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Formulation and Characterization of Vaginal Capsules and Tablets Providing Fast Release of Live Lactobacillus spp.

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Abstract

Live biotherapeutic products (LBPs) are gaining significant medical importance as a new approach in the prevention and treatment of various conditions in the female population. This study investigated the design, development, and evaluation of immediate-release (IR) vaginal dosage forms containing live Lactobacillus spp., with the aim of enabling fast and effective drug delivery in the vaginal environment. Capsule and tablet formulations were developed using different excipients and appropriate manufacturing processes (encapsulation and direct compression) and systematically evaluated for their robustness and performance. The results demonstrated that all capsule formulations exhibited suitable flowability and uniformity of dosage units, with hard gelatine capsules (HGC) providing the most favourable disintegration times compared to hydroxypropyl methylcellulose (HPMC) and pullulan capsules. Tablet formulations based on microcrystalline cellulose (MCC) achieved optimal hardness, friability, and disintegration, especially under lower compression forces, confirming MCC's multifunctional role as a filler, binder, and disintegrant. Importantly, both dosage forms maintained Lactobacillus viability with only minor losses (~0.5 log), preserved the acidic vaginal environment (pH 4.2), and demonstrated robustness under simulated biorelevant conditions. These findings highlight that IR vaginal capsules and tablets can be effectively tailored to deliver live biotherapeutics with rapid onset of action, thereby advancing microbiome-based strategies for women's health.

Key words: vaginal drug delivery, live biotherapeutic products, immediate release, capsules, tablets

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Introduction

Live biotherapeutic products (LBPs) are emerging as a promising class of therapeutics, offering innovative approaches to modulate the human microbiome for improved health and treatment of different diseases (1, 2). Among the various anatomical sites for live bacteria delivery, the vagina presents a particularly appealing target. Its native microbiota, primarily dominated by Lactobacillus species, plays a crucial role in maintaining vaginal wellbeing and female health in general and in protecting against infections and other diseases (3–6).

Direct vaginal administration of live bacteria enables localized therapeutic action, aiming to restore microbial balance, reinforce mucosal defences, and treat various gynaecological conditions such as bacterial vaginosis or candidiasis (7, 8). While sustained-release and mucoadhesive systems are gaining relevance in the field of vaginal drug delivery, immediate-release formulations, particularly tablets and capsules, are still the primary choice within this industrial domain especially for applications requiring rapid onset of action or short-term microbial modulation.

Immediate-release vaginal tablets and capsules offer several advantages: they disintegrate quickly upon contact with vaginal fluids, enabling fast release of viable bacteria; they are easy to administer; and they can be designed to minimize leakage and discomfort. Additionally, they are produced by well-established processes and technologies, which makes these types of dosage forms appealing for industrialization. However, the vaginal environment brings unique formulation challenges due to its moisture content, acidic pH (typically 3.5–4.5 in healthy females), dynamic changes in fluid volume, natural wash out phenomenon, variable microbial composition as well as other physiological variations happening across menstruation, pregnancy, and menopause (9, 10). To ensure efficacy, immediate-release vaginal formulations must balance rapid disintegration with the preservation of bacterial viability during manufacturing (especially during tablet compression), storage and delivery. Excipient selection, process parameters, and environmental conditions all play critical roles in achieving the product's safety, efficacy and robustness.

This study investigates the design and evaluation of immediate-release vaginal capsules and tablets containing live Lactobacillus spp. It explores how formulation and process variables affect overall product performance under both compendial and biorelevant testing conditions. This research contributes to the growing field of microbiome-based therapeutics and highlights the potential of immediate-release vaginal dosage forms in advancing women's health.

Material and Methods

Lactobacillus spp., Drug Substance Complex (DSC), was provided by Bacthera A/S (Hørsholm, Denmark). Anhydrous Lactose (SuperTab®24AN) and Microcrystalline Cellulose (Pharmacel®112) were purchased from DFE Pharma (Goch, Germany). Mannitol (Pearlitol® 200SD) and Sodium starch glycolate (Glycolys®) were supplied by

ROQUETTE (Lestrem, France). N-Vinylpyrrolidone and Vinyl acetate copolymer (Vivapharm® PVP/VA64) was purchased from JRS Pharma (Rosenberg, Germany). L-Ascorbic acid was purchased from VWR International (Leuven, Belgium). Hydrophobic colloidal silica (Aerosil®R 972 Pharma) was kindly provided by EVONIK (Essen, Germany). Magnesium stearate (LubriPrez® 2) was purchased from JRS Pharma (Rosenberg, Germany). Hard gelatin capsules (HGC, Coni-Snap®, size 0), hydroxypropyl methylcellulose (HPMC) capsules (Vcaps® Plus, size 0), and vegetarian pullulan capsules (Capsugel® Plantcaps®, size 0) were all kindly supplied by Lonza-Capsugel (Colmar, France). All other reagents used in the study were of analytical grade and quality.

Blend Preparation

All components were weighed separately. The DSC and all excipients were gathered in an HDPE bottle through a layering principle – half of the filler was deposited on the bottom of the bottle, followed by the other excipients (except magnesium stearate in case of tablet blends) and the DSC, and finally the other half of the filler. The blend was manually pre-mixed for 1 minute, sieved through a 1.18 mm sieve, and automatically blended at 49 rpm for 10 minutes using a 3D powder blender (Turbula®, Switzerland) to ensure homogeneity. For tablets, magnesium stearate was manually sieved through a 0.5 mm sieve and added separately to the rest of the blend. A final mixing step (lubrication) was conducted at 49 rpm for 5 minutes to ensure even distribution of the lubricant throughout the blend.

Flow Properties of Blends

Selected blends were assessed for their flow properties through indirect and direct methods. Indirectly, the flow was assessed through the bulk and tap density (single measurement, 1250 taps, Erweka SMV102, Erweka GmbH, Germany) and consequently by calculating the Carr index and Hausner ratio (Ph. Eur. 2.9.34, method 1) by using a smaller sample size (5 g) and smaller graduated cylinder (25 ml). The direct flow characterization was assessed through the evaluation of the flow rate and angle of repose according to Ph. Eur. 2.9.36 with a smaller sample size (30 g, triplicate) and by using automatic flow meter (PTG-S5, Pharma Test, Germany).

Residual Moisture and Water Activity of Blends

The residual moisture of selected powder blends was evaluated through the loss on drying method by using a halogen oven coupled with precise balance (Mettler Toledo HX204, Mettler Toledo GmbH, Switzerland). Water activity was assessed in accordance with USP 922 by using an automated measuring device (Aqualab 4TE, Aqualab, USA). Both parameters were measured in triplicate.

Capsule Filling

The final mixtures dedicated for capsules were filled into empty shells using a manual device (ProFill 100, Torpac, USA). Filled capsules were primarily assessed for

the filling weight as well as for the uniformity of dosage units by weight variation as per Ph. Eur. 2.9.40.

Tablet Compression

The powder blends were compressed using an automatic single-punch eccentric tablet press (CRP-6, DOTT. BONAPACE & C., Italy), equipped with a 10 mm round concave punch and a load cell. Two compression forces depicted as high (620–650 kg) and low (400–475 kg) were applied to evaluate how variation in this parameter affects overall product performance. Tablets were primarily assessed for weight and uniformity of dosage units by weight variation (Ph. Eur. 2.9.40).

Tablet Hardness and Friability

The robustness of compressed tablets was assessed through determination of the hardness and friability of the units. The first property has been investigated according to Ph. Eur. 2.9.8 (10 units; MT50, SOTAX, Switzerland) while the second one was performed in alignment with Ph. Eur. 2.9.7 (single measurement; sample size as close as possible to 6.5 g; TA120, ERWEKA GmbH, Germany).

pH Assessment of Capsules and Tablets

The influence of the formulation on the pH of simulated vaginal fluid pH 4.2 (SVF, prepared as per Owen and Katz, 1999 (11)) has been assessed by soaking the formulations in 10 ml of tempered medium (37°C) inside a falcon tube which was rotated for 10 minutes. After complete unit disintegration, the pH of the medium was measured (single measurement; Mettler Toledo Seven Excellence pH meter, Mettler Toledo GmbH, Switzerland).

Disintegration of Capsules and Tablets

The disintegration assessment was conducted in classical apparatus (ZT 322, ERWEKA, Germany), through a method adjusted to fit with Ph. Eur. 2.9.2 – Disintegration of Suppositories and Pessaries. One unit was placed in a customized tube (see Figure 1) and 3 such tubes were immersed in 720 mL of SVF (tempered at 37°C), which was used as a disintegration medium. Tubes were manually rotated 180 degrees every 10 minutes to mimic the movement of the Ph. Eur 2.9.2-1 apparatus. The endpoint was reached when no solid residue remained, or if any residue present was soft or frothy, offering no resistance to a glass rod. The measurement was performed in triplicate.

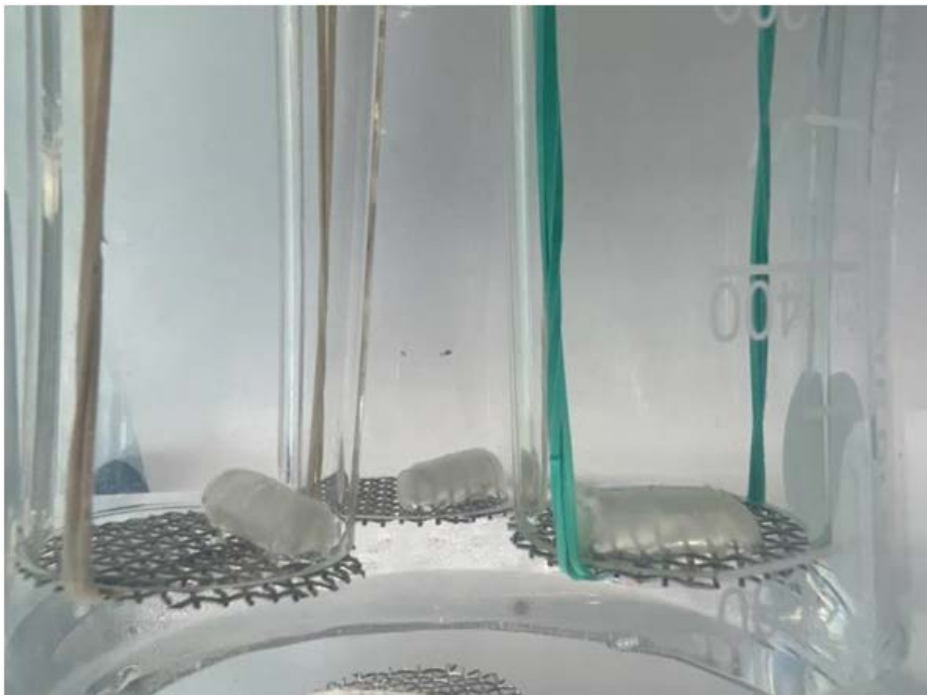

Figure 1. Disintegration method set up to mimic Ph. Eur 2.9.2-1 apparatus
Slika 1. Metoda dezintegracije, prilagođena za oponašanje aparata Ph. Eur. 2.9.2-1

Viability Assessment

The influence of the formulation variations, process parameters and the simulated vaginal conditions on strain viability was assessed through the colony forming unit (CFU) method. Pure DSC, as well as capsule/tablet formulations have been dispersed in 10 ml of De Man- Rogosa – Sharpe (MRS) broth pH 6.2 and Simulated Vaginal Fluid pH 4.2. The tested formulations were dispersed in the media inside 15 ml falcon tubes, which were manually rotated for 30 min, followed by additional 15 minutes of gentle swirl if needed, to achieve optimal strain dispersion. The capsules were placed into sinkers in order to prevent unit floating on the media. The prepared dispersion was used to conduct serial dilution in the range of 10^{-1} up 10^{-6} by using MRS broth as a diluent. 0.5 ml of inoculum of each dilution was placed on a petri dish followed by pouring of molten and tempered (at 40° C) agar-MRS mixture over the inoculum. The prepared plates were further incubated anaerobically at 37°C for 72 hours. After the incubation period, the colonies on each agar plate were manually counted. Only plates with a colony count between 20 and 300 were considered valid, as counts outside this range may not yield reliable data. The measurements were done in triplicate.

The CFU count per gram of sample was determined using the following equation:

$$\mathrm{CFU/g} = \frac{\text{Number of Colonies Counted}}{\text{Volume Plated (mL)} \times \text{Dilution Factor} \times \text{Weight of the Sample (g)}}$$

Obtained CFU/g values were further corelated to unit weight (for formulated product) and the theoretical DSC amount per unit (for DSC).

Results and Discussion

The aim of the conducted research work was to design robust capsule and tablet formulations aimed to deliver an appropriate number of viable cells in an immediate release fashion in simulated vaginal conditions. A detailed evaluation of the dosage forms and comparison between the two types of products was also part of this study.

Capsule Development and Evaluation

Capsule Blend Assessment

Within this part of the research, the influence of formulation composition on capsule blend flowability, residual moisture and water activity was evaluated. Four blend formulations differing in the type of filler and presence/absence of ascorbic acid were investigated and filled into three type of empty capsule shells – HGC, HPMC and Pullulan. The blend compositions are shown in Table I.

Table I Composition of blends (%) aimed to be encapsulated

Tabela I Sastav smeša (%) namenjenih za inkapsuliranje

Formulation blend	DSC	Lactose anhydrous	Mannitol	Ascorbic acid	Na starch glycolate	Hydrophobic colloidal silica	Mg stearate
CB1	30	63.5	0	2.5	3	0.5	0.5
CB2	30	0	63.5	2.5	3	0.5	0.5
CB3	30	66	0	0	3	0.5	0.5
CB4	30	0	66	0	3	0.5	0.5

Powder flowability is one of the crucial parameters for capsule production since it is affecting the blend's processibility and subsequently the uniformity of dosage units. The flowability of the formulations has been assessed indirectly through the Carr index (CI) and Hausner ratio (HR) (through the values of bulk and tapped density) and directly through the flow rate and angle of repose. The results of the flow properties of the four evaluated formulations are shown in Table II.

Table II Flow properties of the formulated blends aimed to be encapsulated

Tabela II Protočnost formulisanih smeša namenjenih za inkapsuliranje

Formulation blend	Carr index (%) and flow description	Hausner ratio and flow description	Angle of repose (°) and flow description	Flow rate (g/sec)
CB1	13 (good)	1.14 (good)	27 ± 1.41 (good)	3.99 ± 0.64
CB2	10 (excellent)	1.12 (good)	25 ± 0.47 (good)	3.23 ± 0.66
CB3	9 (excellent)	1.09 (excellent)	26 ± 2.49 (good)	4.75 ± 0.44
CB4	9 (excellent)	1.10 (excellent)	21 ± 0.47 (excellent)	3.49 ± 0.16

The results of the flowability evaluation demonstrated that all four blends have excellent to good flow characteristics, making them well-suited for the subsequent capsule filling process. Variation in the filler type as well as the presence/absence of ascorbic acid did not affect the flow properties drastically. These findings indicate that including fillers with inherently good flow properties, such as the agglomerated anhydrous lactose and spray dried mannitol, as well as introducing suitable amounts of glidants and lubricant, will provide formulations which are not only optimal for uniform dosing, but also ensure efficient production by reducing the risk of flow-related issues such as inconsistent filling, bridging and adherence to the surfaces.

The chosen formulations have been further evaluated for residual moisture and water activity. The results of this set of measurement are presented in Table III.

Table III Residual moisture and water activity of the formulated blends aimed to be encapsulated

Tabela III Rezidualna vlaga i aktivnost vode formulisanih smeša namenjenih za inkapsuliranje

Formulation blend	Residual moisture (%)	Water activity
CB1	2.03 ± 0.12	0.36 ± 0.006
CB2	2.08 ± 0.09	0.37 ± 0.006
CB3	1.99 ± 0.04	0.36 ± 0.000
CB4	1.98 ± 0.10	0.36 ± 0.005

The average moisture content and water activity is similar for all four blends, with no trends observed when either different type of filler (anhydrous lactose and mannitol) is used or when ascorbic acid is present/absent in the formulation. Maintaining moisture content and water activity levels low enough is crucial in assuring stability and microbiological purity of the product over the shelf life. Appropriate residual moisture

levels are product-specific and should be evaluated within stability studies and correlated with strain viability trends. Water activity (AW) values are critical for potential microbiological contamination, and when above 0.9 and 0.6, they promote bacterial and fungal growth, respectively (12). The current formulations exhibit suitable AW values that do not support the growth of pathogenic microorganisms. However, for maintaining a stable product, AW values above 0.25 normally tend to be unfavourable for bacterial strains (13). Further monitoring of the AW values and their correlation with strain viability should be done during stability testing. Adjustments in the formulation (such as incorporating excipients with lower water content/AW) or manipulation at low relative humidity could be a viable solution in tackling high residual moisture and/or AW.

Capsule Assessment

Formulated blends were further filled into three types of capsules – HGC, HPMC and pullulan with the final goal of comparing them and finding the optimal shell for immediate release of the living bacteria. The capsules were evaluated for the uniformity of dosage units (UDU), influence on the pH of healthy SVF (pH 4.2) and disintegration. The results of these tests can be seen in Table IV.

Table IV Influence of blend composition and shell type on capsule robustness and performance

Tabela IV Uticaj sastava smeše i tipa omotača kapsule na robusnost i performanse kapsule

Formulation capsule	Capsule weight (mg)	UDU (AV)	pH of a slurry	Disintegration time (min)
CB1 – HGC	530 ± 17	9.4	4.37	23.2 ± 1.83
CB1 – Pullulan	551 ± 16	8.5	4.27	45.7 ± 6.13
CB1 – HPMC	538 ± 9	4.5	4.18	37.7 ± 2.49
CB2 – HGC	530 ± 10	4.9	4.39	24.3 ± 0.94
CB2 – Pullulan	518 ± 10	5.5	4.27	38.3 ± 4.49
CB2 – HPMC	532 ± 3	2.5	4.27	27.0 ± 7.07
CB3 – HGC	525 ± 6	3.9	4.48	28.0 ± 2.89
CB3 – Pullulan	528 ± 7	4.2	4.39	35.3 ± 1.25
CB3 – HPMC	538 ± 7	4.0	4.43	38.3 ± 6.89
CB4 – HGC	527 ± 4	2.1	4.48	22.6 ± 2.05
CB4 – Pullulan	519 ± 8	5.1	4.38	43.3 ± 3.32
CB4 – HPMC	531 ± 4	2.1	4.41	37.4 ± 2.64

As shown in Table IV, the AV values of all tested formulations in all types of capsules remained well below the upper limit of 15, indicating uniformly distributed DSC across evaluated units. The capsules based on blends containing spray-dried mannitol (blends CB2 and CB4) generally exhibited slightly lower AV values compared to those units containing agglomerated anhydrous lactose in the blend (blends CB1 and CB3). This difference may be attributed to the more spherical particles with more uniform distribution and reduced fines of the first filler compared to the more irregular particle shape with more fines of the second filler (14, 15). An additional trend was observed regarding the presence or absence of ascorbic acid. The formulations containing this excipient tended to have slightly higher AV values than those without it. Ascorbic acid, being an irregularly shaped material, could contribute to slightly less uniform flow and, consequently, to capsule filling. Furthermore, the products based on HPMC capsule shells showed the best overall outcome for UDU, which might be connected to the lower electrostatic charge of HPMC compared to gelatine and pullulan (16).

Designed capsule formulations were also tested for their influence on the pH of SVF (pH 4.2) mimicking healthy vaginal conditions. The results shown in Table IV suggest that regardless of the composition and capsule shell type, none of the formulations caused a major shift in the primary pH value of the media. A slight general trend is seen in formulations containing ascorbic acid which tended to keep the pH value closer to the initial one. All these findings suggest that the formulations successfully preserved the acidic environment essential for supporting a healthy vaginal flora.

In terms of disintegration, the HGC based products consistently provided the fastest and compendially compliant (not more than 30 minutes; European Pharmacopeia) disintegration times in comparison to the formulations encapsulated in the other two types of capsule shells. It is also the HGC based formulations that provided disintegration rates lower than 30 min, which is the upper test limit set in the European Pharmacopoeia. These results are well aligned with the previously reported ones claiming a faster disintegration of HGC compared to HPMC and pullulan capsules due to a higher solubility/dissolution rate of gelatine compared to the other two polymers (17, 18). Additionally, it was demonstrated that HGC disintegration is less dependent on mechanical stress presence compared to the other two types of capsule shells, where mechanical stress triggers faster unit decay. This attribute is of extensive importance for drug application in the vagina, where milder mechanical stress is present compared to the stomach (17).

Based on the obtained results, the formulations filled into HGC were determined as the optimal ones and were further assessed for strain viability in MRS (pH 6.2) and SVF (pH 4.2). The results are shown in Table V.

Table V Viability of the strain incorporated in different formulation blends encapsulated in HGC

Tabela V Viabilnost soja inkorporisanog u različite formulacione smeše inkapsulirane u tvrdim želatinskim kapsulama

DSC/Formulation	CFU/cps (MRS)	Log10 CFU/cps (MRS)	CFU/cps (SVF)	Log10 CFU/cps (SVF)
DSC	4.10E + 08 ± 3.22E + 07	8.62 ± 0.12	5.00E + 08 ± 2.87E + 07	8.70 ± 0.08
CB1 – HGC	1.34E + 08 ± 2.19E + 07	8.13 ± 0.07	5.33E + 08 ± 3.92E + 08	8.73 ± 0.31
CB2 – HGC	1.10E + 08 ± 1.62E + 07	8.04 ± 0.06	1.56E + 08 ± 1.98E + 06	8.19 ± 0.01
CB3 – HGC	1.08E + 08 ± 5.84E + 06	8.03 ± 0.02	1.51E + 08 ± 1.25E + 07	8.18 ± 0.04
CB4 – HGC	1.30E + 08 ± 8.64E + 06	8.11 ± 0.03	1.27E + 08 ± 2.60E + 07	8.11 ± 0.10

As can be seen, the DSC demonstrated similar viability in both MRS (growth medium) and SVF, indicating that the strain is not losing viability when introduced in the acidic simulated vaginal environment and is well-suited for incorporation into vaginal formulations. Regarding the impact of the formulation on the viability, a comparison between the capsules and DSC rehydrated in MRS showed approximately 0.5 log reduction in viability. This loss is considered as minor, suggesting that the used excipients and process fit the purpose of the formulation, yet the reduction could relate to slight incompatibility or extensive application of stress during blend preparation and should be further evaluated. Regarding the viability under simulated vaginal conditions, the CFU counts were slightly higher in SVF compared to MRS broth across all formulations. However, this difference is considered negligible and likely due to experimental variability, indicating comparable viability in both media. Based on these findings, it can be concluded that the designed formulations are well suited to fit the purpose of immediate vaginal delivery of a viable Lactobacillus spp.

Despite the better performance of HGC-based formulations compared to the HPMC and pullulan-based ones, knowing the fact that gelatine might undergo cross-linking, contains high moisture content, and has lower oxygen permeability, further product investigation through well-planned stability studies should be performed.

Tablet Development and Evaluation

Tablet Blend Assessment

Beside capsules, the aim of this study was to evaluate the suitability of tablets as dosage forms aimed to immediately deliver Lactobaciluss spp. in the vaginal cavity. As in case of the capsule development, a similar approach was applied for the tablet formulations. Three blend formulations differing in the type and amount of filler(s) were prepared and assessed. In this case, all compositions contained ascorbic acid, which was shown not to affect the processability of the capsule blends and could additionally have a positive effect on the strain viability through the antioxidant, pH modifying and prebiotic activity (19, 20). Additionally, higher amounts of glidant and lubricant were incorporated into the blends to ease the tableting process. The tablet blend compositions are shown in Table VI.

Table VI Composition of the blends (%) aimed to be compressed into tablets

Tabela VI Sastav smeša (%) namenjenih za tabletiranje

Formulation blend	DSC	Microcrystaline cellulose	Lactose anhydrous	Mannitol	Ascorbic acid	Na starch glycolate	PVP/VA	Hydrophobic colloidal silica	Mg stearate
TB1	30	59.5	0	0	2.5	3	3	1	1
TB2	30	39.75	19.75	0	2.5	3	3	1	1
TB3	30	39.75	0	19.75	2.5	3	3	1	1

The flowability of the blends for tableting was also assessed indirectly and directly. The results for the Carr index (CI), Hausner ratio (HR), angle of repose and flow rate are shown in Table VII.

Table VII Flow properties of the formulated blends aimed to be compressed into tablets

Tabela VII Protočnost formulisanih smeša namenjenih za tabletiranje

Formulation blend	Carr index (%) and flow description	Hausner ratio and flow description	Angle of repose (°) and flow description	Flow rate (g/sec)
TB1	10 (excellent)	1.11 (excellent)	37 ± 0.5 (moderate)	2.19 ± 0.08
TB2	10 (excellent)	1.11 (excellent)	37 ± 0.8 (moderate)	2.31 ± 0.02
TB3	14 (good)	1.17 (good)	36 ± 0.5 (moderate)	1.97 ± 0.16

The assessment of the tablet blends demonstrated discrepancy between the indirectly and directly determined flowability. According to the angle of repose, all blends have moderate flowability which was additionally supported with the lower flow rate compared to the capsule blends. This trend could be connected to the incorporation of microcrystalline cellulose (MCC) as a filler and PVA/VA as a binder. The fibrous nature of the first one and the small particle size of the second one might hinder the powder flow. Yet their presence could be crucial in providing the optimal compressibility of the formulations and is thus required. The slightly lower flowability of the formulation based on MCC and mannitol could be due to the denser packaging tendency of the particles of these two fillers, shown through the higher values of the Carr index and Housner ratio compared to the other two formulation blends.

The blends were also evaluated for their inherent residual moisture and water activity (AW). The values for these stability and contamination-indicating parameters are presented in Table VIII.

Table VIII Residual moisture and water activity of the blends aimed to be compressed into tablets

Tabela VIII Rezidualna vlaga i aktivnost vode smeša namenjenih za tabletiranje

Formulation blend	Residual moisture (%)	Water activity
TB1	3.70 ± 0.28	0.33 ± 0.04
TB2	3.11 ± 0.23	0.33 ± 0.02
TB3	3.06 ± 0.15	0.36 ± 0.04

As seen in Table VIII, residual moisture and AW values are similar between the three blends for tableting. Compared to the capsule blends, the tablet ones have slightly higher residual moisture, which might be due to the presence of MCC, an excipient having inherently higher water content and is more hygroscopic compared to lactose and mannitol (21). The AW values are well below the threshold promoting microbiological contamination, but above the value of 0.25, which relates to appropriate Lactobacillus spp. viability upon storage. As in case of the capsule blends, further monitoring of the residual moisture and AW values and their correlation with product stability could be seen beneficial and might trigger a necessity in formulation and/or process adjustment.

Tablet Assessment

The designed blends have been further compressed into tablets using two different compression forces per blend – high and low. Tablets were assessed for weight, uniformity of dosage units (UDU), hardness, friability, influence on the pH of healthy SVF (pH 4.2) and disintegration. The results of this set of tests are shown in Table IX.

Table IX Influence of composition and compression force on tablet robustness and performance

Tabela IX Uticaj sastava i sile kompresije na robusnost i performanse tableta

Formulation tablet	Average compression force (kg)	Weight (mg)	UDU (AV)	Hardness (N)	Friability (%)	pH of a slurry	Disintegration time (min)
TB1-High	650	333 ± 1	3.1	83.5 ± 6.0	0.14	4.23	19.0 ± 1.4
TB1-Low	475	325 ± 4	9.0	43.4 ± 6.3	0.15	4.22	14.3 ± 1.2
TB2-High	620	364 ± 4	9.8	53.5 ± 6.4	0.54	4.22	40.2 ± 2.7
TB2-Low	400	345 ± 6	13.0	24.0 ± 3.7	1.40	4.24	27.0 ± 1.4
TB3-High	633	326 ± 3	2.6	53.8 ± 3.9	0.36	4.26	31.3 ± 0.9
TB3-Low	435	330 ± 5	11.3	21.1 ± 6.8	1.72	4.22	24.3 ± 3.7

As can be seen in Table IX, both the formulation composition and the compression force affect the tablet properties. Increasing the compression force led to an increase in hardness and disintegration time and decreased the friability. Additionally, increasing the compression force led into obtaining units with lower variability in weight (AV) and hardness. This could be due to the more uniform compaction process as well as high robustness of the obtained units (less prone to dusting and defecting). As for the influence of the formulation, it can be noted that when compressed under similar forces, the formulation based on MCC alone provided better performance than the formulations containing a mix of MCC and anhydrous lactose or mannitol. This outcome can be attributed to the plastic nature of MCC, which is providing extensive compressibility, even at lower forces, leading into obtaining tablets with high hardness and low friability (22). On the other hand, the incorporation of the generally brittle fillers – anhydrous lactose and mannitol – led to poorer compressibility of the blends and subsequently softer and more friable tablets (23, 24).

In terms of disintegration, it was once again the formulation containing only MCC as filler outperforming the ones containing mixed fillers despite the higher hardness of the tablets of the former. This formulation was also the only one providing suitable results according to the European Pharmacopeia requirements upon application of a higher compression force. This outcome can be connected to the fact that, besides being filler and binder, MCC could act as a disintegrant through capillary action and swelling (22, 24). On the other hand, the presence of the soluble mannitol and anhydrous lactose might prolong the time required for unit decay due to the dissolution momentum introduced in the whole disintegration pattern (25). When comparing to the capsule formulations, tablets provide more favourable disintegration times, especially when lower compression forces are applied. When produced by the usage of lower pressure values, all tablet formulations suited the upper disintegration limit of 30 minutes, set by the European Pharmacopeia. This could be again connected with the disintegration action of MCC, shape recovery on decompression, as well as the absence of a barrier in the capsule shell.

As for the impact of the composition and compression force on the pH of the SVF (pH 4.2), regardless of the variations, the values remained almost identical to the primary one, suggesting that none of the formulations will jeopardize the healthy environment in the vagina.

As the results point out, the MCC-based formulation as the optimal one was further evaluated for strain viability in MRS (pH 6.2) and SVF (pH 4.2). The results were compared to the pure DSC, as well as to the uncompressed blend and can be seen in Table X.

Table X Viability of the strain incorporated in tablets based on MCC as filler and compressed at low and high forces

Tabela X Viabilnost soja u tabletama zasnovanim na mikrokristalnoj celulozi (MCC) i komprimovanim pri niskim i visokim silama

DSC/Formulation	CFU/cps (MRS)	Log10 CFU/cps (MRS)	CFU/cps (SVF)	Log10 CFU/cps (SVF)
DSC	3.7E + 08 ± 1.77E + 07	8.57 ± 0.41	/	/
TB1 (blend)	3.0E + 08 ± 2.71E + 07	8.48 ± 0.62	/	/
TB1-High	1.17E + 08 ± 8.59E + 06	8.07 ± 0.59	9.04E + 07 ± 1.84E + 07	7.96 ± 0.16
TB1-Low	1.11E + 08 ± 5.59E + 06	8.04 ± 0.41	1.02E + 08 ± 7.19E + 06	8.01 ± 0.56

Based on the viability results, it can be seen that there was only a minor difference between DSC and the blend, suggesting that the chosen excipients are compatible with the strain. Upon tableting, strain viability showed a minor drop (0.5 log), regardless of whether a high or low compression force was applied. The observed trend suggests that the strain is slightly sensitive to compression and that process parameters should be adjusted in a way that the product remains sufficiently robust, but also shows minimal loss in viable cells upon tableting. The marginal drop in viability is in alignment with the previous studies suggesting that the usage of plastic filler-binder (such as MCC) showing partial elastic recovery upon decompression, leads into substantial cell viability preservation (26). Lastly, both tablet units showed only a minor loss in viability when dispersed in SVF (pH 4.2), relative to the results in the MRS broth (pH 6.2), suggesting that regardless of the compression force, the chosen formulation keeps the strain unaffected in the simulated vaginal environment. According to the viability results shown for both capsules and tablets, similar trends can be observed.

Conclusion

This study confirmed the feasibility of formulating immediate-release vaginal dosage forms containing Lactobacillus spp., with both capsules and tablets showing promising performance in terms of robustness, disintegration, and bacterial viability. Hard gelatine capsules were identified as the most suitable capsule type, offering adequate properties and superior disintegration compared to formulations encapsulated into HPMC and pullulan shells. In parallel, tablets formulated with microcrystalline cellulose (MCC) provided an optimal balance of hardness, friability, and disintegration, and outperformed the formulations containing a mixture of MCC and another brittle filler (anhydrous lactose and mannitol). The multifunctional properties of MCC – acting as filler, binder, and disintegrant – were critical to the favourable outcomes observed.

From a microbiological perspective, both dosage forms preserved bacterial viability with only minor losses (~0.5 log) during processing, while maintaining stability in simulated vaginal fluid without altering its acidic pH. This preservation of both strain activity and vaginal conditions is essential for ensuring therapeutic efficacy and supporting the restoration of healthy microbiota. These findings emphasize the need for rational formulation design, including excipient selection and process parameter optimization, to achieve products that combine rapid release with microbial stability.

Overall, the study contributes valuable insights to the formulation science of live biotherapeutic products (LBPs) and supports the use of immediate-release vaginal delivery systems as a promising strategy for rapid microbial restoration and the treatment of gynaecological conditions.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution

AA and HH contributed to study conceptualization. HH contributed to leading the design and execution of the experimental work. All authors contributed to conducting the experiments. AA and HH led the result gathering and interpretation. AA edited the draft manuscript. All authors contributed to the draft review and fine tuning. All authors have read and agreed with the published version of the manuscript.

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Formulacija i karakterizacija vaginalnih kapsula i tableta sa brzim oslobađanjem živih sojeva Lactobacillus spp.

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Kratak sadržaj

Živi bioterapijski proizvodi (LBP) stiču sve veći medicinski značaj kao novi pristup u prevenciji i lečenju različitih stanja u ženskoj populaciji. U ovoj studiji razmatrana je formulacija i karakterizacija vaginalnih farmaceutskih oblika sa trenutnim oslobađanjem (IR) koji sadrže žive sojeve Lactobacillus spp, sa ciljem obezbeđivanja brze i efikasne vaginalne primene. Formulacije vaginalnih kapsula i vaginalnih tableta razvijene su korišćenjem različitih ekscipijenasa i postupaka izrade (kapsulacija i direktna kompresija), a zatim je izvršena njihova karakterizacija i procenjene performanse. Rezultati su pokazali da sve formulacije punjenja kapsula imaju odgovarajuću protočnost i ujednačenosti doziranog oblika, pri čemu su tvrde želatinske kapsule (HGC) imale povoljnije vreme raspadanja u poređenju sa kapsulama od hidroksipropil metilceluloze (HPMC) i pululana. Formulacije tableta na bazi mikrokristalne celuloze (MCC) imale su optimalnu čvrstinu, friabilnost i raspadljivost, posebno pri nižim silama kompresije, čime je potvrđena multifunkcionalnost MCC-a kao punioca, vezivnog sredstva i dezintegratora. Važno je istaći da su oba farmaceutska oblika obezbedila očuvanu vitalnost sojeva Lactobacillus uz minimalne gubitke (~0,5 log), kiselu vaginalnu sredinu (pH 4,2) i pokazala robusnost u simuliranim biorelevantnim uslovima. Ovi nalazi ukazuju na to da vaginalne kapsule i vaginalne tablete sa trenutnim oslobađanjem mogu biti pogodan oblik živih bioterapijskih proizvoda sa brzim početkom delovanja, čime se doprinosi napretku strategija zasnovanih na mikrobiomu u zaštiti zdravlja žena.

Ključne reči: vaginalna primena lekova, živi bioterapijski proizvodi, trenutno oslobađanje, kapsule, tablete