

## Enteric coating of granules containing the probiotic *Lactobacillus acidophilus*

HASSAN PYAR  
KOK-KHIANG PEH\*

School of Pharmaceutical Sciences  
Universiti Sains Malaysia, 11800 Minden  
Penang, Malaysia

In the present study, a capsule formulation composed of enteric coated granules of *Lactobacillus acidophilus* ATCC 4962 was developed using Eudragit L30D-55 as enteric polymer. Optimization of the capsule formulation was achieved with a maximum viable cell count after 2 h of incubation in acid medium and disintegration time of 1 h in buffer pH 6.8. The amount of Eudragit L30D-55 in the capsules correlated with gastric juice resistance. The best protective qualities against artificial gastric juice were observed when capsules were prepared from granules composed of *L. acidophilus*, corn starch, lactose monohydrate, polyvinylpyrrolidone and coated with 12.5 % (m/V) of Eudragit L30D-55. Capsule formulation of *L. acidophilus* in edible broth medium suspension serves as a cheap alternative to the expensive freeze-drying procedure for preparing *L. acidophilus*. In addition, the enteric coating using Eudragit L30D-55 could protect probiotics from the acidic gastric environment and enhance the bioactivity of probiotics along with replacement of pathogenic microbes in human intestine.

**Keywords:** *Lactobacillus acidophilus*, Eudragit L30D-55, enteric coating, probiotic, viable cells

Accepted December 12, 2013

Uses of *Lactobacillus* species have attracted much attention as probiotics due to their natural occurrence in the human gastrointestinal system. As a probiotic, *Lactobacillus acidophilus* is important in the dairy and nutraceutical industries due to its application in the maintenance of human and animal health. Probiotics are live microbial supplements that provide many benefits to human health such as favorably alter the intestinal micro-flora balance, inhibit the growth of harmful bacteria, promote good digestion, boost immune function, prevent and treat atopic and allergic diseases, lower blood cholesterol levels, exert anticancer effects, prevent symptoms of lactose intolerance and increase resistance to infection (1, 2). Probiotic bacteria maintain a healthy balance of intestinal flora by producing compounds such as organic acids and hydrogen peroxide that increase in-

---

\* Correspondence; e-mail: kkpeh@usm.my

testinal acidity and inhibit reproduction of numerous harmful bacteria. In addition, they also produce substances called bacteriocins, which act as natural antibiotics destroying undesirable microorganisms (3).

Wet granulation is a size enlargement process in which a liquid is used to achieve agglomeration of solid particles in a formulation (4). This granulation method affects the physical properties of granules, such as size, hardness, density and porosity. This also influences the reproducibility of preparations by affecting granular flowability, compressibility of powders, disintegration time and dissolution rate (5). Granulation parameters must be controlled to ensure the manufacture of granules with the desired particle size (6). Further, enteric coating is aimed at protecting the formulations from gastric fluid in the stomach and consequent release in the intestinal region (7). Probiotics have been prepared as dietary supplements in the form of tablets, capsules and freeze-dried preparations (8), but the expense of freeze-drying is a distinct disadvantage. Use of edible medium would bypass the use of the freeze-drying procedure (which is expensive) and allow direct mixing of the bacteria and metabolites to form granules. In this research, polymers were used with an intention to protect *L. acidophilus* ATCC 4962 against the harmful effects of gastric acidity and in order to ensure its viability and safe release at the target, *i.e.*, intestine. Hence, the main objective was to evaluate the suitability of available coating polymers for enteric-coating of granules containing *L. acidophilus* ATCC 4962.

## EXPERIMENTAL

### *Bacterial culture and growth conditions*

*Lactobacillus acidophilus* ATCC 4962 was purchased from the American Type Culture Collection (USA). The culture was stabbed in De-Man Rogosa Sharpe Agar (MRSA) medium (Difco, USA) and stored at 4 °C. The strain was activated by two subcultures in 100 mL MRS broth prior to experimental use.

### *Cultivation of L. acidophilus in edible broth medium with skim milk*

Food-grade broth medium was prepared according to Pyar and Peh (9). The food-grade broth medium (per liter of distilled water) contained 5.0 g peptone water (Oxoid, UK), 5.0 g yeast extract (Fluka, Germany), 0.5 g bile salts (Sigma-Aldrich, Germany), 0.5 g L-cystine HCl (Fluka, Switzerland), 2.0 g NaHCO<sub>3</sub> (R & M Chemicals, UK), 0.1 g NaCl (Sigma-Aldrich, USA), 0.01 g CaCl<sub>2</sub>·2H<sub>2</sub>O (Merck, Germany), 0.01 g MgSO<sub>4</sub> × 7 H<sub>2</sub>O (AJAX Chemicals, Australia), 0.04 g K<sub>2</sub>HPO<sub>4</sub> (Merck, Germany) and 20.0 g glucose (R & M Chemicals, UK). The medium was prepared by suspending all ingredients in 1.0 L of distilled water and boiling it until the ingredients were completely dissolved. The pH of the mixture was adjusted with 1 mol L<sup>-1</sup> HCl to 6.0 ± 0.1. The mixture was autoclaved at 121 °C for 15 min and cooled to 45 °C prior to use. Skim milk powder (Sunlac, New Zealand), at concentrations of 0.5, 0.8, 1.0, 1.2, 1.6, 1.8, 2.0, 2.2, 2.5, 2.7 and 3.0 % (*m/V*), was added to edible broth medium. *L. acidophilus* ATCC 4962 was cultivated for 36 h at 37 °C

at an agitation speed of 100 rpm. A 1 mL sample was taken from each flask, followed by serial dilution before the number of colonies was counted using the pour plate method. The plates were then incubated at 37 °C for 72 h and viable counts of each concentration were obtained.

### *Preparation of L. acidophilus granules in edible broth medium with skim milk*

Microcrystalline cellulose (Asahi Chemical Industry, Japan), lactose monohydrate impalpable grade (HMS, Holland) and corn starch (Euro Chemo, China) were first sieved through a 300 µm diameter sieve (Endecotts, UK) to break up lumps. 2 % (*m/m*) povidone water solution (ISP Technology, USA) was added as a binder. All these materials were blended in a planetary mixer (Kenwood, UK) for 5 min. Three different volumes of edible broth medium containing *L. acidophilus* ATCC 4962 and 2.0 % (*m/V*) skim milk were mixed with the powder mass for 10 min. Different formulations of *L. acidophilus* ATCC 4962 granules were prepared as shown in Table I. The wet mass was screened through a 1.70 mm diameter sieve (Endecotts, UK) and dried in an oven (Memert, Germany) at  $38 \pm 2$  °C for about 18 h until constant weight was reached. Dried granules were screened through a 1.00 mm diameter sieve (Endecotts, UK). Finally, magnesium stearate (Uitgest, Holland) at a concentration (1 %, *m/m*) and aerosil (Uitgest, Holland) at a concentration (0.5 %, *m/m*) were added and mixed with the granules. Granules within a size range of 0.71–1.00 mm diameter were used in capsule preparation.

### *Characterization of formulations*

The parameters studied to characterize the formulations were, bulk density, tap density, moisture content, compressibility, flowability and viable count.

**Bulk and tapped densities.** – Bulk density ( $\rho$ ) was calculated by measuring the mass (*m*) per unit volume of granules (*V*). The mass of the volume was recorded and the corresponding volume of granules was measured using a graduated measuring cylinder. For tapped density measurement, the granules were tapped 200 times, which was sufficient for the granules to reach a constant volume using graduated glass measuring cylinders (10).

Table I. Formulations of granules containing *L. acidophilus* ATCC 4962 in edible broth medium

Ingredient	Formulation		
	F(1)	F(2)	F(3)
<i>L. acidophilus</i> and 2 % ( <i>m/V</i> ) skim milk in edible broth medium (mL)	35	52	65
Corn starch (g)	30	25	25
Lactose monohydrate (g)	38	35	25
Povidone (g)	2	2	2
Microcrystalline cellulose (g)	30	38	48

*Carr's compressibility index.* – Carr's index was calculated from bulk and tapped densities using the following equation (11):

$$\text{Carr's Index (\%)} = \frac{\rho_t - \rho_i}{\rho_i} \times 100 \% \quad (1)$$

where:  $\rho_f$  is tapped density and  $\rho_i$  is the bulk density.

*Moisture content measurement.* – The moisture content of granules for the different formulations was monitored and measured using an infra-red moisture analyzer (Mettler Toledo Delta range, PM 480, Switzerland). Five grams of powder was heated for 10 min at 105 °C and reweighed (12).

*Flowability test.* – Carr's compressibility index is widely used as a marker of powder flowability (13). Therefore, Carr's compressibility index was calculated in the present study.

*Total viable count.* – To determine the viable count of *L. acidophilus* ATCC 4962 present in the granules, 1 g of granules was rehydrated with 9 mL of food-grade broth medium and the suspension was serially diluted with broth medium (14). Serial dilutions of the *L. acidophilus* ATCC 4962 suspension were incubated aerobically at  $37 \pm 1$  °C for 72 h in food-grade agar medium, and viable count was determined by the pour plate method using a colony counter (Technical Lab Instrument, USA).

### *Enteric coating of granules*

To achieve suitable processing conditions for the top-spray fluidized bed coater in granule coating (Uni-Glatt, Germany), different coating parameters were optimized, such as inlet temperature, fluidization, air flow rate, atomizer pressure and spray rate, using one-at-a-time strategy by varying one parameter each time while keeping the others constant. Various inlet temperatures were studied. By accurate control of temperature, the optimum temperature between the granule drying time and viable bacterial cell count was selected.

Optimum temperature was then fixed and different fluidized air flow rates were selected to optimize the flow rate. The best fluidized air flow rate was fixed and the pressure was optimized. Further, two spray rates were tested after fixing all the other parameters and the spray rate that assured little formation of agglomerates was selected. A batch of 200 g of granules (F3) was allowed to fluidize in the coating chamber until the inlet air temperature reached the required temperature. The granules were coated with 100 mL aqueous coating solution containing Eudragit L30D-55 (Pharma Polymer, Germany), at concentrations 5.0, 7.5, 10.0, 12.5 and 15.0 % (m/V), plasticized with 5 % (V/V) of triethylcitrate (Merck, Germany). Coated granules were allowed to fluidize for 10 min to ensure complete drying after spraying. Coated granules of 500 mg were then filled manually into hard gelatin capsules (Halagel (M), Malaysia) of size zero.

### *In vitro dissolution studies*

The *in vitro* dissolutions of *L. acidophilus* ATCC 4962 capsules were studied under simulated gastric and intestinal fluids. Simulated gastric fluid and intestinal fluid were prepared according to the method described in the United States Pharmacopoeia (28).

Artificial gastric juice was prepared by dissolving 2.0 g of sodium chloride and 3.2 g of purified pepsin (pepsin is derived from porcine stomach mucosa, with an activity of 800 to 2500 units per mg of protein) (Sigma, Germany) in 7.0 mL of hydrochloric acid and sufficient water to 1000 mL. The pH of the solution was 1.1–1.2.

Artificial gastric juice 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). To evaluate the gastric juice resistance, capsules were incubated at 37 °C for 1 or 2 h and the differences of microbial cell counts were determined (15).

Simulated intestinal fluid was prepared by dissolving 6.8 g of monobasic potassium phosphate (Merck, Germany) in 250 mL of water, which was mixed and 77 mL of 0.2 mol L<sup>-1</sup> sodium hydroxide (R & M Chemicals, UK) and 500 mL of water were added. 10.0 g of pancreatin (Sigma, Germany) was added and topped up with water to 1000 mL. The resulting solution was adjusted with 0.2 mol L<sup>-1</sup> sodium hydroxide to pH of 6.8 ± 0.1 (16).

The dissolution study was performed according to the USP dissolution test apparatus II paddle method (model PT-DT7, Pharma Test Apparatebau GmbH, Germany). 900 mL of dissolution medium was maintained at 37.0 ± 0.5 °C and the rotation speed was set at 100 rpm. A 5 mL sample was withdrawn after 2 h and the release of *L. acidophilus* ATCC 4962 from the capsule in dissolution medium (simulated intestinal fluid) was estimated using the pour plate method. The test was run in triplicate.

### Statistical analysis

The results were treated statistically using SPSS software (version 13.0, USA). Student's independent *t*-test and one-way analysis of variance were used to analyze the results. When there was a statistically significant difference, a post-hoc Tukey's honestly significant difference test was used. A statistically significant difference was considered when  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Growth of *L. acidophilus* in edible broth medium with skim milk

Skim milk has been used as a growth medium for *L. acidophilus* (17). *L. acidophilus* requires different amino acids to grow. These amino acids are present in skim milk but they alone are not sufficient to support maximum growth rates (18). Combination of skim milk (contains amino acids) with edible broth medium containing glucose (the carbohydrate source), yeast extract (the nitrogen source) and minerals stimulates the growth of *L. acidophilus* (19). The profile of growth and viable counts of *L. acidophilus* ATCC 4962 are shown in Fig. 1. Viable counts increased by increasing the concentration of skim milk and reached a maximum of 9.73 log cfu mL<sup>-1</sup> at a 2.0 % concentration. The results of viable counts for different skim milk concentrations were statistically significant ( $p < 0.05$ ). There was no significant difference with a further increase from 2.0 to 2.2 % ( $p > 0.05$ ). Above 2.2 %, there was a significant decline in viable counts. The increase in viable counts of bacteria with an increase in skim milk concentration was obviously due to the larger supply of amino acids to sustain and enhance the growth of bacterial cells. Reduction in

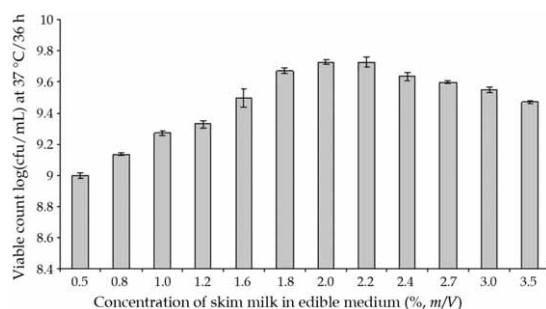


Fig. 1. Viable count of *L. acidophilus* in edible broth with skim milk. Results are expressed as mean  $\pm$  SD; each data point is the average of three independent replicated measurements,  $n = 3$ .

the growth rate with further increase in skim milk concentration beyond the optimum 2.2 % was possibly due to the osmotic effects, which could have killed the bacteria.

### Characterization of different formulation types

The results of bulk density, tapped density, moisture content, compressibility, flowability and viable counts of different *L. acidophilus* ATCC 4962 formulations are shown in Table II. Interparticulate interactions correlate with the powder flow and a comparison of bulk and tapped densities. Comparison between bulk and tapped densities was often used as an index of the ability of powder to flow, such as Carr's compressibility index or the Hausner ratio (20). The results of bulk and tapped densities of the granules provide useful information about the volume of the final dosage form and can affect the dosing of the final granules into final capsules (21).

Carr's index measures the flow properties of powders; the smaller is Carr's index, the better are the flow properties. Powders that form hard compacts under applied pressure without exhibiting any tendency to capping or chipping can be considered compressible. Carr's index between 5–15 % indicates excellent flowability of the granules (22). Carr's index obtained for all the formulations was between 5.08 and 9.37 % and therefore the flow properties of the granules were excellent.

Table II. Characterization of different *Bacillus acidophilus* ATCC 4962 formulations

Parameters <sup>a</sup>	Formulations		
	F(1)	F(2)	F(3)
Bulk density (g mL <sup>-1</sup> )	0.448	0.482	0.47
Tap density (g mL <sup>-1</sup> )	0.476	0.511	0.510
Moisture content (%)	1.97 $\pm$ 0.14	1.87 $\pm$ 0.13	1.92 $\pm$ 0.28
Carr's index (%)	6.0	5.78	8.01
Flowability	Excellent	Excellent	Excellent
Viable count (cfu/g)	9.23 $\pm$ 0.01	9.43 $\pm$ 0.05	9.51 $\pm$ 0.02

<sup>a</sup> Mean  $\pm$  SD,  $n = 3$ .

Moisture content is a critical parameter for the granulation process with optimum binding force. As the moisture content of a powder increases, adhesion and cohesion tend to increase (23). Granules having a moisture content above the critical range affect the frictional properties and become sticky and hard. The moisture content results of all formulations investigated in this study were within the acceptable range (1.87–1.97 %). According to the literature, the acceptable limit for moisture content is between 0.7 and 2.0 % (24).

Viable counts were conducted for all the *L. acidophilus* ATCC 4962 formulations. Results of all the *L. acidophilus* ATCC 4962 formulations were above 9.0 log cfu/500 mg, which is sufficient for the humans (25). Formulation F (3) was selected for enteric coating.

### Coating process and capsulation of granules

Enteric coating is important to protect *L. acidophilus* ATCC 4962 against the highly acidic environment in the stomach (26). To optimize and achieve suitable coating conditions, a series of experiments were carried out using granules with *L. acidophilus* ATCC 4962.

The coating parameters, which included the inlet air temperature, fluidized air flow rate, atomizer pressure and spray rate, were critical during optimization, since these parameters could affect the viability of probiotics during coating (27). Observations obtained in the optimization of coating parameters are summarized in Table III.

The selected formulation (F3) was coated successfully using Eudragit L30D-55, which ensured that the oral dosage form would pass through the stomach without release and deliver the drug into the small intestine (28).

To improve the plasticity of granules, triethyl citrate was introduced into the coating. This plasticizer does not only affect the film formation process by lowering the glass transition temperature but also the film properties such as water vapor transmission and accelerated drug release by leaching into the dissolution medium creating pathways for drug diffusion (release of probiotics) (29).

Table III. Evaluation of coating parameters

Parameter	Condition	Observation
Inlet temperature (°C)	50–60	reduced viable bacterial cell count
	28–30	granules agglomerated
	36–38	granules dried and enhanced viable bacterial cell count
Fluidizing air flow rate (m <sup>3</sup> h <sup>-1</sup> )	120–140	granules fragmented
	90–110	good fluidization
	60–80	granules could not be fluidized
Atomizing air (bar)	1.4	granules became powdery
	1.0	good fluidization
	0.8	agglomeration occurred
Spray rate (mL min <sup>-1</sup> )	1.5	agglomeration occurred
	1.0	no agglomeration



### In vitro release studies

The *in vitro* release results of *L. acidophilus* ATCC 4962 coated with different levels of Eudragit L30D-55 are shown in Table IV. Formulation F(3) showed release at the coating level below 7.5 % and no release at the coating level of 7.5 % and above. The coating level below 7.5 % was not sufficient to protect *L. acidophilus* ATCC 4962 from the highly acidic stomach environment. The lower enteric coating level required by formulation F(3) could be attributed to the presence of higher content of skim milk and the use of skim milk that was not exposed to freeze drying. However, it was observed that once formulation F(3) was in simulated intestinal fluid, *L. acidophilus* ATCC 4962 was readily released, with the dissolution of the enteric coat under the alkali pH environment. Numbers of viable counts of *L. acidophilus* ATCC 4962 at 12.5 and 15 % (*m/V*) Eudragit L30D-55 coating were found to be more or less equal (9.1 log cfu/cap). Therefore, no significant difference was observed between the two different concentrations of coating. This coating level would be critical to ensure that probiotics survived during their passage through the human gastrointestinal tract and reached the small intestine as viable organisms for potential health effects (30). In the present study, the addition of Eudragit L30D-55 was essential to guarantee protection against gastric juice. The preparation demonstrated resistance to gastric juice, which enabled the delivery of approximately  $10^9$  viable probiotic bacteria to the intestine within 1–2 h. These results are in agreement with other researchers who found that effects of *L. acidophilus* were very beneficial when colonizing the human intestine at doses of  $10^6$  cfu and above and replacing proteolytic microbes.

Table IV. *Lactobacillus acidophilus* ATCC 4962 release in simulated gastric fluid

Formulation	Coating level (%)	Viable count
F(3)	5.0	3.83 log CFU/cap
	7.5	no growth
	10.0	no growth

### CONCLUSIONS

It can be concluded from the present work that the probiotic *L. acidophilus* ATCC 4962 can be incorporated directly into the preparation of granules without the laborious process of freeze-drying. Enteric coating of the granules using Eudragit L30D-55 protected the release of *L. acidophilus* ATCC 4962 in acidic media. In contrast, the release of these bacteria from capsules was shown to be very prompt in alkyl pH. It is believed that the coating of granules with enteric polymers would protect the probiotic from the acidic environment and improve the survival rate of the microorganism also in human intestine.

*Acknowledgments.* – The present study was financially supported by a grant from Universiti Sains Malaysia (1001/PFARMASI/843084) and USM Graduate Assistance Scheme.



## REFERENCES

1. M. Kumar, R. Nagpal, R. Kumar, R. Hemalatha, V. Verma, A. Kumar, C. Chakraborty, B. Singh, F. Marotta, S. Jain and H. Yadav, Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases, *Exp. Diabetes Res.* **2012** (2012) 1–16; DOI: 10.1155/2012/902917.
2. E. Johansen, K. Soerensen, M. Curic-bawden and M. Junge, Use of lactic acid bacteria for preparing fermented food products with increased natural sweetness, PCT/EP2013/058655, 31.10.2013; IPC:C12N 1/20 (2006.01) (**2013**) WO2013160413.
3. S. Messaoudi, M. Manai, G. Kergourlay, H. Prévost, N. Connil, M. Chobert and X. Dousset, *Lactobacillus salivarius*: bacteriocin and probiotic activity, *Food Microbiol.* **36** (2013) 296–304; DOI: 10.1016/j.fm.2013.05.010.
4. M. Cavinato, M. Bresciani, M. Machin, G. Bellazzi, P. Canu and A. Santomaso, Formulation design for optimal high-shear wet granulation using on-line torque measurements, *Int. J. Pharm.* **387** (2010) 48–55; DOI: 10.1016/j.ijpharm.2009.11.032.
5. B. Ennis, *Theory of Granulation: An Engineering Perspective*, in *Handbook of Pharmaceutical Granulation Technology* (Ed. D. Parikh), 3<sup>rd</sup> ed., Taylor and Francis Group, New York 2009.
6. D. Daniher, L. Briens and A. Tallevi, End point detection in high-shear granulation using sound and vibration signal analysis, *Powder Technol.* **181** (2008) 130–136; DOI: 10.1016/j.powtec.2006.12.003.
7. P. Sinko, Martin's *Physical Pharmacy and Pharmaceutical Sciences*, 5<sup>th</sup> ed., Lippincott Williams & Williams Publishers, Philadelphia 2006.
8. J. E Silva, C. Sousa, P. Costa, E. Cerdeira, M. Amaral, J. Sousa, A. Gomes, M. Pintado, D. Rodrigues, T. Rocha-Santos and A. Freitas, Development of probiotic tablets using microparticles: viability studies and stability studies, *AAPS PharmSciTech.* **14** (2013) 121–127; DOI: 10.1208/s12249-012-9898-9.
9. H. Pyar and K. Peh, Effect of cryoprotective agents on survival and stability of *Lactobacillus acidophilus* cultured in food-grade medium, *Int. J. Dairy Technol.* **64** (2011) 578–584; DOI: 10.1111/j.1471-0307.2011.00715.x.
10. E. Abdullah and D. Geldart, The use of bulk density measurements as flowability indicators, *Powder Technol.* **102** (1999) 151–165; DOI: 10.1016/S0032-5910(98)00208-3.
11. V. Nalluri and M. Kuentz, Flowability characterisation of drug excipient blends using a novel powder avalanching method, *Eur. J. Pharm. Biopharm.* **74** (2010) 388–396; DOI: 10.1016/j.ejpb.2009.09.010.
12. *The United States Pharmacopoeia*, Pharmacopoeial Convention, Asian edition, Web com. Ltd., 2011, Canada.
13. S. Badawy, M. Meening, M. Gorko and D. Gilbert, Effect of process parameters on compressibility of granulation manufactured in a high-shear mixer, *Int. J. Pharm.* **198** (2000) 51–61.
14. G. Font de Valdez, G. de Giori, A. de Ruiz Holgado and G. Oliver, Effect of the rehydration medium on the recovery of freeze-dried lactic acid bacteria, *Appl. Envir. Microbiol.* **50** (1985) 1339–1341.
15. D. Priyanka, A. Anishaparkin, G. Joseph and C. Anandharamakrishnan, Microencapsulation of *Lactobacillus plantarum* (mtcc 5422) by spray-freeze-drying method and evaluation of survival in simulated gastrointestinal conditions, *J. Microencapsul.* **28** (2011) 568–574. DOI: 10.3109/02652048.2011.599435.
16. R. Mokarram, S. Mortazavi, M. Habibi-Najafi and F. Shahidi, The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice, *Food Res. Int.* **42** (2009) 1040–1045; DOI: 10.1016/j.foodres.2009.04.023.
17. Z. Mousavi, S. Mousavi, S. Razavi, M. Hadinejad, Z. Emam-Djomeh and M. Mirzapour, Effect of fermentation of pomegranate juice by *Lactobacillus plantarum* and *Lactobacillus acidophilus* on

- the antioxidant activity and metabolism of sugars, organic acids and phenolic compounds, *Food Biotechnol.* **27** (2013) 1–13; DOI: 10.1080/08905436.2012.724037.
18. V. Monnet, S. Condon, T. Cogan and J. Gripon, *Metabolism of Starter Culture*, in *Dairy Starter Culture* (Ed. T. Cogan and J. Accolas), VCH publishers, New York 1996, p. 47.
  19. W. Sandine, *Commercial Production of Dairy Starters Cultures*, in *Dairy Starter Culture* (Ed. T. Cogan and J. Accolas), VCH publishers, New York 1996, p. 191.
  20. I. Valaei, S. Hassan-Beygi, M. Kianmehr and J. Massah, Investigation of avalanche time and Carr's index of poultry litter powder as flowability indices, *Cercetari Agronomice in Moldova* **45** (2013) 15–27; DOI: 10.2478/v10298-012-0061-2.
  21. G. Amidon, P. Secreast and D. Mudie, *Developing Solid Oral Dosage Forms: Pharmaceutical Theory and Practice*, Academic press, Burlington 2009.
  22. K. Ghosh and B. Jasti, *Theory and Practice of Contemporary Pharmaceutics*, Philadelphia 2010.
  23. A. Faqih, A. Mehrotra, S. Hammond and J. Muzzio, Effect of moisture and magnesium stearate concentration on flow properties of cohesive granular materials, *Int. J. Pharm.* **336** (2007) 338–345; DOI: 10.1016/j.ijpharm.2006.12.024.
  24. P. Müller, S. Antonyuk and J. Tomas, Influence of moisture content on the compression behavior of granules, *Chem. Eng. Technol.* **34** (2011) 1543–1550; DOI: 10.1002/ceat.201100262.
  25. S. Choi, L. Liu, K. Yamamoto, K. Igawa, M. Mochizuki, T. Sakai, R. Echigo, M. Honnami, S. Suzuki, I. Chung and N. Sasaki, Development and evaluation of tetrapod-shaped granular artificial bones, *Acta Biomater.* **8** (2012) 2340–2347; DOI: 10.1016/j.actbio.2012.02.019.
  26. P. Seitavuopio, J. Heinämäki, J. Rantanen and J. Yliruusi, Monitoring tablet surface roughness during the film coating process, *AAPS PharmSciTech.* **7** (2006) E1–E6; DOI: 10.1208/pt070231.
  27. B. Rambali, L. Baert, D. Thoné and L. Massart, Using experimental design to optimize the process parameters in fluidized bed granulation, *Drug Dev. Ind. Pharm.* **27** (2001) 47–55; DOI: 10.1081/DDC-100000127.
  28. V. Sinha, A. Singh, R. Kuma, S. Singh, R. Kumria and R. Bhinge, Oral colon specific drug delivery of protein and peptide drugs, *Crit. Rev. Ther. Drug Carrier Syst.* **24** (2007) 63–92; DOI: 10.1615/CritRevTherDrugCarrierSyst.v24.i1.30.
  29. G. Kibria, A. Roni, S. Absar and U. Jalil, Effect of plasticizer on release kinetics of diclofenac sodium pellets coated with Eudragit RS 30 D, *AAPS PharmSciTech.* **9** (2008) 1240–1246; DOI: 10.1208/s12249-008-9163-4.
  30. H. Abukawa, M. Papadaki, M. Abulikemu, J. Leaf, J. Vacanti, L. Kaban and J. Troulis, The engineering of craniofacial tissues in the laboratory: a review of biomaterials for scaffolds and implant coatings, *Dent. Clin. North Am.* **50** (2006) 205–216; DOI: 10.1016/j.cden.2005.11.006.