Research Article

DEVELOPMENT OF VAGINAL TABLETS CONTAINING PROBIOTIC AND PREBIOTIC

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ABSTRACT

Vaginosis, which is characterized by increased pH, does not promote the development of probiotic which are administered by means of therapeutic preparations. Non-physiological pH favors the development of the pathological flora which is associated with long-lasting therapy and frequent recurrences of the inflammatory condition. In our study we have undertaken the task of restoring the physiological pH of the vaginal fluid. For this reason, organic acid as prebiotic was incorporated in to probiotic formulation. This section deals with formulation and evaluation of multifunctional vaginal tablets containing Prebiotic and Probiotic for synergistic activity. In the first phase of study prebiotic granules containing organic acid was prepared and evaluated for flow and compaction properties. In second phase of study two kinds of vaginal tablets containing probiotic (Lactobacilli) and Prebiotic (organic acid) were formulated and evaluated: a) Bilayer tablets with prebiotic and probiotic in separate layers and b) compression coated tablets with inner core of probiotic and outer coat of prebiotic. Prebiotic from the combined formulation release first, which restores normal vaginal pH and creates favorable condition to growth of probiotic. The effect of prebiotic on growth of probiotic was studied and results indicate that growth rate of probiotic increases in presence of prebiotic.

Keywords: Vaginosis, vaginal pH, vaginal tablet, prebiotic, probiotic, organic acid.

INTRODUCTION

Bacterial vaginosis is referred when the normal flora of lactobacilli sp. is replaced by pathogenic bacteria[1]. The first line of treatment to this condition includes course of antibiotics. However, in use of antibiotics there is always a chance of recurrence.[2] Therefore; the better alternative is to restore the normal vaginal flora.

In normal healthy vaginal condition the lactobacilli sp. are capable of fermenting the glycogen, derived from vaginal mucosa, to lactic acid. This results in to vaginal pH between 4 to 4.5.[3] It is reported that in post menopause condition or use of contraceptives, or in some systemic diseases the vaginal pH increases which is detrimental for the growth of lactobacilli sp. this results in growth of opportunistic pathogens that are counteracted by lactobacilli sp.[4]

The background that lactobacilli sp. flora of vagina defends the host against pathogens, has increased considerable interest in either formulating lactobacilli sp. or organic acids that can controls the pH of vagina in to suitable dosage form.[5] Several marketed formulations in the form of powder, gel or tablets are available containing lactobacilli sp. and similarly gel preparations containing organic acid.

In this work for the first time an attempt is made to formulate a unit dosage form that will contain both prebiotic (organic acid) and probiotic (lactobacilli sp.).

MATERIALS AND METHODS

Material

The lyophilized *Lactobacillus* species as probiotic was obtained as a gift from Uni Sankyo Ltd., Chiplun, India. Organic acid as Prebiotic was synthesized in our laboratory. DeMan–Rogosa–Sharpe (MRS) medium was purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Tablet excipients such as lactose monohydrate, sodium starch glycolate, magnesium stearate and talc were purchased from S. D. Fine Chemicals, Mumbai, India. All other chemicals were analytical grade and, where required, bacteriological grade.

Methods

Preparation of Prebiotic granules

Prebiotic granules were prepared by wet granulation method using starch paste as binder.[6] Weighed amount of lactose monohydrate, sodium starch glycolate and organic acids (as prebiotic) were mixed well for 10 minutes at constant RPM in tumbling blender. Above blend were converted to cohesive mass using starch paste as granulating fluid. Granules were prepared by forcing cohesive mass through sieve. Wet granules were dried in oven for 1 hrs at 80°C. Sized granules were lubricated and stored in closed plastic container.

Pre-formulation studies on Prebiotic granules

Measurement of powder flow properties:

The loose bulk density and tapped bulk densities were determined by using a density measuring apparatus. An amount of the sample (5 g) was placed in a measuring cylinder and the volume (bulk volume) is measured after applying three taps. For measurement of tapped volume (20 g) of the material were transferred to a 100 ml graduated cylinder. The unsettled apparent volume is noted. The cylinder is tapped at a rate of 300 drops/min over a fixed drop distance of 14±2mm. After the first 500

drops, the volume of the material in the cylinder is measured. Further tapping (750 and then 1250 drops successively) is applied until the difference between two volumes following successive tapping is less than 2.0%. This final volume is taken as the tapped volume. True density (ρ0) was determined by liquid displacement method. Bulk density, tapped density, relative density, porosity, Carr's index (%) and Hausner's ratio were calculated as in Equations 1 – 6. [7]

$$\text{Bulk density } (\rho b) = (\text{weight (gm)})/(\text{bulk volume } (cm^3)) \quad \text{Eq 1}$$

$$\text{Tapped density } (\rho t) = (\text{weight (gm)})/(\text{tapped volume } (cm^3)) \quad \text{Eq 2}$$

$$\text{Relative density } (\rho r) = (\text{bulk density } (\rho b))/(\text{true density } (\rho 0)) \quad \text{Eq 3}$$

$$\text{Carr's Index} = ((\rho t - \rho b))/((\rho t)) \times 100 \quad \text{Eq 4}$$

$$\text{Hausner Ratio} = \text{true density } (\rho t))/\text{bulk density } (\rho b) \quad \text{Eq 5}$$

$$\text{Porosity } (\varepsilon) = (1 - \rho r) \times 100 \quad \text{Eq 6}$$

Angle of repose of the test materials was assessed by the fixed funnel method and computed by Equation 7.

$$\text{Angle of repose } \theta = \frac{\text{Tan}^{-1}h}{r} \quad \text{Eq 7}$$

Where h is height and r is diameter of powder pile

Measurement of Compaction Properties:

Compaction behavior was evaluated by using following parameters

Heckels plot: Granules were compacted to tablets at different compression force (F) from 1-6 ton using 12mm diameter flat punch on rotary tablet press. This compression force was correlated with compression pressure (P) using equation 8. Relative density (D) of compact at each applied pressure was calculated using equation 9. Plot of pressure (P) vs. ln(1/1-D) gives heckels plot; equation 10. [8]

$$\text{Pressure } (P) = \frac{4 \times \text{force}(F)}{\pi D^2} \quad \text{Eq 8}$$

Where D is diameter of cylindrical tablet

$$\text{Relative density } (Dr) = \frac{\pi r^2 h}{w\rho 0} \quad \text{Eq 9}$$

Where r, h and w are radius, thickness and weight of tablet and ρ0 is true density of granule

$$\ln\left(1 - \frac{1}{Dr}\right) = kP + A \quad \text{Eq 10}$$

Where Dr = relative density, P = compression pressure, and k, A are constant

Elastic recovery: For determination of elastic recovery, granules were compressed to tablet using 12mm diameter flat punch at maximum pressure on rotary tablet press. Thicknesses of the tablets were measured immediately after ejection (Hc) and at about 24 h after tablet ejection (He). The elastic recovery of tablet in % was calculated using equation 11. [9]

$$ER\% = \frac{He - Hc}{Hc} \times 100 \quad \text{Eq 11}$$

Tensile strength determination: The force required to fracture the compacts is measured on hardness tester to determine tablet crushing strength.[9] The tensile strength of the compact was calculated from crushing strength using equation 12:

$$T = \frac{2F}{\pi Dt} \quad \text{Eq 12}$$

Where T = tensile strength, F = fracture load, and D, t are diameter and thickness of tablet.

Preparation of Probiotic blend for direct compression

Weighed Lyophilized powder of Probiotic strain was mixed with weighed amount of lactose as diluent, sodium starch glycolate as super disintegrant and talc as glidant. Above mixture was blended in plastic bag for 10 min. Prepared blend was used for preparation of probiotic tablets and bilayer tablets.

Preparation of vaginal tablet containing probiotic strains

Vaginal tablet containing probiotic was prepared by direct compression method.[10] Prepared blend of Probiotic was compressed in to tablet on rotary tablet press using 8mm flat punch at compression force of 3 tons. Weight of tablet was maintained at 200mg ±5%. Prepared tablets were stored at 25°c in air tight plastic container and used further for preparation of compression coated tablet.

Preparation of multifunctional vaginal tablets

Multifunctional vaginal tablets containing Probiotic and prebiotics were prepared in the form of compression coated and Bilayer tablets.

Compression coated tablet

Compression coated tablet with inner core of probiotic and outer coat of Prebiotic was prepared by subsequent compression method. In first step the probiotic tablets were prepared as per method and specification mentioned in section 1.3.5. The prebiotic granules were prepared as per method specified in section 1.3.1. The compression coating of prebiotic granules was applied on probiotic tablet as depicted in Figure1. The tablets were produced on rotary tablet press using 12mm flat punch with operation parameter as mentioned below;

Tablet weight-750mg±5%

Compression speed-10 RPM

Compression force-4

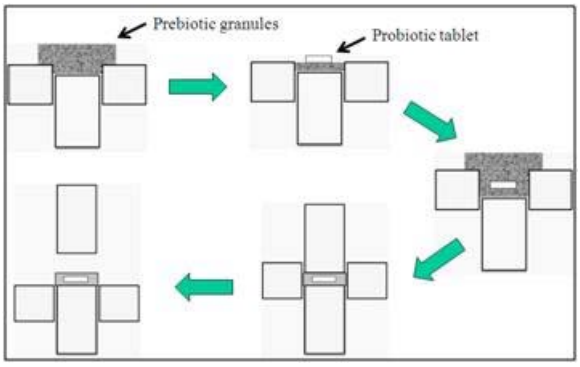


Figure 1: Compression process for preparation of compression coated tablet

Bilayer tablet

For preparation of Bilayer tablet a blend for probiotic layer was prepared as per section1.3.3 and granules for prebiotic layer were prepared as per method specified in section 1.3.1. The quantity of Prebiotic granules (550mg) for first layer was compressed lightly using a rotary tablet press equipped with 12mm round, flat and plain punches. Over this compressed layer, the required quantity probiotic blend (200mg) for second layer was placed and compressed to obtain hardness in the range of 3-4 $kgcm^{-2}$ to form a Bilayer tablet.

Evaluation of vaginal tablet containing Probiotic strains and Prebiotic

Hardness: hardness of tablet was measured using Monsanto hardness tester.[11,12] Fracture load at which tablet breaks in diametric compression test was noted as hardness of tablet from scale of tester. The mean value was calculated from triplicates.

Friability: Friability was investigated using Roche-type Friabilator.[11,12] As per I.P. protocol 20 tablets were weighed and placed into the apparatus and 100 revolutions are performed at speed of 25 rpm. Percent friability is calculated using the following equation 13.

$$\% \text{ Fraibility} = \left(\frac{W_0 - W}{W_0}\right) \times 100 \quad \text{Eq 13}$$

Disintegration time: The disintegration test of vaginal prebiotic tablet was modified from the method described in BP[13] by using tablet disintegrator. The tablet to be tested was placed in a cylindrical glass container with perforated ends and immersed in 1,000 ml of citric acid/phosphate buffer solution pH 4.4 maintained at 37 ± 0.5°C. The cylindrical glass container was moved up and down in the buffer. The time at which disintegration starts and time at which tablet disintegrates completely was noted. The mean values were calculated from six parallel measurements.

Viability assessment: Viability of the probiotic strains after compression was determined by serial dilution plate count method.[10] One tablet was vortexed for 5 min. in 100 ml sterile saline solution to release the bacteria from the tablet. A series of dilution was prepared from this stock solution and was plated out using MRS agar as growth medium. After 48 h incubation at 30°C the colony forming units were counted and cfu/tablet was calculated from colony count. Experiment was performed in triplicate.

Assay for acid content: assay of the tablets for acid content was performed using acid base back titration. One tablet was placed in conical flask containing standardized 20 ml of 1 N NaOH solution. Heated gently for 20 minutes and added phenolphthalein solution as an indicator. While hot it was titrated with standardized 1N H_2SO_4. Burette reading was noted as (A) when color changes from pink to colorless. Experiment was repeated on five other tablets. Blank was performed using placebo tablets (without acids) in triplicates and mean was calculated and noted as (B). Assay of tablet were calculated using equation 14.

$$\% \text{ Assay} = \frac{(B - A) \times 90.08}{50} \times 100 \quad \text{Eq 14}$$

Where, B is blank reading, A is assay reading, 90.08 is factor and 50 is label claim.

Time required for attaining pH 4: the tablet to be tested was placed in cylindrical glass container containing 7 ml of deionized distilled water maintained at 37 ± 0.5°C. pH of the liquid in the glass container was measured at interval of 30 sec using pH meter. The time at which pH of liquid reached to pH 4 were noted as time required to attain pH 4. The experiment was performed in triplicates at mean was calculated.

Effect of Prebiotic on growth of Probiotic strains

Effect of prebiotic on growth of probiotic was determined for Bilayer and compression coated tablets.[10] Combined formulation containing probiotic and acidifier in separate layer was made to dissolve in 9 ml sterile nutrient broth media to release the bacteria and acids. The resulting media was then incubated for 48 hrs at 37°C. A series of dilution was prepared from this grown culture and was plated out using MRS agar as growth medium. After 48 hrs incubation at 37°C the colony forming units were counted and cfu/tablet was calculated from colony count. Similar experiment was performed on conventional probiotic tablet which does not contain organic acid. Effect on growth was established by comparing viable count after incubation obtained for combined formulation containing prebiotic and probiotic with conventional probiotic tablet.

RESULTS AND DISCUSSION

Pre-formulation studies on prebiotic granules

Measurement of powder flow properties

The physical properties of prebiotic granules are summarized in table 1. These derived properties of powder are important measures in characterization of powder flow behavior during high speed compression.[14-16] Static angle of repose less than 25-30° indicates good flow and slightly cohesive nature of prepared granules. Further

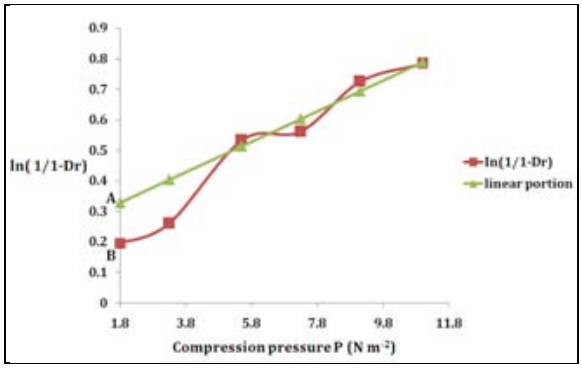
values of Car's index and Hausner ratio indicates good flow and utility of prepared granules for high speed compression process.

Table 1: Derived Properties of Prebiotic Granules Related to Flow

S. No.	Measures	Values (n=6)
1	Bulk density	0.584 ±0.012
2	True density	1.348±0.06
3	Relative density	0.452±0.035
4	Tapped density	0.688±0.038
5	Porosity	0.549±0.033
6	Hausner ratio	1.18±0.08
7	Cars index	11.76±1.75
8	Angle of repose	27.2±1.33

Measurement of Compaction Properties

The heckels plot for prepared prebiotic granules and heckel constants derived from the plot are shown in figure 2. The heckels plot for prebiotic granules shows linearity above compression force 3 ton (pressure 5.5 Nm^{-2}). The nonlinear portion below 3 ton force indicates initial packaging rearrangement and mechanism of consolidation, predominantly fragmentation. The value of portion A-B indicates degree of fragmentation. The slope of linear portion, K, can be correlated to the crushing strength of compact: lager value of K usually indicated harder compact. The value of K for prebiotic granule would be expected to form harder compact. [17]



Heckel constants: a) Portion A-B =0.133b) K=0.105

Figure 2: Heckels plot and Heckle constants for prebiotic granules

Other compaction properties of prebiotic granules are shown in table 2. The value of elastic recovery indicates slightly elastic nature of granules. The higher value of crushing strength and tensile strength are indicative of good mechanical strength of compact prepare from prebiotic granules.

Table 2: Other compaction properties for Prebiotic Granules

Measures	Values
Elastic recovery	2.08±0.3
Crushing strength	4
Tensile strength	14

Preparation and evaluation of Multifunctional vaginal tablets

Vaginal tablets containing prebiotic and probiotic were prepared successfully in the form of compression coated (figure 3) and Bilayer tablet. Prepared tablets were evaluated for various official (disintegration time, assay, weight uniformity, content uniformity and friability) and non-official (hardness, time to start disintegration and time required for attaining pH 4) test. The results of various official and non-official tests are within the prescribed official limits and satisfy predefined acceptance criteria for quality, table 3 and table 4.

Figure 3: Compression coated tablets with inner core of probiotic and outer coat of prebiotic

Table 3: Official tests of vaginal tablet

Tablet type	DT Min ±SD	Assay of acid %±SD	Friability %±SD	Weight variation	Content uniformity
Compression coated	15.2±0.76	96.82±1.02	0.14±0.33	Within limits	Within limits
Bilayer	16.8±0.57	97.12±1.54	0.14±0.33	Within limits	Within limits

Table 4: Non-official tests of vaginal tablet

Tablet type	Hardness Kg/cm^2 ±SD	Time to start disintegration Min± SD	Time required for attaining pH 4 Min± SD	Viability after compression
Compression coated	4.8±0.72	4.12±0.34	10.8±0.48	$2.4±0.38×10^8$
Bilayer	5.4±0.67	4.84±0.57	11.6±0.76	$1.9±0.46×10^8$

Effect of Prebiotic on growth of Probiotic

Effect of prebiotic on growth of probiotic was determined. Viable count of lactobacilli after 48 hrs of incubation at 37°C for Bilayer, compression coated and conventional probiotic tablet were shown in table 5. Bilayer and compression coated tablets shows grater viable count after 48 hrs of incubation than conventional probiotic tablets. This suggests that prebiotic present in Bilayer and compression coated tablets, creates favorable environment for growth of lactobacilli by acidifying media to normal vaginal pH 4.

Table 5: Effect of prebiotic on growth of Probiotic

Formulation	Viability after 48 hrs of incubation
Plain probiotic tablet	$5.8\pm0.19\times10^{6}$
Compression coated tablet	$6.4\pm0.14\times10^{10}$
Bilayer tablet	$4.2\pm0.27\times10^{10}$

CONCLUSION

Combined formulation of Prebiotic and Probiotic in the form of compression coated and bilayer tablet showed synergistic activity and are better than conventional Probiotic formulations in relation to colonization of Probiotic in vagina. The compression-coated tablet provides a means of compression coating of Prebiotic to inner core of Probiotic that not only protects Probiotic from environmental condition such as moisture, but also enables Prebiotic to release first from the outer coating. Multifunctional vaginal tablets in the form of compression coated and bilayer tablet may serve as valuable therapy for treatment of vaginal urinary tract infections.

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