli. Canadian Journal of Microbiology 2005; 51(6), 483–489. doi:10.1139/w05–031
54. **Reitermayer D., Kafka T. A., Lenz Ch. A., Vogel R. F.** Interrelation between Tween and the membrane properties and high pressure tolerance of Lactobacillus plantarum. BMC Microbiology 2018; 18(1), 72. doi:10.1186/s12866-018-1203-y
55. **Malheiros P. S., Sant'Anna V., Todorov S. D., Franco B. D. G. M.** Optimization of growth and bacteriocin production by lactobacillus sakei subsp. Sakei 2a. Brazilian Journal of Microbiology, 2015; 46(3), 825–834. doi:10.1590/S1517–838246320140279
56. **Coelho L. F., Lima C. J. B., Rodovalho C. M., Bernardo M. P., Contiero J.** Lactic acid production by new Lactobacillus plantarum LMISM6 grown in molasses, optimization of medium composition. Braz. J. Chem. Eng. 2011; 28(1). doi:10.1590/S0104-66322011000100004
57. **Realdon N., Dal Zotto M., Morpurgo M., Franceschinis E.** Effects of surfactant characteristics on drug availability from suppositories. Pharmazie 2008; 63(6), 459–463. doi:10.1691/ph.2008.7378
58. **Hanaee J., Javadzadeh Y., Taftachi S., Farid D., Nokhodchi A.** The role of various surfactants on the release of salbutamol from suppositories. Farmaco 2004; 59(11), 903–906. doi:10.1016/j.farmac.2004.07.006
59. **Kale V., Patil M., Yadav S.** Preparation and evaluation of Novel Vaginal Pessaries of Lactobacilli. Int. J. Drug Dev. & Res. 2012; 4(3), 97–103.
60. **Pashayan M. M.** Formulation and investigation of vaginal double layer suppositories containing lactobacilli and herbal extracts. The New Armenian Medical Journal 2011; 2, 54–59.
61. **Franceschinis E., Realdon N.** Effect of the surfactant on the availability of piroxicam as a poorly hydrosoluble drug from suppositories. Pharmazie 2012; 67(1), 37–45. doi:10.1691/ph.2012.1092
62. **Chale G. R., Jeughale A. S.** Suppository, a review. World Journal of Pharmaceutical Research 2019; 8, 1968–1982.