International Journal of Pharmaceutics 370 (2009) 54–60

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Development of tablets containing probiotics: Effects of formulation and processing parameters on bacterial viability

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ARTICLE INFO

Article history:
Received 24 February 2008
Received in revised form 7 November 2008
Accepted 11 November 2008
Available online 18 November 2008

Keywords:
Probiotic
Tablet
Viability
Stability
Sodium alginate
HPMCP

ABSTRACT

The probiotic products available in the market nowadays are mostly in the form of liquid or semisolid formulations which show low cell viability after oral administration, mainly because the bacteria do not survive the harsh conditions in the stomach. The development of suitable dry dosage forms enable higher bacterial survival and consequently is the main aim of the present study. An anticipated advantage is that due to the low water-activity lyophilized bacterial cells will preserve their viability. Further, by a proper selection of a tablet forming matrix, it is foreseen that the entrapped bacteria are protected against the low pH in the stomach. In this study, the effects on bacterial survival in tablets were investigated concerning compression force, matrix forming excipients such as hydroxypropyl methylcellulose phthalate (HPMCP) or other swelling agents. The results showed that the proportion of matrix forming excipients in tablets and the compression force affected the properties of probiotic tablets in terms of tensile strength and disintegration as well as the survival of the bacteria. The tensile strength of the tablets increased with increase of HPMCP content. Tablets manufactured with high compression force showed a slow disintegration time and high bacterial cell viability (more than 80%). Incorporation of sodium alginate in the tablets resulted in higher cell survival in simulated GI fluid (>90%) and a suitable disintegration time (approximately 5 h). By a proper design of the formulation, tablets with a fast disintegration time and a high preservation of bacterial cell viability were developed.

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1. Introduction

A probiotic is generally defined as a live microbial food supplement which beneficially affects the host by improving its intestinal microbial balance (Fuller, 1991). Several authors have shown that the regular consumption of viable probiotic microorganisms can be effective in improvement of lactose tolerance (Kim and Gilliland, 1983; Hove et al., 1999), reduction of cholesterol levels (Anderson and Gilliland, 1999; Nguyen et al., 2007), and control of gastrointestinal infections (Saavedra et al., 1994; McFarland et al., 1995), caused either by virus (Colbère-Garapin et al., 2007) or bacteria (Shah, 2007). Further, it has been reported that the colonization of some strains of probiotics can lower the severity of acute diarrhea in children (Szymański et al., 2006) and can prevent radiation-induced diarrhea in patients with gynecologic cancers treated with pelvic radiotherapy (Giralt et al., 2006). Probiotics can influence intestinal physiology directly or indirectly through the modulation of the endogenous microbiota or the intestinal immune system (Marteau et al., 1993). The intestinal flora and the immune system play an important role in the modulation of carcinogenesis and could explain the use of probiotics in the prevention of tumor development. A number of studies reported a stimulation of the immune function (Cross et al., 2002; De Moreno de LeBlanc et al., 2008) and a suppression of cancer through the consumption of these microorganisms (Aso et al., 1995; Cross, 2002). Several authors have shown that probiotics may decrease the fecal concentrations and activities of certain enzymes such as β-glucuronidase, azoreductase, and nitroreductase, which are involved in the activation of mutagens (Orrhage et al., 1994; Commane et al., 2005) and decrease secondary bile salts that may be involved in colon carcinogenesis (Ohkawara et al., 2005; Wollowski et al., 2001; Gonet-Surówka et al., 2007). Other benefits of probiotics are an antidiabetic effect (Matsuzaki et al., 1997; Yadav et al., 2007) and the improvement of glucose tolerance (Östman et al., 2005). A new therapeutic approach for the treatment of inflammatory bowel diseases is also based on the administration of probiotic bacteria (Lammers et al., 2003) with suitable prebiotics, referred to as 'synbiotic' (Geier et al., 2007). Probiotics can also inhibit several cariogenic pathogens in oral cavity (Meurman, 2005). Some probiotic products have been developed and showed beneficial inhibition

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doi:10.1016/j.ijpharm.2008.11.004

effects on salivary mutans *streptococci* and *lactobacilli* (Caglar et al., 2007).

Presently, probiotic products containing specific probiotic strains are developed in different formulations, such as fermented milk (Lavermicocca, 2006), chewing gum (Caglar et al., 2007), sachets (Cruywagen et al., 1996) and capsules (Bruno and Shah, 2003). However, these products show limited stability of the probiotic microorganisms. Takahashi et al. (2004) reported that among several species of probiotics, only one strain of *Bifidobacterium longum* could survive in fermented milk for 2 weeks. Moreover, the number of viable bacteria that enter the intestinal tract is not controlled with these formulations because the bacteria do not survive the low pH in the stomach. It was demonstrated that the bifidobacteria in the form of fermented milk to humans showed survival rates only 7–30% (Berrada et al., 1991; Bouhnik et al., 1992). Therefore, there is a need for formulations that protect the bacteria from the harsh conditions in the stomach. In the pharmaceutical field, acid labile drugs are formulated in tablets which are able to protect them from these harsh conditions and deliver the active substances into the intestinal tract. Tablets can be easily designed to control the release and enhance the adhesion and colonization of the probiotic microorganisms to the epithelial mucosa of human host by using the proper kinds of tablet excipients (Maggi et al., 2000). The probiotic tablets with suitable excipients and optimum compression force were reported to ensure high stability of *Lactobacillus acidophilus* in artificial gastric juice (Stadler and Viernstein, 2003). In addition, tablets have advantages above other dosage forms. These are accurate dosage, ease of administration, good patient acceptance and suitability for large-scale production. Based on this knowledge, we investigated 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 used hydroxypropyl methylcellulose phthalate (HPMCP) as tablet forming matrix because this polymer is insoluble in gastric fluids (pH ~ 1.5) but dissolves rapidly in the upper small intestine where the pH is around ~5.5 according to the manufacturer's instructions (Shin-Etsu Chemicals Ltd., Tokyo, Japan).

2. Materials and methods

2.1. Bacterial cultures

Lactobacillus fermentum 2311 isolated from fermented leaves of tea (*Camellia sinensis* Linn.) was used as the model probiotic bacteria in described experiments. Cultures of *L. fermentum* 2311 were grown in MRS broth (Merck, Damstadt, Germany) at 37 °C under anaerobic conditions. Cultures were harvested at the beginning of stationary phase and collected by centrifugation. The harvested cells were suspended in 10% skimmed milk solution. The cultures were then frozen at −20 °C for about 12 h and subsequently freeze-dried by a freeze dryer model Christ 1–4 (Chirst; Osterode, Germany) for 24 h. The lyophilized probiotic bacteria (LAB) were carefully ground into fine powders and stored at 4 °C in closed containers for further experiments in tableting process. The number of probiotic cells in LAB powder was between 10^{10} and 10^{11} CFU/g.

2.2. Chemicals

Hydroxypropyl methylcellulose (Metolose®) and hydroxypropyl methylcellulose phthalate (Hypromellose Phthalate; HPMCP 55) were purchased from Shin-Etsu Chemicals Ltd. (Tokyo, Japan), sodium alginate was from Fluka (Buchs, Switzerland), and apple pectin from Roth-Lactan (Graz, Austria). Magnesium stearate and talcum were supplied by Kwizda (Vienna, Austria). Hydrochloric acid (37%) was from Merck (Darmstadt, Germany). Other solvents were of analytical grade and chemicals were of the highest grade available.

2.3. Tablet preparation

Tablets were prepared by direct compression using a single punch tablet press (Korsch EKO, Berlin, Germany) connected to a computerized compression force analyzer, under constant environmental conditions (35% RH, 20–22 °C). An exactly weighed quantity of powder mixture containing LAB powder and HPMCP was filled into a die of 10 mm diameter and under a determined pressure ranging from 2 to 20 kN tablets with a plane surface were formed. The powder contained also a suitable amount of magnesium stearate as lubricant and talcum as antiadherant (0.1% and 0.9%, w/w, respectively). Sodium alginate, apple pectin and Metolose® were used as swelling agents replacing partly HPMCP. Tablet formulations, investigated in this study are summarized in Table 1.

2.4. Test of bacterial viability in tablets

2.4.1. Exposure of tablets to a test medium

According to the methods described by Chan and Zhang (2005) with some modification, the test tablets were transferred into 600 ml of 0.04N hydrochloric acid (pH 1.5) or phosphate buffer (PBS pH 6.8: K_2HPO_4 3.4 g/l; Na_2HPO_4 3.53 g/l). The USP paddle method for dissolution testing was applied by using a paddle speed of 100 rpm at 37 °C. After the end of the incubation period, the medium was removed and the viable cells inside the non-disintegrated tested tablets were determined.

2.4.2. Viability assay of cells inside the tablet

According to the method of Ferreira et al. (2005) with some modification, each tablet was broken and dispersed in 600 ml of phosphate buffer (PBS, pH 6.8). A serial dilution of this suspension was made until a suitable cell density was obtained. The cell suspension was then spread onto the pre-dried MRS agar (Merck, Darmstadt, Germany) plates. The plates were then incubated at 37 °C for 48 h. This plating procedure was carried out in triplicates. 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

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

2.5. Tablet evaluation

The probiotic tablets obtained were evaluated for their disintegration, tensile strength and friability according to USP. Disintegration of the tablets was examined by means of a disintegration apparatus (PharmaTest PTZ-AUTO). The tablets were placed separately in the test chamber, and then immersed in PBS pH 6.8 as the disintegration medium at 37 °C for 5 h. The tablet mechanical strength was determined by using PharmaTest PTB 311 tester. 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 (Kiekens et al., 2000). The tablet friability was measured by using a friabilator (PharmaTest PTF E).

2.6. Stability of probiotic tablets

For stability testing the tablets were kept in tight light resistant containers at 10 and 30 °C for 6 months. The stability of bacterial

Table 1
Probiotic tablet formulations.

Formulation no.	Compression force (kN)	Composition (mg)					Final weight (mg)
		LAB	HPMCP 55	Alginate	Pectin	Metolose®	
1	5	25	200	–	–	–	227
2	5	33	192	–	–	–	227
3	5	50	175	–	–	–	227
4	5	75	150	–	–	–	227
5	5	100	125	–	–	–	227
6	20	100	40	–	–	–	141
7	20	100	55	–	–	–	156
8	20	100	70	–	–	–	171
9	20	100	85	–	–	–	186
10	20	100	100	–	–	–	202
11	2	100	125	–	–	–	227
12	10	100	125	–	–	–	227
13	20	100	125	–	–	–	227
14	2	100	100	–	–	–	202
15	5	100	100	–	–	–	202
16	10	100	100	–	–	–	202
17	5	100	70	–	–	–	171
18	5	100	42	28	–	–	171
19	5	100	42	–	28	–	171
20	5	100	42	–	–	28	171

cells in terms of cell viability in the tablet along the storage period was investigated monthly. The method used to determine cell viability was the plating procedure described above under Section 2.4.2.

2.7. Data analysis

Values are expressed as mean ± standard deviation. The data were analyzed with the statistical software Statgraphics plus 3.0. One-way analysis of variance (ANOVA) and multiple range tests (Fisher's least significant difference procedure, LSD) were used to determine whether or not data were significantly different. The level of confidence was set to be 95%.

3. Results and discussion

The effects of tablet formulation such as concentration of probiotic cells and tablet excipients (e.g. polymer matrix and swelling agents) as well as tablet processing conditions, such as compression force, 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 conditions for tablet storage.

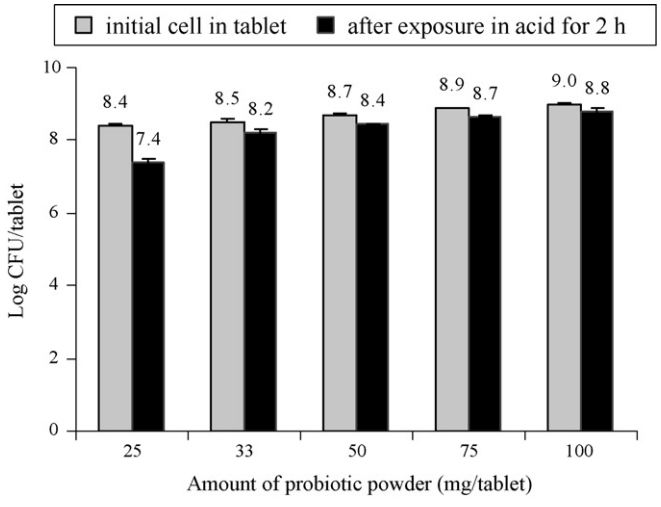

Fig. 1. Effect of probiotic concentration in tablets on cell viability ($n = 3$).

3.1. Effect of probiotic bacteria/polymer ratio on bacterial survival in tablets

Tablets with different ratios of bacteria to HPMCP 55 (formulation nos. 1–5) were prepared with a compression force of 5 kN. Bacterial survival inside the tablets after exposure to an acidic medium of pH 1.5 for 2 h was investigated according to Blanquet et al. (2004). The pH of the stomach is acid (pH 1.5–5.5) and transient time is about 2 h. Vizoso Pinto et al. (2006) showed that the viability of *L. johnsonii* LA1 in an artificial gastric electrolyte solution containing lysozyme and pepsin at pH 2.5 was 87.5% whereas the viability was <70% at pH 2.5 without the enzymes. This we took to indicate that pH had a stronger bactericidal effect than the enzymes. Therefore in our study, we did not add enzymes to the gastric mimicking solutions. As seen in Fig. 1, the percentage of surviving cells in the tablets was higher when the quantity of the bacteria in the tablet was increased ($P < 0.05$). Possibly, at higher bacterial loading the bacteria are better protected against outer stresses due to cell–cell interactions. Additionally, the polymer might adversely interact with the bacterial cell surface causing cell destruction. This obviously occurred in the tablets with low concentration of bacterial cells which in turn had high polymer content, so that lower cell viability was obtained. The tensile strength of the tablets after punching and before immersing into the acidic medium was determined. It was found that the tablets obtained exhibited tensile strengths of $0.42 \pm 0.09\ N/mm^2$ to $1.23 \pm 0.19\ N/mm^2$ when the polymer content was increased from 55% (w/w) to 89% (w/w) (Table 2). According to these results, the amount of 100 mg of probiotic powder which showed highest cell survival was used for further studies. The same trend was observed when the tablets were prepared at higher compression force (20 kN) while the amount of bacteria in each tablet was fixed at 100 mg/tablet (formulation nos. 6–10) but the percentage of HPMCP 55 was varied as shown in Table 1. It was found that the tensile strength of the tablets increased from $1.71 \pm 0.19\ N/mm^2$ to $2.75 \pm 0.29\ N/mm^2$ ($p < 0.05$) when the polymer content was increased from 28% (w/w) to 50% (w/w) as shown in Table 3. The results also indicate that microorganisms in tablets prepared with higher tensile strength, which resulted from a higher compression force, show slightly increased

Table 2
Probiotic survival and tensile strength of probiotic tablets prepared with a compression force of 5 kN (formulation 1–5 from Table 1 content of probiotic and polymer was varied but tablet total weight was fixed).

Polymer content (mg)	Survival of LAB (%) in the tablets after 2 h incubation in 0.04 N HCl*	Tensile strength** (N/mm^2)
200	20.5 ± 3.5^{d}	1.23 ± 0.19^{a}
192	49.8 ± 2.9^{c}	0.97 ± 0.14^{b}
175	56.1 ± 2.8^{c}	0.87 ± 0.08^{c}
150	62.3 ± 2.9^{b}	0.60 ± 0.13^{d}
125	85.2 ± 2.4^{a}	0.42 ± 0.09^{e}

Values within a column with different superscript are significantly ($P < 0.05$).
* Mean ± SD ($n = 3$).
** Mean ± SD ($n = 10$).

survival rates after incubation in acidic medium. This effect of tensile strength or compression force on the cell survival in the medium is considered to be due to some advantage properties of tablets obtained from a higher compression force. To clarify this, the influence of compression force on tablet property was further investigated.

3.2. Influence of compression force on cell survival and tablet properties

In this study, tablets each containing 100 mg bacteria and 125 mg HPMCP 55 prepared by using different compression forces of 2, 5, 10 or 20 kN (formulation nos. 5, 11, 12 and 13) were firstly subjected to the acidic medium (0.04 N HCl, pH 1.5) for a period of 2 h then the medium was changed to PBS pH 6.8. It was found that most tablets showed no disintegration after 2 h in the gastric mimicking acidic medium. The viability of bacterial cells inside the non-disintegrated tablets after acidic immersion was then determined. It was shown that cell survival in the tablets prepared at a compression force higher than 5 kN was substantially higher than that in tablets prepared with 2 kN (80% and 50%, for a compression force of 5 and 2 kN, respectively) as shown in Fig. 2. The non-disintegrated tablets from the acidic medium demonstrated only some extent of disintegration in higher pH medium. Results indicated that in PBS (after 2 h in acidic medium) the tablets manufactured with high compression force, particularly higher than 5 kN, hardly disintegrated in a period of 1 h while those obtained from 2 kN disintegrated in less than 30 min as shown in Fig. 3. The effect of compression force on tensile strength of the probiotic tablets was also investigated. In this study, tablet formulation nos. 10, 14, 15 and 16 were used. It was found that the tensile strength of the tablets was increased when the compression force was increased from 2 to 20 kN, as shown in Table 4. Increase in compression force significantly increased the tensile strength ($P < 0.05$). In addition, the increase of compression force from 5 to 10 kN had no significant

Table 3
Probiotic survival and tensile strength of probiotic tablets prepared with a compression force of 20 kN (formulation 6–10 from Table 1 content of probiotic was fixed but content of polymer was varied).

Polymer content (mg)	Survival of LAB (%) in the tablets after 2 h incubation in 0.04 N HCl*	Tensile strength** (N/mm^2)
40	84.4 ± 4.0^{b}	1.71 ± 0.19^{d}
55	85.9 ± 2.2^{b}	2.43 ± 0.24^{c}
70	87.4 ± 1.8^{ab}	2.63 ± 0.45^{c}
85	86.3 ± 2.9^{ab}	2.70 ± 0.28^{b}
100	89.3 ± 3.0^{a}	2.75 ± 0.29^{a}

Values within a column with different superscript are significantly ($P < 0.05$).
* Mean ± SD ($n = 3$).
** Mean ± SD ($n = 10$).

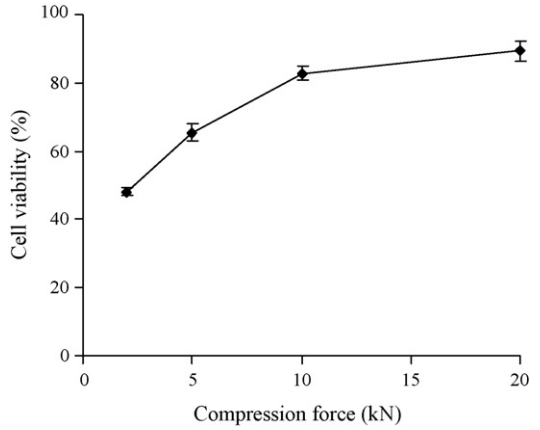

Fig. 2. Effect of the compression force on cell viability inside the probiotic tablets ($n = 3$).

effect on the survival rate of LAB under experimental condition. Increasing the compression force to 20 kN improved the tablet efficacy in protecting bacterial cells against acidic challenge. It was also noted that the probiotic tablets obtained from an extremely high compression force of 20 kN demonstrated a significant low friability (<1%). This result was in good agreement with the results of other authors (Sawicki and Łunio, 2005) which reported previously that tablets with greater hardness showed lower friability. As the cells inside these tablets showed high survival when the tablets were immersed in the medium, the friability was considered to be one of the important tablet properties to protect the cells inside the tablet from the contact fluid. The hardness is also essential for protection of tablets from friableness, particularly during the process of handling. The results indicated that HPMCP 55 as tablet excipient with sufficient compression force allows the preparation of probiotic tablets with suitable properties in terms of hardness and friability. Our results demonstrate that the probiotic tablets with a high tensile strength, a low friability and long disintegration time gave the highest probiotic cell viability.

3.3. Effect of swelling agents on bacterial survival and tablet properties

To increase gastric juice resistance of tablets rapidly disintegrating in the intestine, tablets prepared by a low compression force (5 kN) and low amounts of HPMCP 55 and additional swelling agents were considered for further experiments. The polymer con-

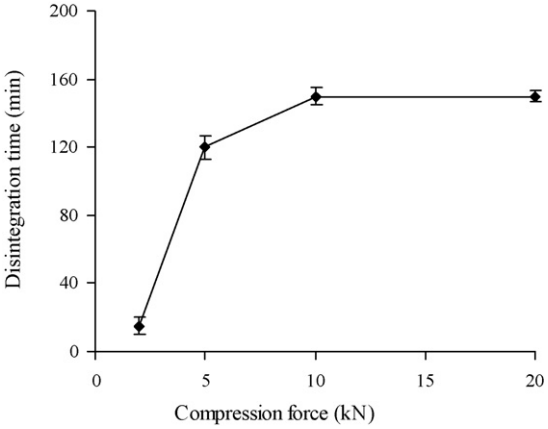

Fig. 3. Effect of the compression force on disintegration time of the probiotic tablets ($n = 6$).

Table 4
Effect of compression force on tablet properties and probiotic survival.

Compression force (kN)	Tensile strength* (N/mm²)	Survival of LAB (%) in tablets after 2 h incubation in 0.04 N HCl**	Friability
2	0.09 ± 0.02[d]	–***	>1%
5	0.26 ± 0.01[c]	82.3 ± 1.4[b]	<1%
10	0.98 ± 0.04[b]	82.8 ± 2.0[b]	<1%
20	2.75 ± 0.29[a]	89.3 ± 2.5[a]	<1%

Values within a column with different superscript are significantly ($P < 0.05$).
* Mean ± SD ($n = 10$).
** Mean ± SD ($n = 3$).
*** Whole tablet was disintegrated in acid medium.

Table 5
Effects of swelling agents on cell viability and tablet properties.

Swelling agent	Tensile strength* (N/mm²)	Survival of LAB (%) in tablets after 2 h incubation in 0.04 N HCl**	Disintegration time (h)
None**	0.42 ± 0.20[d]	72.7 ± 2.5[b]	1.25
Sodium alginate	1.38 ± 0.08[b]	90.2 ± 2.2[a]	>5***
Apple pectin	1.17 ± 0.04[c]	48.9 ± 1.0[d]	1
Metolose®	1.80 ± 0.04[a]	60.2 ± 0.9[c]	1

Values within a column with different superscript are significantly ($P < 0.05$).
* Mean ± SD ($n = 10$).
** No replacement of any swelling agent.
*** There were some small pieces sticking to the stainless sieve after 5 h of the test.

tent of 70 mg/tablet was chosen as a minimum polymer content ensuring relatively high survival rates as well as fast disintegration in artificial intestinal medium (data not shown). To investigate the influence of additional excipients on stability towards acid, the HPMCP 55 content was partially replaced (40%) with sodium alginate, apple pectin or Metolose®.

In line with expectations, the tensile strength of the tablets increased substantially from 0.42 ± 0.20 N/mm² to 1.80 ± 0.04 N/mm² as shown in Table 5, due to a different swelling agent added to the formulations (nos. 17–20, respectively). All tablets were not disintegrated in acidic medium during the period of 2 h. Furthermore, cell viability assay of the tablets after acidic incubation indicated that swelling agents provided significantly different levels ($P < 0.05$) of cell protection as seen in Table 5. Tablets with sodium alginate provided the highest cell survival whereas apple pectin or Metolose® did not show cell protection effects. Moreover, viability of the cells in tablets containing apple pectin or Metolose® was lower than in those without swelling agent. It was observed that these tablets were swelling faster in the acidic test medium than those containing sodium alginate or those without a swelling agent. This fast swelling indicates the high efficiency at water uptake of the excipient. The rapid uptake of artificial gastric juice into the tablets brings the probiotic cells in contact with the acid faster, resulting in a higher death rate of the bacteria. After 2 h immersion in acidic medium, the tablets were tested for disintegration by using PBS (pH 6.8) as a medium. It was shown that the disintegration time of the tablets with apple pectin and Metolose® was shorter than that of tablets without swelling agents (1.25 and 1 h, respectively). Our findings contrast with those of Ashford et al. (1993, 1994) who reported on the advantageous use of pectin in dry coating processes. The reasoning used by these authors was that the gelatinization of pectin diminishes the penetration of water into the dosage form. The dry coated dosage form has been reported to reach the upper colon 6–8 h after ingestion (Ashford and Fell, 1994). However, in our hands the formulation containing apple pectin showed the least stability. This agrees with the report of Zaleska et al. (2000) who concluded that apple pectin and whey protein were unsuitable for making films.

The disintegration time of the tablets containing sodium alginate was substantially longer (>5 h). However, only small pieces of less than 10% of these tablets were found to stick tightly to the stainless sieve after 5 h of the test. It was noted that more than 90% of the tablet mass passed the sieve after 5 h of incubation. More interestingly, sodium alginate showed the strongest cell protection activity with more than 90% retention of cell viability, as compared to the other formulations (cell viability 50–70%). Stadler and Viernstein (2003) reported that probiotic tablets prepared from compaction of a mixture of *L. acidophilus* La-5, hydroxypropyl methylcellulose acetate succinate and sodium alginate were the best protective formulation against artificial gastric juice. This report along with our results supported the use of sodium alginate as one of the essential excipients in probiotic tablets. In general, sodium alginate hydrocolloids show good stability between pH 3 and 10 and they are rather resistant to bacterial and enzymatic degradation. The hydrogel barrier formed around the tablet containing sodium alginate may retard the permeation of the acidic fluid into the tablets (Chan and Zhang, 2002; Calinescu et al., 2005). This could explain the observed excellent survival of bacteria in the alginate tablets after exposure to pH 1.5 for 2 h. Moreover, the transient time of the ingested food from the stomach to the intestinal tract is about 2–6 h (Blanquet et al., 2004). Therefore, the tablets should ideally protect probiotic bacteria until this time and then release viable bacteria in the intestinal tract. In this study, the tablets with sodium alginate as swelling agent showed the highest retention of cell viability with an acceptable disintegration time (data were shown in Fig. 4). Based on these data, it is suggested that probiotic bacteria in tablets containing sodium alginate can multiply with no significance during

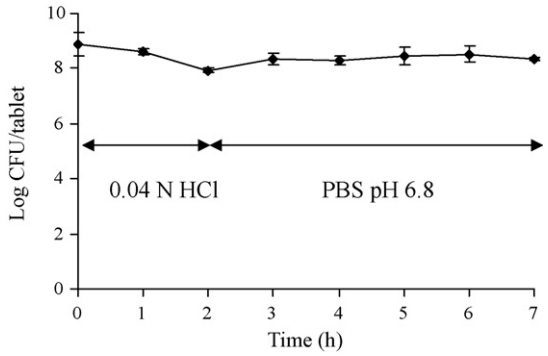

Fig. 4. Cell viability inside the HPMCP 55 – sodium alginate-based probiotic tablets after immersing in fluid media ($n = 3$) at two pH-values.

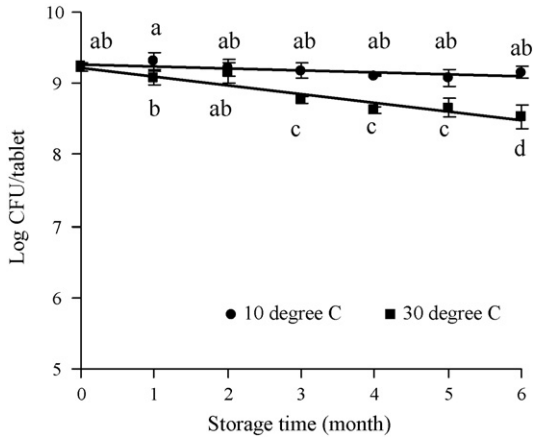

Fig. 5. Cell viability inside the HPMCP 55 – sodium alginate-based probiotic tablets after storage at 10 and 30 °C ($n = 6$). Values with different superscripts are significantly ($P < 0.05$) different and the levels containing a group of means within which there are no statistically significant differences.

incubation in intestinal fluid (pH 6.8). In addition, acceptable values of friability (≤1%) were obtained for all batches of probiotic tablets with sodium alginate, indicating good mechanical properties. The HPMCP 55-based probiotic tablet formulation with the addition of sodium alginate was considered to be the best formulation and selected for stability testing during storage.

3.4. 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. As the probiotic tablet formulation containing HPMCP 55 and sodium alginate showed the best property in terms of cell protection in acidic media and suitable disintegration in artificial intestinal fluid condition as well as acceptable friability, this formulation was subjected to stability testing. In this study, two different temperatures (10 and 30 °C) were selected to evaluate the stability of the tablets. The selected temperatures represent the common cool storage as in a household refrigerator and ambient room temperature, respectively.

It was found that storage at 30 °C caused significant decreases in cell viability ($P < 0.05$). After 6 months, the loss of viability was observed to be less than 1 log unit, as shown in Fig. 5. Importantly, however, no significant decrease in viability was observed during storage of the tablets at 10 °C for 6 months ($P < 0.05$). It has been reported that the storage stability of freeze-dried *Lactococcus lactis* encapsulated in a calcium alginate matrix was higher than that of the freeze-dried free cells (Champagne et al., 1992). Chan and Zhang (2002) developed tablets with a core consisting of *L. acidophilus* ATCC 4356 cells surrounded with sodium alginate and claimed that they had 10 times higher stability than the free cell powders after 30 days storage at 25 °C. The results in our study indicated the effect of temperature on viability of the lyophilized probiotic cells distributed homogeneously inside the tablets. The 20 °C difference of 30 to 10 °C affected the cell stability to the extent of about 1 log CFU.

4. Conclusion

This 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. Importantly, the storage stability of the tablets is very good with almost full preservation of the number of viable cells when the tablets were stored at 10 °C for 6 months. These results open the possibility to treat patients who might benefit from probiotic therapy with tablet formulations.

Acknowledgement

Authors are grateful to the Thailand Research Fund for financial support through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0152/2546).

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