Formulation Direct Compression Tablet of Probiotic as Vehicle for Oral Delivery

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A B S T R A C T

There has been an explosion of probiotic health-based products. Many reports indicated that there is poor survival of probiotic bacteria in these products. For oral delivery of probiotic, providing living cells with a physical barrier against adverse environmental conditions is considerable interest. The main goal of the study was to develop tablets made out of functional polymers in order to protect probiotic bacteria from gastric acidity, thus providing an easily manufacturing scale-up dosage form to deliver probiotics to the human intestinal. Tablets were produced by direct compression using Microcrystalline Cellulose (Avicel®) as main exipient. To optimize the formulation, using survival rate after both compressing and exposed pH: 2 acid medium were evaluated. Storage stability of *lactobacilluse acidophiluse* tablets was also performed by evaluation of viable cells throughout 3 months at 4 ºC. The highest viability was found in formulation 7 and 8 with %90.37 and 90.27% after compression pressure. Increasing amount of Avicel® in the tablet increase bacterial viability against pressure. 72.51% Survival was showed is related to formulation 3 after exposure acid. It can be concluded that The best protective qualities against artificial gastric juice were observed when tablets were prepared from compaction mixtures of lactic acid bacteria (LAB), sodium alginate, and Xanthan. A decrease of approximately one logarithmic cycle was observed after 3 month storage for formula 3 while untreated cell decrease 3.97 log. This preparation method and tablet formulation can be employed for intestinal delivery to ensure maximum viable cell release at intestine or colon and this product remains stable until the time of consumption.

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Introduction

Probiotics are defined as living microorganisms which may, on administration, colonize the human intestine and beneficially affect health [1]. During the last 2–3 decades, attempts for improving the human health status, are focusing on ways for modulating the indigenous intestinal flora by live microbial adjuncts, now called "probiotics" [2]. **Several beneficial functions have been suggested for probiotic include:**
Reduced antibiotic associated diarrhea; Antibiotic-associated diarrhea can be attributed in part to imbalances in intestinal micro flora. Therefore, probiotic preparations are used to prevent this diarrhea [3] .The therapeutic effect of probiotics in various models of experimental colitis and in several clinical trials in patients with inflammatory bowel disease (IBD) or related disorders [4]. Effect of probiotics Anti-inflammatory Lactobacillus strains promotes the formation of a desirable anti-inflammatory environment in the peripheral blood of IBD patients, and showed no harmful effects in patients or control subjects [5]. Probiotics may have a beneficial effect on the severity and duration of symptoms of respiratory tract infections (RTIs) but do not appear to reduce the incidence of RTIs [6]. Results show that probiotic products containing Lactobacillus GG may reduce respiratory infections and their complications among children attending day care centers [7]. Lactic acid bacteria on the risk for colorectal cancer can therefore be anticipated although definite proof remains to be presented [8] and they might suppress the growth of bacteria that convert proarcinogens into carcinogens, thereby reducing the amount of carcinogens in the intestine [9]. There has been considerable interest in the effect of probiotics on human lipid metabolism, and numerous studies have focused on the potential hypocholesterolemic activity of probiotics in human [10] and can be anticipated to induce a lowering of circulating cholesterol concentrations, thus diminishing the risk of CHD [11]. Microbes from many different genera are being used as probiotics. Lactic acid bacteria (LAB) are the most important probiotic

microorganisms typically associated with the human gastrointestinal tract. These bacteria are Gram-positive, rod-shaped, non-spore-forming, catalase-negative organisms that are devoid of cytochromes and are of non-aerobic habit but are aero-tolerant, fastidious, acid-tolerant and strictly fermentative; lactic acid is the major end-product of sugar fermentation [12]. A number of probiotic product snare marked and they exist in different forms include fermented food products and drug forms such as granules, beads, tablets, microencapsulation or gelatin capsules. Food products containing probiotics will emerge such as energy bars, cereals, juices, infant formula and cheese, as well as disease-specific medical foods [13]. Furthermore, many of the microorganisms included in these products are not viable and have not been selected either for specific beneficial properties or for their ability to survive in the gastrointestinal tract. Probiotics survival will depend on such factors as the final product pH, the presence of other microorganisms, the storage temperature and the presence or absence of microbial inhibitors in the substrate [14]. Many studies have been done in order to solve the problem. Furthermore, the addition of cry protectants during the freeze drying of lactobacilli has been used to help overcome inactivation during drying and stabilization during storage [15].Ascorbic acid added to the microbial suspensions before drying was found to favored the stability of the cells during long term storage, and therefore should be considered for the production of freeze-dried cultures that will eventually be included as probiotics in pharmaceutical preparations [16].Two processes of enclosure of probiotic Lactobacillus plantarum CMU-FP002, probiotic granules and calcium alginate beads, were studied. The beads contained more survival cells than granules. Furthermore, the beads formulated from 1.5% (w/v) sodium alginate solutions had the highest survival cells (9.30 log cfu/g) [17]. Microencapsulation was used to study an alternative technological way to obtain a probiotic concentrate able to be incorporated in the tablets. The potential of tablets based on a combined CAP and croscarmellose sodium matrix to deliver viable cells of L. paracasei L26 in

simulated GI fluids namely in the colon with very good extension of cell survival [18]. In the another study, gastric juice resistant microcapsules of L. Acidophilus LA14 and B. lactis BI07 were developed, using 0.5%(w/v) of XG or 1% of CAP within 3% (w/v) alginate solution. These formulations were also stable for a long time in presence of bile salts, maintaining a viable count of 10^9 CFU/g over 24 h (viability of 98%) [19] Probiotic cell containing powders were first compressed into a pellet, which was then encapsulated with the coating material by further compression. Results indicated significant improvement in survival of encapsulated cells when exposed to acidic media of pH 1.2 and 2. The encapsulated cells showed 10^4–10^5-fold increment in cell survival when compared to free cells under the test conditions [20].From the resent research work it may be concluded that matrix tablets coated with 10 %shellac solution that contain probiotics may be employed successfully for colon specific delivery to ensure maximum drug release at colon even in patients with disturbed gastrointestinal micro flora [21].HPMC-HV with a more continuous bacterial release and suitable disintegration time could be a suitable retarding polymer for tablet formulations [22].It was found that the amount of HPMCAS in the tablet correlates with gastric juice resistance. As HPMCAS also leads to a decrease of disintegration time in intestinal fluid, slight amounts of this excipient were preferred. The best protective qualities against artificial gastric juice were observed when tablets were prepared from compaction mixtures of LAB, HPMCAS and sodium alginate [23]. Succinylated ß-lactoglobulin revealed to be a suitable natural excipient to form tablets containing probiotic bacteria and promote their survival against gastric conditions. In fact, it was possible to produce rigid tablets by direct compression with a bacterial content as high as 10^9CFU [24]. The recent study shows that it is possible to prepare tablets using HPMCP 55 as matrix forming material from which living probiotics are released in simulated GI fluids The extent of cell survival (up to 80%) depends on the formulation and processing parameters [25].Pro- and prebiotics-loaded bioadhesive microparticles made of hyaluronic acid sodium salt, HA and low

methyl pectin have successfully been developed for a pharmaceutical use. The operating conditions allowing the preparation of favorable microparticles for probiotic encapsulation have been determined through an experimental design [26].Concerning the dry-coated tablets, the (carboxymethyl high amylose starch, CM-HAS) external layer afforded a better protection of bacteria, with an enhanced number of CFU of *L. rhamnosus* delivered in simulated intestinal conditions. In this context, the CM-HAS could be an efficient excipient for dry-coated formulations for colon delivery [27].Of all tablets tested, sodium alginate was found to be the most suitable excipient for tablets containing probiotic bacteria and for the promotion of their survival within gastric conditions [28].Based on this knowledge, we investigate in the present study whether it is possible to design tablet formulations for probiotics that protect them from degradation at low pH and deliver them to the intestinal tract in viable form. We investigated the effect of simulated gastric fluid and the amount of coating material on survival of encapsulated cells. The release time of the cells was also monitored in order to make a predictive indication of the site of cell release in the human gastro-intestinal tract.

Material and method

Strain and culture condition

Lactobacillus acidophilus **ATCC 4356** (PTCC 1643) was purchased from Persian Type Culture Collection (PTCC), Tehran, Iran. LAB regenerated into MRS broth (Merck, Darmstadt, Germany) from the lyophilized vial and several stock cultures were prepared in PBS and stored at -70ºc.

Cells production and storage conditions

10ml of *L. Acidophilus* stock vials were grown in 300 ml of MRS broth (Merck, Damstadt, Germany) at 37 ◦C for 20h under anaerobic conditions and incubated without shaking. Cultures were harvested at the beginning of stationary phase and collected by centrifugation (5000g, 15min, 4ºC), washed once with Nacl 0.9%. Washed cell were

freeze driedby Freeze Dryer (brand Christ, model Alpha 2-4 LD).A serial dilution of washed cell suspension was made in 0.1% sterile peptone water until a suitable cell density was obtained and subsequently plated in triplicate in MRS agar plates. These plates were incubated at 37 ºC for 24 h. This plating procedure was carried out in triplicates. Colonies of bacteria were counted and converted to log CFU (colony forming units).The number of probiotic cells in the suspension obtained was $1.55 \times 10^{13} \pm 1.2 \times 10^{13}$ CFU/1ml.The lyophilized probiotic bacteria (LAB) were carefully ground into fine powders and stored at 4ºCin closed containers for further experiments in tableting process. The number of probiotic cells in LAB powder was $0.94 \times 10^{10} \pm 1.6 \times 10^{9}$ CFU/10mg.

Chemicals

Hydroxy propyl methyl cellulose (HPMC) and sodium alginate and Microcrystalline Cellulose Avicel® was from Merck (Darmstadt, Germany) , and cellulose acetate phthalate (CAP), glycerol, calcium chloride, sodium hydroxide pellets and hydrochloric acid (37%) were purchased from Sigma–Aldrich (Germany). Xanthan gum (XG) was supplied by ACEF Spa (PC, Italy). Sodium dihydrogen phosphate and MRS agar and saline solution were supplied by Merck (Darmstadt, Germany). Lactose and starch were purchased from Sigma–Aldrich.

Tablet preparation and analysis

A single punch tableting machine (Korsch AR 400, Germany) was used for preparation of tablets, with circular shape of die and punch. The possible adverse effects of pressing on lactobacilli's viability were investigated by comparing the viability of bacteria in tablets. An exactly weighed quantity of powder containing the freeze-dried probiotic cell and the tablet formulation material was filled into a die of 10 mm diameter and under a determined pressure 50 MP tablets with a plane surface were formed. The half of the total amount of the tablet formulation material was first used to fill a 10 mm die. The freeze-dried probiotic cell containing powders was then carefully positioned

at the center of the die before the rest of the tablet formulation material was poured on top of it. The thickness was measured using a digital vernier caliper. Tablet formulations, investigated in this study are summarized in Table 1.

Physical properties of tablets

To determine the physical properties of the tablets, the following tests were performed:

Mean weight

For this purpose 10 tablets were individually and randomly weighed on an analytical balance (brand Rad wag, model AS 220/C2) to determine their mean weight: a 5% deviation was considered acceptable [29].

Hardness and tensile strength

Hardness was determined by resistance to crushing or rupture under continuous pressure and was measured using a hardness tester (brand dr schleuniger, model 8m). The result obtained was the mean of the resistance of the 10 tablets and was expressed in Newton (N)(29). The minimum value recommended for hardness is 30 N. The tensile strength (σ) was calculated by the following equation

$$\sigma = \frac{2P}{\pi Dt}$$

where P is the measured crushing force, D is diameter and t is the thickness of the tablet (28).

Friability

This parameter was determined by shock and friction. A friabilometer (brand erweka, model Ta 200) was used. For this measurement, samples of 10 tablets were submitted to a 25 rpm rotation speed for 4 minutes; a weight loss maximum value of 1 % was considered acceptable [30].

Disintegration

The probiotic tablets obtained were evaluated for their disintegration and tensile strength according to USP. Disintegration of the tablets was examined

by means of a disintegration apparatus brand erweka (model zt 510). The tablets were placed separately in the test chamber, and then immersed in PBS pH 6.8 as the disintegration medium at 37 ◦C.

Table 1. Probiotic tablet formulations.

Formulation No	composition(mg)							
	LAB	Avicel®	sodium Alginate	CAP	xanthan	HPMC	Lactose	Starch
1	10	150	50	-	-	-	-	-
2	10	100	50	50	-	-	-	-
3	10	100	50	-	50	-	-	-
4	10	100	50	-	-	50	-	-
5	10	100	50	-	-	-	50	-
6	10	100	50	-	-	-	-	50
7	10	100	50	25	-	25	-	-
8	10	100	50	-	25	25	-	-
9	10	100	50	-	-	25	25	-
10	10	100	50	-	-	25	-	25

Test of bacterial viability in tablets

Bacterial viability after tableting

In according [31] method, after compression, each table was immediately broken and dispersed in 100 mL of phosphate buffer (pH 6.8). This suspension was made in 0.1% sterile peptone water until a suitable cell density was obtained and subsequently plated in triplicate in MRS agar plates. These plates were incubated at 37 ºC for 24 h. Colonies of bacteria were counted and converted to log CFU (colony forming units).The survival of probiotic cells reported as percentage viability was calculated according to the following equation

$$\text{Viability (\%)} = \frac{\text{CFU after exposure to the compression}}{\text{CFU before exposure to the compression}} \times 100$$

Exposure of tablets to in simulated gastric fluids (SGF)

The SGF was prepared by adjusting the pH of a phosphate buffer solution (PBS) pH=2, prepared according to USP XXII method, to the required pH by addition of HCl. All tests were carried out according to USP 2 paddle method at100 rpm, 37 ºC with 600 ml of SGF. After the end of the incubation period, The acidic medium was removed and the viable cells inside the non-disintegrated tested tablets were determined according to the methods described by (20)with some modification.

$$\text{Viability (\%)} = \frac{\text{CFU after exposure to The asidic medium}}{\text{CFU before exposure to The acidic medium}} \times 100$$

Cell release time

All dissolution tests were carried out according to USP 2 paddle method at 100 rpm, 37 ℃ with 600 ml of simulated intestinal fluid (SIF). The tablets were first exposed to the respective SGF for 2 h and subsequently transferred to simulated intestinal fluid for dissolution. The simulated

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intestinal fluid was prepared from phosphate buffer solution (PBS) pH 6.8. They were allowed to dissolve completely into the dissolution medium.The time taken for total dissolution to occur (including 2 h in SGF) was recorded.

Storage stability

Tablets were stored in amber bottles at 4 ºC for a period of 3 months. Sample tablets were taken out after 3 month, dissolved in 100 mL SIF and bacterial viability was assessed following the aforementioned protocol.

Data analysis

Statistical analyses of survival were performed by the ANOVA methodology using as independent variable the heating or storage time. Differences were considered significant at $p < 0.05$.Values is expressed as mean ± standard deviation.

Table 2. Tablet evaluation and cell release time.

formulation no	The tensile strength(N/mm2)	mean weight	total release time(h)(±0.05)
1	5.19±0.76	214.75±7.71	3.57
2	6.51±1.23	206.25±3.59	3.66
3	5.65±0.36	213.25±5.67	4.01
4	5.37±0.39	209.5±4.12	3.67
5	5.23±0.41	206.25±7.53	3.98
6	6.57±1.02	205.25±4.03	3.46
7	5.44±0.68	211.75±7.70	3.63
8	5.3±0.28	211.5±6.24	2.01
9	5.31±0.14	211.25±3.94	3.72
10	5.26±0.37	206.5±3.69	3.57

Values within a column with different superscript are significantly ($P < 0.05$).
Mean ± SD (n =10).

Results and discussion

The effects of tablet formulation such as concentration of probiotic cells and tablet excipients (e.g. polymer matrix) as well as tablet processing conditions, such as compression pressure, on tablet properties and survival of the probiotic bacteria were investigated. The results of these studies were then evaluated in order to find out a suitable probiotic tablet formulation prepared with proper compression force. The developed probiotic tablets were then subjected to a stability test in order to identify suitable formulation.

Tablet preparation

A total of twenty nine formulations were developed with compression forces ranging from 50-70 MP. Of these, only ten kinds of tablet (Table1) were selected for the subsequent experiments, based on the results of disintegration, tensile strength (Table 2) and friability. Working pressure was established at 50MPa for all of tablets.

Tablet evaluation

The selected conditions allowed for the creation of rigid (friability < 1%) and easy-to-manipulate tablets. Moreover, tensile strengths ranging between 1.05N/mm2 and 3.44 N/mm2 arecalculated (Table2).

Bacterial viability after tableting

The effect of the compression pressure on the viability of probiotic bacteria in the tablets, investigated in this study are summarized in Table 3. Tablets manufactured with high compression force showed a slow disintegration time and high bacterial cell viability (more than 80%) [25]. A decrease of 1 log cycle was observed after the compaction with a 9.8 kN force; however, the number of viable cells for different compaction forces was of the same order of magnitude showing no increase of detrimental effects for compaction forces higher than 9.8 kN [18]. The loss of viability appears to be dependent to the strain and also to the formulation or the process conditions. The Lcr35 strain was compressed at different pressures to evaluate the impact of tableting on its viability and on its biological and genetic properties. Genomics analysis demonstrated that the compression had no effect on the genetic profile of the *Lactobacillus rhamnosus* Lcr35strain [32].

Tablets contained 10 mg of probiotic bacteria ($0.94 \times 10^{10} \pm 1.6 \times 10^9$ CFU/tablet). A decrease of 1 log cycle was observed after the compaction with a 50MP pressure; however, the number of viable cells for different compaction forces was of the same order of magnitude showing no increase of detrimental effects for compression pressure higher than 50 MP.

The results of the analysis of formulations Nos. 1–6 (applying 50 mg of alginate and 50 mg of different of polymers), show that tablets containing HPMC have suitable formulation. The examination of formulations Nos. 7–10 (prepared with 25mg HPMC and difference amount of polymers). The highest viability was found in formulation 7 and 8 with %90.37 and 90.27% after compression pressure. In fact, the size of the powder, particles and their physical behavior under pressure, namely brittle fracturing and/or plastic deformation, have a major influence on the extent of bacterial death. Cell mortality is connected to the tablet's initial porosity so that when porosity is decreased, cell mortality is logically increased [33]. The dependence of tablet porosity and tensile strength on compression speeds showed that HPMC K4M is consolidated by plastic deformation. At all compression speeds, an increase in moisture content reduced the percentage elastic recovery of HPMC compacts due to greater tablet consolidation [34].

Table 3. Survival of LAB after compressing.

formulation no	number of cell after compressing (CFU/tablet)	number of cell after compressing (LogCFU/tablet)	Survival (%)
1	$1.36 \times 10^8 \pm 2.7 \times 10^7$	8.13 ± 0.08	81.54
2	$2.97 \times 10^7 \pm 1.02 \times 10^7$	7.46 ± 0.15	74.82
3	$4.41 \times 10^7 \pm 0.83 \times 10^7$	7.64 ± 0.08	76.62
4	$4.90 \times 10^8 \pm 1.13 \times 10^8$	8.68 ± 0.10	87.06
5	$1.50 \times 10^8 \pm 6.71 \times 10^6$	8.17 ± 0.01	81.94
6	$1.27 \times 10^8 \pm 6.01 \times 10^7$	8.07 ± 0.21	80.94
7	$1.05 \times 10^9 \pm 1.41 \times 10^8$	9.01 ± 0.05	90.37
8	$1.07 \times 10^9 \pm 5.30 \times 10^8$	9.00 ± 0.22	90.27
9	$3.32 \times 10^8 \pm 1.87 \times 10^8$	8.48 ± 0.25	85.05
10	$1.30 \times 10^8 \pm 3.53 \times 10^7$	8.1 ± 0.11	81.24

Values within a column with different superscript are significantly (P < 0.05).
Mean ±SD (n = 3).

Viability of the probiotic tablets after acid challenge

Stability of probiotic cells in term of cell viability is one of the major indexes which indicate the efficiency of pharmaceutical excipients and dosage forms to protect the cells in gastrointestinal tract (GI). The formation of a hydrogel around the cell tablet is thought to be the basis for cell protection. To obtain better acid stability the HPMC content was partially replaced (40%) with sodium alginate, calcium alginate, apple pectin or Metolose in Fazeli M study (23). Sodium alginate, which can form gels after being hydrated, has been exploited as the prime coating. The formation of a hydrogel barrier by the compacted sodium alginate layer has shown to retard the permeation of the acidic fluid into the cells. This contributed to the enhanced cell survival (20). That's why all the Tablets Containing 50 mg Sodium alginate. The viability of the probiotics was reduced less than 1 log for formulation 2, 3 and 6 (Table 4). Fig. 1 shows the permeation of the acidic fluid into the tablets. The acidic fluid needs to permeate through the gel layer before reaching the bacterial cells. Hence the deleterious effect of acid could be minimized as the improvement in survival of encapsulated cells when exposed to acidic medium of pH 2 and below is evident. In formula 3 acid penetrated into the layer of gel before reaching the probiotic bacteria, and the gelatinous layer blocked penetration of acid into the cell, thus the lowest mortality associated. With the formulation 7 and 8 high acid penetration in layer of gelatin caused high cell death.

Fig. 2 shows the highest survival of LAB after tableting and exposure acid is related to formulation 3 (Survival (%) was 72.51). The best formulation of probiotics is formulation 3 which has the most viability amount of bacteria.

Table 4. Survival of LAB after exposure PH:2 for 2h.

formulation no	number of cell after acid challenge (CFU/tablet)	number of cell after acid challenge(LogCFU/tablet)	Survival (%)
1	$0.89×10^7±0.70×10^5$	6.95±0.01	69.7
2	$5.55×10^6±3.46×10^6$	6.69±0.29	67.1
3	$1.7×10^7±5.09×10^6$	7.23±0.12	72.51
4	$1.1×10^6±1.41×10^5$	6.03±0.05	60.48
5	$2.9×10^6±1.41×10^5$	6.46±0.02	64.79
6	$1.85×10^7±6.36×10^5$	7.2±0.01	72.21
7	$2.40×10^6±0.84×10^6$	6.3±0.15	63.18
8	$2.00×10^6±1.41×10^6$	6.2±0.33	62.18
9	$3.55×10^6±6.36×10^5$	6.5±0.07	65.19
10	$6.20×10^6±2.54×10^6$	6.7±0.18	67.2

Values within a column with different superscript are significantly (P < 0.05).
Mean ± SD (n = 3).

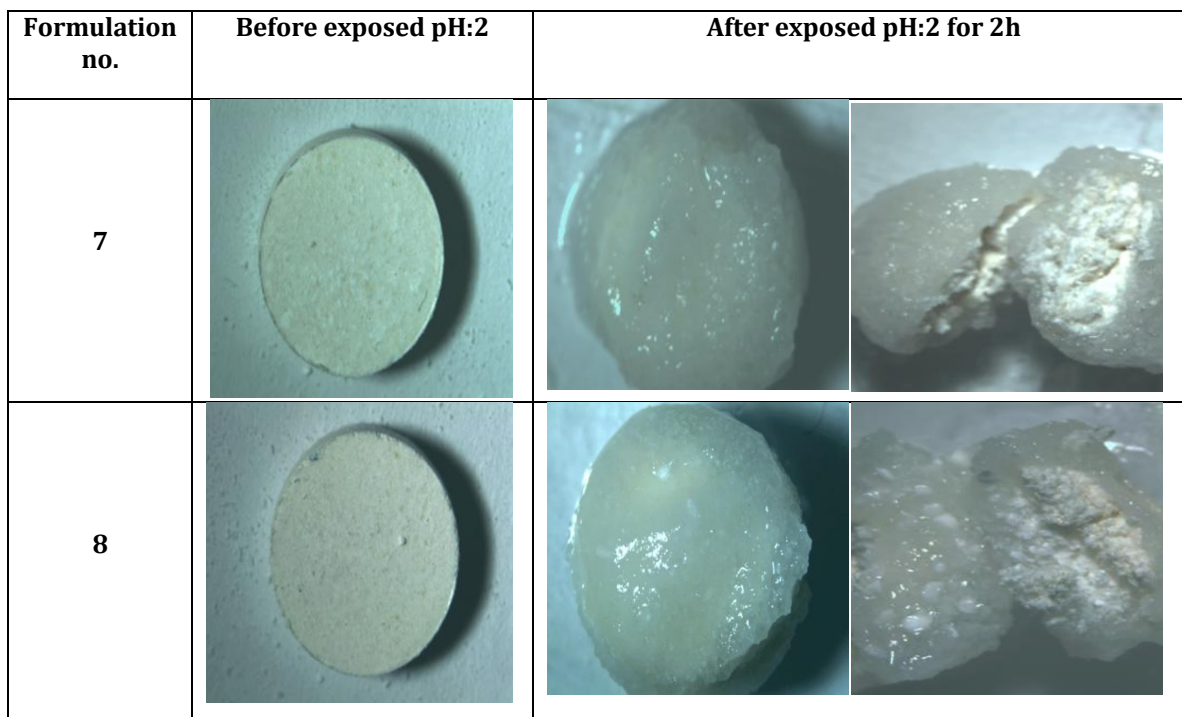| Formulation no.	Before exposed pH:2	After exposed pH:2 for 2h	
7			
8			

Fig. 1. The permeation of the acidic fluid into the cells before and after exposure pH: 2 for 2h
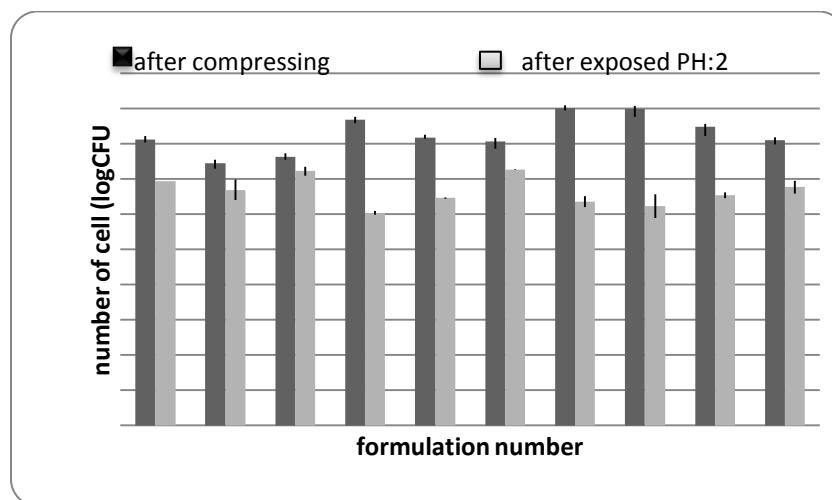

Fig. 2. Survival of LAB after compressing and exposed pH: 2 (n=3).

Comparison of total release time

The release time of encapsulated cells could be used as an indicator of the site of cell release in human gastro-intestinal tract. Since, the mean residence time of a dosage form in the stomach is about 2 h, and the mean transit time of a dosage form in the small intestine of a healthy subject is about 3–4 h, it could be deduced that the cells encapsulated by the formulation and method described above would probably be released in the region between the near end of the small intestine and the beginning of the colon. Therefore, the tablets should ideally protect

probiotic bacteria until 2h in pH:2 and then release viable bacteria in the intestinal tract.

In this study, the time releases of all the tablets were 4h. About 3–4 h (table 2) that this may be due the fact all tablets contains sodium alginate. It has been seen that the PH of the medium may influence alginate gel. Formulation 8 has the highest rates of liquid permeation. In fact the highest release time were found for formulation 3

and 5 (table2). The best formulation of probiotics is formulation 3 which has the most viability amount of bacteria after compressing and exposed pH: 2**.** Finally, in conclusion to our studies, we suggest that Tablets are considered as a good choice of GI LAB delivery because there is less chance of cell mortality during GI transition. Formulation 3 is able to deliver LAB in oral form.
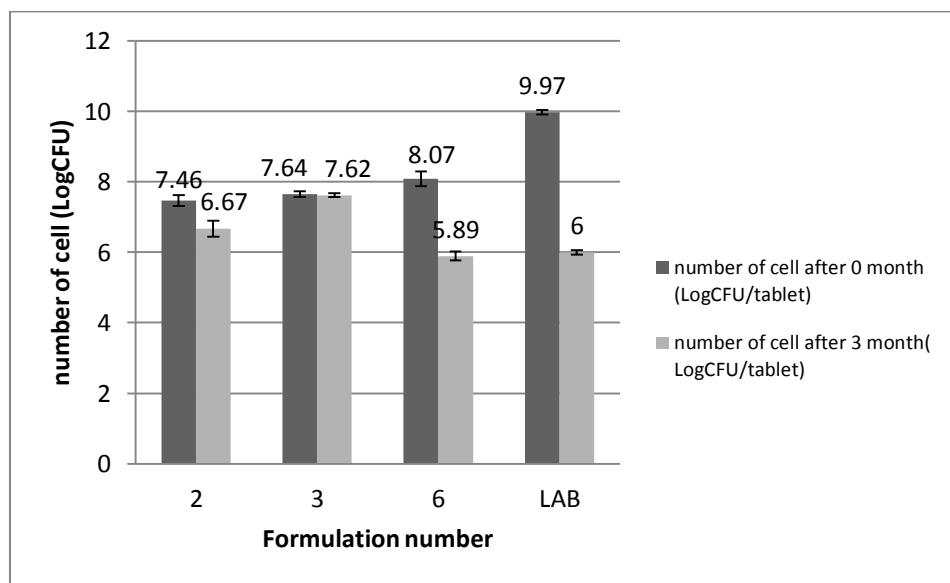

Fig. 4. Storage Stability of the probiotic tablets and LAB (n=3)at 4 ºC after 3 months.

Stability of the probiotic tablets

Stability of probiotic cells in term of cell viability is one of the major indexes which indicate the efficiency of pharmaceutical excipients and dosage forms to protect the cells with long shelf life. It was found that storage at 30 °C caused significant decreases in cell viability. After 6 months, the loss of viability was observed to be less than 1 log unit. Importantly, however, no significant decrease in viability was observed during storage of the tablets at 10 °C for 6 months [25].In contrast, during storage at 4°C the ability to reduce the pH of SVF

Throughout the time of storage was observed. As previously observed, lyophilized *P. pentosaceus* SB83.

Were more stable at 4°C than room temperature [35].This capacity only decreased slightly with time (Storage at a temperature of 10 or 20 °C for 6months results only leads a slight loss of viable cells. A temperature of 30 °C results in a microbial cell count reduction of less than one log unit [23]. Probiotics bacteria in FDP stored in the refrigerator evidenced as light decrease of viability during time and after 6 months the cell viability was around 85–88%.Whenthe microcapsules are stored at5 °C, the results highlighted a viability of about 82–83% (108

CFU/g)after 6 months and of about 68–70% (107 CFU/g) after 9 months [19].

Survival of probiotic is dependent on the intrinsic properties of the strain and the excipients used for the preparation of tablets [28].

Fig. 3 represents the evolution of the number of viable bacteria within formulation 2, 3, 6 and LAB. The stability of the encapsulated bacteria higher than free cell containing powders after 3 month storage at 4°C. No significant differences were reported between the number of cells at time zero and after 3 months of storage at 4°Cfor formulation 3.

Conclusion

Of all tablets tested, Formulation number 3 was found to be the most suitable for tablets containing probiotic bacteria and for the promotion of their survival within gastric conditions. The viability of the probiotic was reduced during tableting by less than 1 logarithmic cycle for formulations 7and 8. The highest survival of LAB exposure acid is in formulation 2, 3. Also formulation 3 was found to be very stable over a 3 months period when stored at 4 ºC. In addition, in vitro tests indicate that the probiotic cells shall most likely be released in an appropriate site within the human gastro-intestinal tract. Further studies are needed to elucidate if the same process could also be suitable for other probiotic strains.

Conflict of Interests

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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