Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 256 (2003) 117–122
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Optimization of a formulation containing viable lactic acid bacteria

M. Stadler, H. Viernstein*

Institute of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Received 20 July 2002; received in revised form 22 November 2002; accepted 15 December 2002

Abstract

In the present study, gastric juice resistant tablet formulations of lactic acid bacteria (LAB) were developed, using hydroxypropylmethylcellulose acetate succinate (HPMCAS) as well as alginates, apple pectin and Metolose® as matrix forming components. To optimize the formulation—using survival rate in acid medium, and disintegration time in intestinal fluid as test parameters—tablets were modified with respect to LAB content, amount of applied excipients per tablet, and compaction forces. A decrease of viable cells of not more than one log unit after 2 h of incubation in acid medium was desired, as well as a disintegration time of 1 h in phosphate buffer pH 6.8.

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.

Keywords: Gastric juice resistant tablets; Lactic acid bacteria; Formulation

1. Introduction

At the beginning of the 20th century, Eli Metchnikoff (Metchnikoff, 1907) proposed consumption of milk fermented with a flora of human intestinal origin, such as *Lactobacilli*, and claimed that the proverbial longevity of Bulgarians resulted from the consumption of such milks. The organism he recommended was *Bacillus bulgaricus*, which was later identified as *Bacillus acidophilus*. More recently, the name of this organism was changed to *Lactobacillus acidophilus* (Gilliland, 1980).

During the past decade there has been increasing interest in the hypothesis that the long-term consumption of a healthy diet may reduce an individual's risk of certain age-related disorders, including obesity, osteoporosis, hypertension and some cancers. Consumers have attempted to identify certain products as being protective with regard to the food's impact on their overall diet and health (Halpern et al., 1991). The use of the term "probiotic" to describe food supplements specifically designed to improve health, however, dates from 1974 when Parker used it to describe growth promoting feed supplements. He defined the term as "organisms and substances which contribute to intestinal microbial balance" (Fuller, 1991). The resident flora has an important protective function. Any potential pathogen or new colonizer must overcome the nonspecific chemical and physical defense mech-

* Corresponding author. Tel.: +43-1-4277-55402; fax: +43-1-4277-9554.
E-mail address: helmut.viernstein@univie.ac.at (H. Viernstein).

0378-5173/03/$ – see front matter
doi:10.1016/S0378-5173(03)00068-1

anisms of the gastrointestinal tract, such as gastric acidity and peristaltic gut movements (Salminen and Deighton, 1992). Although the gut flora is relatively stable, it may be influenced by several dietary and environmental factors. Preparations containing viable LAB of human origin appear to have value in restoring normal microbial function and alleviating symptoms in some patients suffering from gastrointestinal infections and other conditions. Besides the control of intestinal infections, LAB impart further nutritional and therapeutic benefits. Among these are improved digestion of lactose (Marteau et al., 1993; Hove et al., 1999), control of some types of cancer (Goldin, 1998; Aso et al., 1995) and control of serum cholesterol levels (De Ross et al., 1999, Anderson and Gilliland, 1999).

A prerequisite for any effect of ingested bacteria is a successful implantation in the gastrointestinal tract. So bacteria must remain viable during gastric transit. Based on the acid instability of LAB it is essential to consume these microbes with food or to protect them by encapsulation.

2. Methods

Lyophilized batches of the lactic acid bacteria strain (LAB) *Lactobacillus acidophilus* La-5 were supplied by Chr. Hansen BioSystem's (Horsholm, Denmark), HPMCAS by Shin-Etsu Chemicals Ltd. (Tokyo, Japan). Calcium alginate, sodium alginate, and Avicel® were purchased from Fluka (Buchs, Switzerland), pectin (apple pectin, food grade) from Roth-Lactan (Graz, Austria). Magnesium stearate and talcum were supplied by F.Joh. Kwizda (Vienna, Austria).

Tablet processing took place on an instrumented single punch tablet press (Korsch EKO, Berlin, Germany), providing constant environmental conditions (35% RH; 20–22 °C). All tablets were compacted biplan with a diameter of 10 mm.

Degradation of lyophilized viable LAB cells during the formulation process and tablet preparation as well as the survival in artificial gastric juice were monitored using a standard plate count technique (Mersch-Sundermann, 1998).

The loss of bacteria due to tabletting was evaluated by calculation from numbers of viable cells in the mixed powders prior to compaction and in the tablets made thereof.

Artificial gastric juice (0.04 M HCl) was studied to evaluate resistance against acidic conditions at a temperature of 37 °C for 1 or 2 h of incubation, using a dissolution tester (apparatus 2, USP XXIV). For evaluation of the gastric juice resistance, tablets were incubated in 0.04 M HCl at 37 °C for 1 or 2 h and the differences of the microbial cell counts before and after treatment were determined.

Tablet disintegration time was studied using phosphate buffer (pH 6.8; 37 °C). Tablet friability was tested with a friabilator (Pharma-Test; Type PTFE), tablet hardness with Pharma-Test PTB 311.

Storage stability of tablet formulations was examined at three different temperatures (10, 20, and 30 °C) during a period of 6 months.

Table 1 gives an overview of all examined tablet formulations; tablets contained 1% talcum and magnesium stearate as lubricants. Formulations Nos. 6–53 contained 100 mg of LAB.

Formulations Nos. 1–5 were prepared with a constant tablet weight (227.2 mg), but different amounts of LAB (25, 33.3, 50, 75, 100 mg) and of HPMCAS (200, 192, 175, 150, 125 mg) to investigate the influence of different ratios of LAB to HPMCAS on the gastric juice resistance.

To examine the influence of different amounts of HPMCAS on the survival rate of LAB during gastric transit, formulations Nos. 6–11 were manufactured

Table 1
Overview of all examined tablets (Nos. 1–53)

Nos.	kN	HPMC	Na-alg	Ca-alg	Pectin	Metolose
1–5	5	×				
6–11	20	×				
12–17	10	×				
18–23	5	×				
24–29	2	×				
30–32	5	×	×			
33–35	2	×	×			
36–38	5	×		×		
39–41	2	×		×		
42–44	5	×			×	
45–47	2	×			×	
48–50	5	×				×
51–53	2	×				×

kN, compaction force during the tabletting process; Na-alg, sodium alginate; Ca-alg, calcium alginate.

applying HPMCAS amounts of 40, 55, 70, 85, 100 mg as well as 125 mg, at a constant compaction force and with a constant amount of LAB.

The influence of different compaction forces (10, 5, 2 kN) on gastric juice resistance was studied by analysis of tablets Nos. 12–29.

Tablets Nos. 30–53 were manufactured to investigate the influence of sodium alginate, calcium alginate, apple pectin and Metolose® on the survival rate in acid medium by replacing part of the HPMCAS content (40, 55, 70 mg) with each of these additional excipients, using two different compaction forces (5 and 2 kN).

3. Results and discussion

The results of the analysis of formulations Nos. 1–5 (applying different HPMCAS amounts and different LAB contents) show that tablets containing high amounts of HPMCAS (200 mg) and small amounts of LAB (25 mg) surprisingly sustain nearly the same loss of viable cells during incubation in gastric juice as tablets containing much lower amounts of the gastric juice resistant matrix component HPMCAS (125 mg) and a high LAB content (100 mg). This illustrates that the ratio between these two test variables only slightly influences the survival rate in acid medium, when a constant tablet weight is used.

In further tests, tablets with 100 mg of LAB were manufactured to reach high microbial cell counts per tablet (10^9–10^{10} CFU/tablet, depending on the compaction force applied).

The examination of formulations Nos. 6–11 (prepared with different HPMCAS amounts and a constant compaction force) demonstrated that the amount of HPMCAS had great influences on both the gastric juice resistance and the disintegration time in the intestinal fluid. The higher the HPMCAS amount, the better the gastric juice resistance; however, a high HPMCAS amount (a high tablet weight) resulted in slow disintegration in the intestinal fluid.

Fig. 1 shows the decrease of the gastric juice resistance by lowering the HPMCAS amount per tablet, while Fig. 2 illustrates the acceleration of the disintegration time in the intestinal fluid by lowering this parameter. The formulation with the lowest amount of HPMCAS (No. 6) required at least 65 min for disintegration.

To shorten the disintegration time in intestinal fluid, tablets (Nos. 12–29) were prepared using lower compaction forces (10, 5, and 2 kN).

The results of these experiments (tablets containing different HPMCAS contents and different compaction forces) indicate the need of both, a low amount of HPMCAS (<85 mg) and a low compaction force (<10 kN) to achieve disintegration in the intestinal fluid within 1 h. For attaining tablets with high gastric

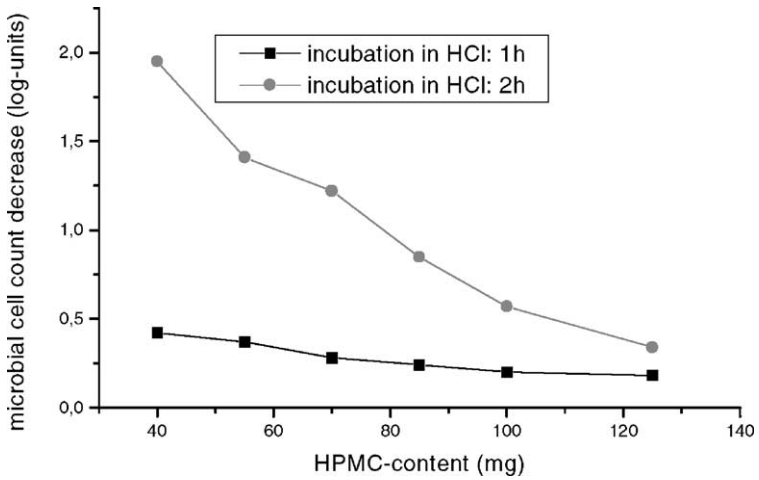

Fig. 1. Correlation between gastric juice resistance and HPMCAS content (dissolution tester, 0.04N HCl/37 °C).

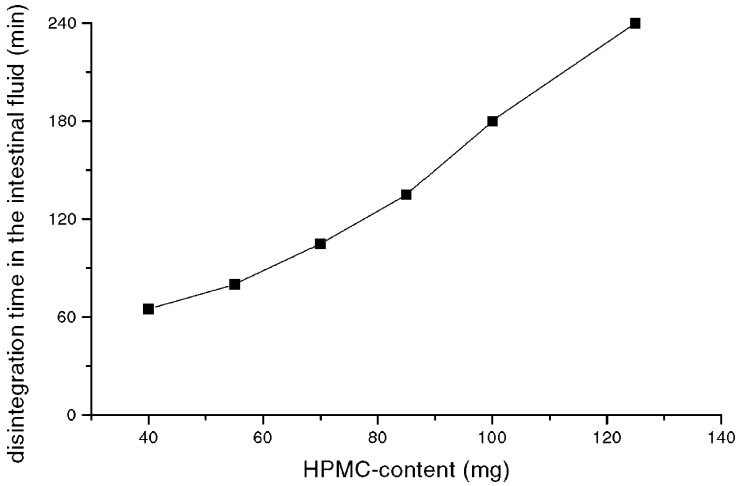

Fig. 2. Dependence of the disintegration time in the intestinal fluid upon the HPMCAS content (dissolution tester/phosphate buffer pH 6.8/37 °C).

juice resistant qualities, a high amount of HPMCAS is needed, especially when tablets are compacted by low compaction forces.

Due to the countervailing effects (good gastric juice resistance being accompanied by slow disintegration time in phosphate buffer pH 6.8), none of the tablet formulations manufactured so far (Nos. 1–29) meet the combined requirements of loss of only one log unit of viable cells after 2 h of incubation, and disintegration within 1 h in simulated intestinal fluid.

To increase gastric juice resistance of tablets disintegrating within less than 1 h in the intestine, tablets compacted by a low compaction force (5 and 2 kN) and low amounts of HPMCAS (40 g, 55, and 70 mg) were most suitable for further experiments. To obtain better acid stability the HPMCAS content was partially replaced (40%) with sodium alginate, calcium alginate, apple pectin or Metolose®. As seen in Figs. 3 and 4, replacement of part of the HPMCAS with sodium alginate had a positive effect on gastric juice resistance. Tablets prepared by a compaction force of 2 kN and containing a very low amount of HPMCAS (40 mg at a tablet weight of 141 mg) lose 6 log units of viable cells after incubation for 2 h in 0.04 M HCl. On the other hand a cell count reduction of about one log unit was achieved by replacing 24 mg of the HPMCAS amount with sodium alginate.

Addition of calcium alginate also increased resistance against artificial gastric juice, while apple pectin and Metolose® did not increase survival rates in acid medium.

The results indicate that optimal gastric juice resistance can be achieved with tablets containing HPMCAS and sodium alginate as excipients.

Formulations Nos. 31, 32, 34 and 35 are to a large extent gastric juice resistant and disintegrate within less than 1 h in the intestine. These are considered the

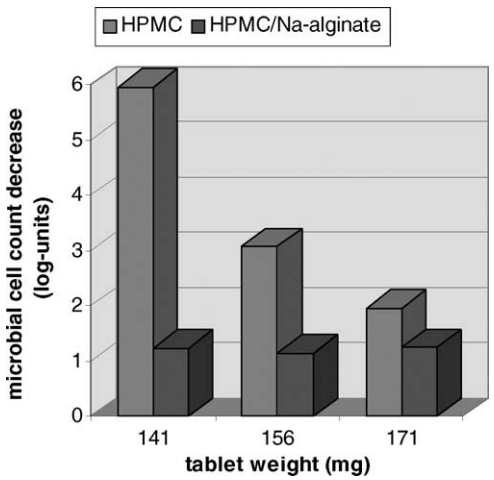

Fig. 3. Comparison of gastric juice resistance after an incubation for 2 h of tablets containing different excipients (compaction force: 2 kN). Dissolution tester/0.04N HCl/37 °C.

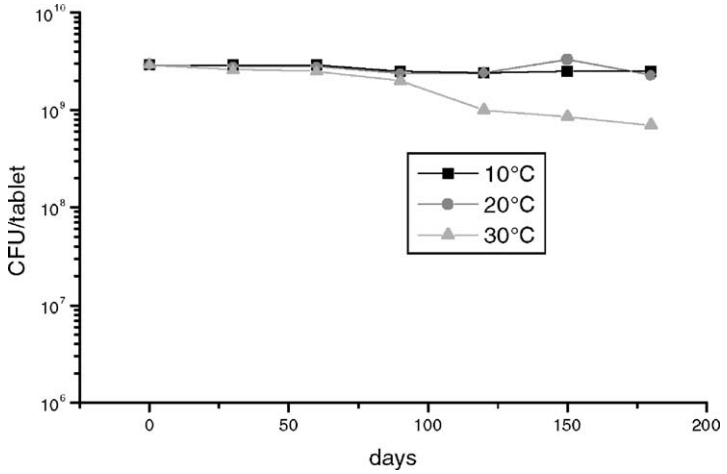

Fig. 4. Storage stability of tablet No. 35 (HPMCAS: 33 mg; Na-alginate: 22 mg; compaction force: 5 kN).

best formulations and are recommended for application.

For further characterization, tablet hardness, friability, the survival rate in intestinal fluid as well as storage stability of one of the optimized formulations (No. 31) have been tested.

A friability <1% and an average tablet hardness of 26 N was measured.

Concerning the incubation in intestinal fluid (incubation time: 6 h), no significant microbial cell count reduction was observed.

Storage at a temperature of 10 or 20 °C for 6 months 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.

4. Conclusion

In the present study, the preparation was demonstrated of gastric juice resistant tablets, that enable the delivery of approximately 10^8 viable bacteria to the intestine within 1 h.

For aiming acid stability, the ratio of LAB to HPMCAS was not decisive when a constant tablet weight was used. Using a constant amount of LAB and varying the amounts of HPMCAS and the compaction force, it was found that a high content of HPMCAS as well as a medium or high compaction force are needed to achieve gastric juice resistance. When tablets are to be prepared with low amounts of HPMCAS, the addition of sodium alginate is essential to guarantee protective qualities against artificial gastric juice.

Acknowledgements

The author would like to thank Chr. Hansen A/S, Denmark, for the kind supply of lactic acid bacteria.

References

Anderson, J.W., Gilliland, S.E., 1999. Effect of fermented milk (yokurt) containing *Lactobacillus acidophilus* L1 on serum cholesterol in hypercholesterolic humans. J. Am. Coll. Nutr. 18, 43–50.

Aso, Y., Akazan, H., Kotake, T., Tsukamoto, T., Imai, K., Maito, S., 1995. Preventive effect of *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer in a double-blind trial. Eur. Urol. 27, 104–109.

De Ross, N.M., Schouten, G., Katan, M.B., 1999. Yoghurt enriched with *Lactobacillus acidophilus* does not lower blood lipids in healthy men and women with normal to borderline high serum cholesterol levels. Eur. J. Clin. Nutr. 53, 277–280.

Fuller, R., 1991. Probiotics in human medicines. Gut 32, 439–442.

Gilliland, S.E., 1980. Beneficial interrelationships between certain microorganisms and humans: candidate microorganisms for use as dietary adjuncts. J. Food Prot. 42, 164–167.

Goldin, B.R., 1998. Health benefits of probiotics. Br. J. Nutr. 80, 203–207.

Halpern, G.M., Vruwink, K.G., Van de Water, J., Keen, C.L., Gershwin, M.E., 1991. Influence of long-term yoghurt consumption in young adults. Int. J. Immunotherapy 7, 205–210.

Hove, H., Norgaard, H., Mortensen, P.B., 1999. Lactic acid bacteria and the human gastrointestinal tract. Eur. J. Clin. Nutr. 53, 339–350.

Marteau, P., Pochart, P., Bouhnik, Y., 1993. Fate and effects of some transiting microorganisms in the human gastrointestinal tract. Wild. Rev. Nutr. Dietetics 74, 1–21.

Mersch-Sundermann, V., 1998. Medizinische Mikrobiologie. Georg Thieme Verlag, Stuttgart.

Metchnikoff, E., 1907. The Prolongation of Life. William Heinemann, London.

Salminen, S., Deighton, M., 1992. Lactic acid bacteria in the gut in normal and disordered states. Dig. Dis. 10, 227–238.