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The Influence of Selected Parameters on the Size and Shape of Alginate Beads Prepared by Ionotropic Gelation

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Abstract

Many bead biopharmaceutical characteristics are dependent on the bead shape. Furthermore, the shape is one of crucial parameters for incorporation of beads in more complex drug delivery system. Therefore, the aim of this study was to evaluate the influence of various processing parameters such as hardening time, temperature and concentration of calcium chloride solution and drying conditions on size, shape and morphology of alginate beads prepared by ionotropic gelation method. Theophylline was selected as a model drug. It was found that all studied parameters markedly affected bead form, resembling in most cases to ellipsoid spheres. Their sphericity was estimated three-dimensionally by measuring diameters of frontal and lateral side which were perpendicular to each other. Smaller and more spherical beads were obtained at longer hardening time and higher temperature of calcium chloride solution. The freeze-dried beads were the largest and the most spherical. It was demonstrated that optimization of bead shape as well as size and morphology could be achieved by altering processing parameters.

Keywords

alginate beads • ionotropic gelation • shape • sphericity

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Introduction

Alginate is linear naturally occurring polysaccharide, consisted of D-mannuronic (M) and L-guluronic (G) acids [1]. The ability of alginate to form gel in the presence of multivalent ions has been applied to prepare beads by ionotropic gelation method where the dispersion of alginate and material to be encapsulated is added dropwise into multivalent ion solution. The contact of droplets with multivalent ions results in instantaneous formation of gel spheres containing uniformly dispersed material throughout the crosslinked alginate matrix. The size of wet beads is dependent on size of a droplet of polymer dispersion, which is influenced by diameter of a nozzle and viscosity of polymer dispersion. However, the drying may influence the size and shape of dry beads.

The properties of the beads prepared by ionotropic gelation are influenced by formulation and processing parameters. This statement is supported by several papers reporting that drug release and/or encapsulation efficiency is dependent on type of drug and its characteristics [2–5], type and concentration of polymer [6–8], inclusion of various additives [3, 8–16], drug polymer weight ratio [5, 7] as well as on process variables like hardening time [4, 7, 8, 13, 17, 18] , type and concentration of cross-linking agent [6, 16–20], drying conditions [21–24]. However, the influence these parameters on the shape of beads was studied very rarely.

The scope of the present study was to investigate the influence of some processing parameters on size and shape of alginate beads containing theophylline as a model drug, prepared by ionotropic gelation.

Results and discussion

Wet beads were all spherical regardless of the studied processing parameters. However, the shape of wet bead was influenced by the distance between the top of a needle, through which the dispersion was dropped, and hardening solution as well as the stirring speed of the hardening solution. Namely, the beads flattened on contact with the hardening medium if the alginate dispersion was dropped from the height higher than 6 cm. Furthermore, elongation of the beads during hardening

was observed at stirring speed higher than 600 rpm. Therefore, in the present work the stirring speed and the distance between the top of a needle and the hardening solution were fixed at 600 rpm and 6 cm, respectively.

Drying may influence bead size and shape. During drying, the beads shrank significantly and their shape changed. Dry beads had a typical shape with following characteristics: they resembled to flattened, ellipsoid spheres, some of them were completely flattened, some had a hollow in the center and some had regular spherical shape. Therefore, the method for their shape characterization was adapted to their characteristics. First, the shape of bead frontal sides was evaluated. From the mean values of aspect ratio (1.13 ±0.06) and shape factor (0.88 ± 0.06), which were close to 1, it can be seen that they were round-shaped. However, beads are three-dimensional objects and estimation of their shape on the basis of only two-dimensional image is not sufficient. Therefore, the bead shape was additionally examined from two different perspectives (frontal and lateral) which were perpendicular to each other. The sphericity of beads was thus defined as the ratio between the mean values of the lateral and the frontal diameter.

It was found that bead size and shape was significantly affected by all the tested parameters. Increasing the hardening time from 1 to 30 minutes resulted in decrease of the mean values of bead frontal diameter from 1.91 to 1.33 (Fig. 1a). This could be due to lower drug content since a significant decline of theophylline content with prolongation of hardening time was observed (data not shown). The decrease in size as well as improvement of the sphericity of calcium alginate beads with decreasing theophylline content was also observed by Smrdel et al. [4]. Furthermore, more extensive alginate crosslinking after longer hardening time contributed to decrease of the bead diameter [20]. These results are in accordance with findings of other researchers [4, 8, 24]. On the other hand, the mean value of the lateral diameter and sphericity increased with increasing hardening time. Beads isolated after 1 and 3 minutes of hardening in calcium chloride solution had a hollow in the center and were flattened, the estimated sphericity was 0.42 and 0.63, respectively. Obviously, the alginate crosslinking was not completed in that time

and consequently the beads flattened and collapsed during drying. Prolongation of hardening time significantly improved the sphericity; i.e. the sphericity of beads isolated after 30 minutes of hardening was estimated at 0.88.

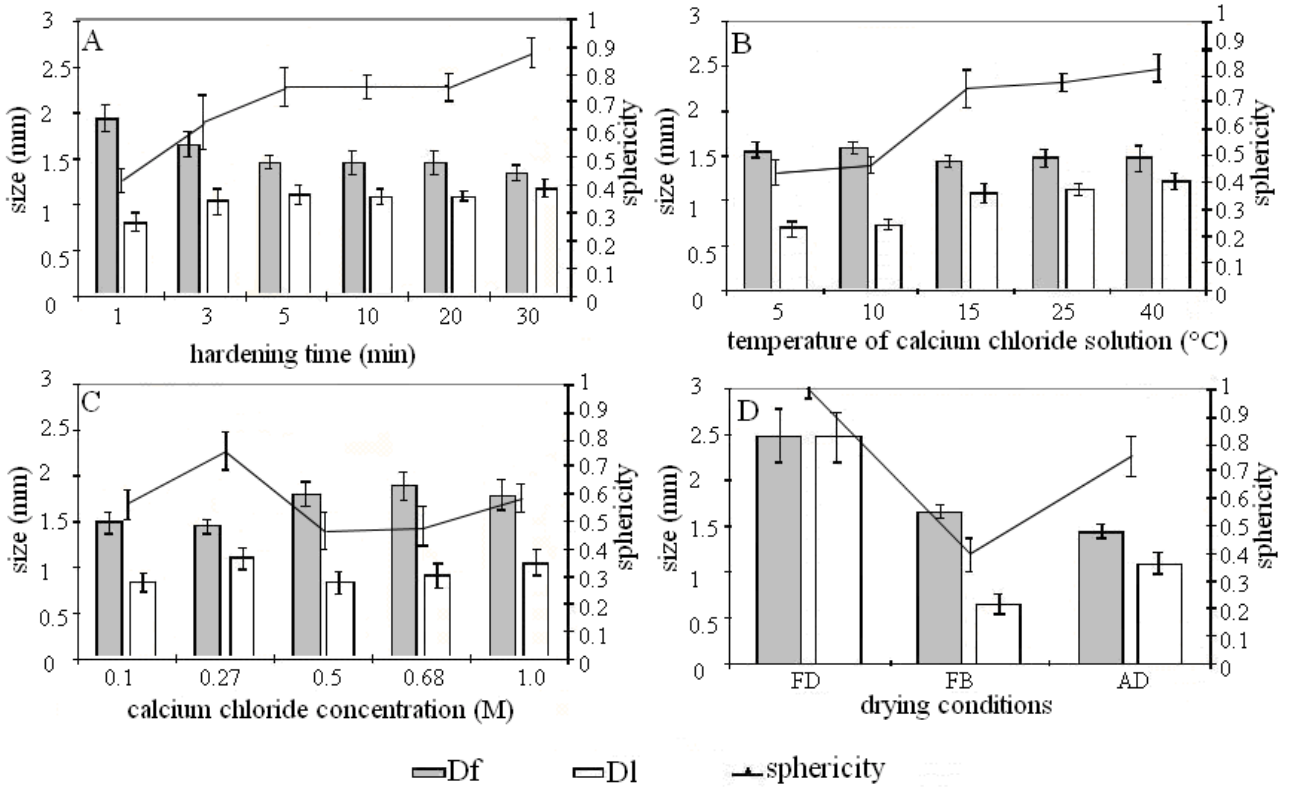

Fig. 1. The effect of (a) hardening time, (b) temperature of calcium chloride solution, (c) calcium chloride concentration and (d) drying conditions (FD-freeze-dried, FB-fluidized-bed-dried, AD-air-dried) on bead frontal diameter (Df), lateral diameter (Dl) and sphericity. The columns represent bead size (left y-axis), the curves represent bead sphericity (right y-axis).

At higher temperature of calcium chloride solution more spherical beads were obtained (Fig. 1b). As mentioned above, the bead shape could be affected by drug content (lower drug content at higher temperature was observed (data not shown)) as well as by degree of alginate crosslinking. Since in our case the hardening time was constant, it is assumed that the rate of alginate crosslinking was also affected by the temperature. Better sphericity at higher temperature of calcium chloride solution was therefore probably due to faster and thus more thorough alginate crosslinking and lower drug content.

It was reported in the literature that by increasing the calcium chloride concentration smaller beads were obtained [6, 18]. Our results are presented in Fig. 1c. The beads hardened in 0.1 and 0.27M calcium chloride solution had significantly lower mean values of frontal diameters than beads prepared using 0.5, 0.68 and 1.0M calcium chloride solution. However, the beads prepared with different calcium chloride concentrations differed significantly in their shape. Some of them were curved and some completely flattened. No correlation between drug content and bead size was observed. However, neither frontal nor lateral diameter neither sphericity alone represents a suitable approach for evaluation of actual size or shape of beads. Thus it is difficult to make any conclusions on the bases of presented parameters.

Drying technique could influence several bead characteristics such as size [11, 16, 21], shape, mechanical properties [11], swelling properties [11, 16], drug release kinetics [24] and even drug migration to periphery of the beads along with water during drying [21, 22]. Concerning the latter, accumulation of drug on the surface of the beads could be accountable for several difficulties, i.e. burst effect in drug release, ineffective taste masking of drugs with unpleasant taste, drug loss due to crumbling of the drug from the bead surface… Drug migration during bead drying can be avoided by freeze-drying which involves sublimation but not evaporation of water molecules [22].

In the present work it was demonstrated that drying conditions had great influence on shape and morphology of beads including surface as well as internal structure characteristics of the beads (Figs. 1d and 2). The freeze-dried beads were the largest as they remained almost of the same size as before drying. Furthermore, they were the most spherical proving by sphericity being approximately 1. Their surface was rough (Fig. 2a) and their internal structure was very porous (Fig. 2b) making them brittle to the touch. Such characteristics of freeze-dried beads are due to the fast sublimation of frozen water from alginate matrix resulting in formation of pores in areas of former ice crystals without having time to shrink.

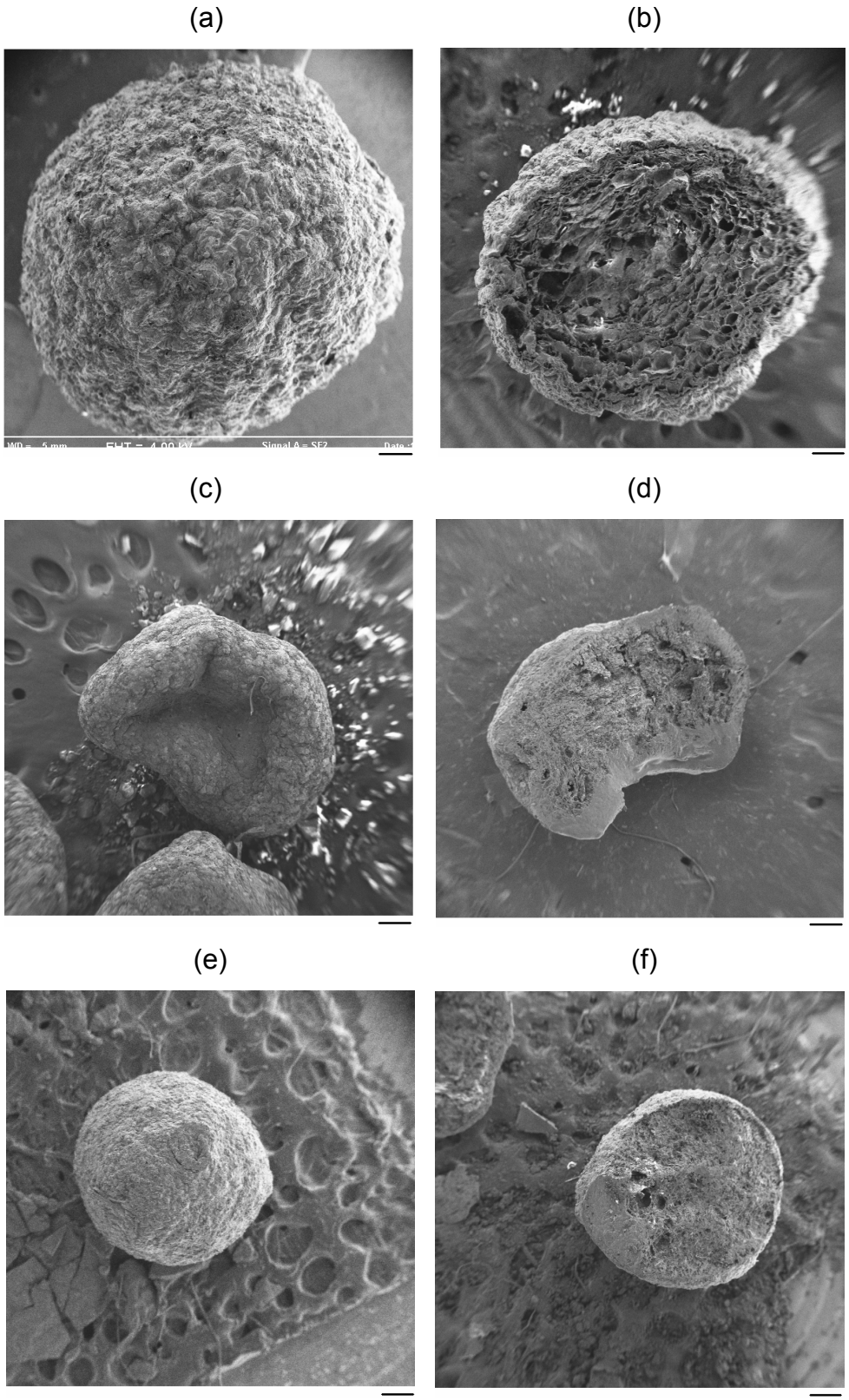

Fig. 2. The scanning electron micrographs of (a) freeze-dried, (c) fluidized-bed-dried and (e) air-dried beads and their corresponding cross-sections (b, d, f, respectively). The bar corresponds to 200 μm.

On the other hand, fluidized-bed-dried and air-dried beads substantially shrank and became smaller during drying. The first ones were folded and irregularly shaped (Fig. 2c). It is supposed that fluidized-bed drying was very rapid resulting in immediate drying of the bead surface. As a consequence of liquid inner part, beads collapsed and particles of irregular shape were formed. The air-dried beads were the smallest (Fig. 1d). They were more spherical than fluidized-bed-dried beads (Fig. 2e) probably due to slower water removal during air-drying resulting in slower and more uniform shrinkage of beads. Similar findings regarding the size of freeze-dried and air-dried beads were reported in the literature [11, 16, 21]. The surface of fluidized-bed-dried as well as of air-dried beads was, however, much smoother than that of freeze-dried. Furthermore, they were not as porous as freeze-dried ones. From the cross-sections of fluidized-bed-dried and air-dried beads (Figs. 2d and 2f, respectively) a dense interior can be seen. This is in accordance with the results of George et al. [16] and Gal et al. [11]. The latter evaluated also mechanical properties of the freeze-dried and vacuum-dried beads and demonstrated that freeze-dried beads had significantly higher porosity and were mechanically weaker than vacuum-dried beads [11]. High porosity and consequently low density of freeze-dried beads provide a potential for development of multiunit gastroretentive drug delivery system due to buoyancy of the beads in gastric fluid [23]. Furthermore, it was reported that due to the porous structure and larger size of freeze-dried beads the water uptake into the beads was greater compared to vacuum-dried [11] and air-dried beads [16] with dense interior. Such difference in water absorption could have a tremendous impact on drug release kinetics. This hypothesis was confirmed by Sriamornasak [24] who demonstrated significantly faster release of bovine serum albumin from freeze-dried than from air-dried calcium pectinate beads. This indicates that owing to changes in bead morphology drying conditions affect drug release from the beads.

This study demonstrates that slight change in processing parameters can cause a significant influence on size, shape and morphology of alginate beads prepared by ionotropic gelation method. It was proven that altering various

processing parameters enables optimization of bead shape which is one of crucial characteristics for further incorporation of beads into more complex drug delivery systems.

Experimental

Preparation of alginate beads

Beads were prepared by dissolving sodium alginate (Protanal LF 120M, Selectchemie AG, Switzerland) in distilled water at concentration of 4% (w/v). Theophylline (Fluka Chemie AG, Switzerland), magnesium stearate (supplied by Lek Pharmaceuticals, Slovenia) and Kollidon K30 (BASF) were added to alginate solution so that the weight ratio of all dry substances was 4:2:1:5, respectively. After stirring on a magnetic stirrer for half an hour homogeneous and bubble-free dispersion was filled into disposable syringe equipped with a 0.9 mm inner diameter needle. Dispersion was then manually added dropwise (80 drops) into 100 ml of 0.27M calcium chloride solution. The distance between the top of the needle and the surface of the hardening medium was 6 cm. Calcium ions crosslinked alginate and the beads were formed. Beads were allowed to harden in gently stirred calcium chloride solution (stirring rate of magnetic stirrer was 600 rpm) and after that they were isolated and washed twice with 800 ml of distilled water. Beads were dried by air-drying at room temperature, in fluidized-bed apparatus or by freeze-drying. Fluidized-bed-drying was performed at 40°C and 0.6 bar of atmosphere using STREA1, Niro-Aeromatic, Switzerland. The freeze-drying was done according to the following procedure: immediately after bead isolation from calcium chloride solution and washing with distilled water the beads were poured over with liquid nitrogen and dried in a vacuum dryer VS – 50 S Kambič, Laboratorial equipment, Slovenia at reduced pressure at room temperature for 4 hours. All beads were additionally dried at reduced pressure at room temperature overnight.

In general the beads were prepared by dropping the dispersion into 0.27M calcium chloride solution, allowed to harden for 5 minutes and air-dried. In order to examine the effect of hardening time (1, 3, 5, 10, 20 and 30 min), concentration

(0.1M, 0.27M, 0.5M, 0.68M and 1.0M $CaCl_2$ solution) and temperature (5, 10, 15, 25 and 40°C) of calcium chloride solution and drying conditions (freeze-drying, fluidized-bed-drying and air-drying) on beads properties, four series of experiments were performed where only the studied parameter was varied. All experiments were made in triplicate.

Characterization of beads

The bead size and shape was estimated using Stereomicroscope Olympus SZX12, Japan, equipped with digital camera (3CCD Color Video Camera, Power HAD, Sony, Japan). Five beads of each sample were examined. First, the bead frontal shape was characterized by aspect ratio and shape factor using image analysis system AnalySIS (AnalySIS® software program, SIS, Germany, 1999). The aspect ratio was defined as ratio between the maximal and the minimal bead diameter, while shape factor, which provides the information about the roundness of the particles, was calculated by following equation (AnalySIS® software program, SIS, Germany, 1999):

$$shape\ factor = \frac{4\pi \times area}{perimeter^2}$$

The closer the values of the aspect ratio and the shape factor were to 1, the rounder the bead was.

Furthermore, the bead frontal diameters were measured in three directions. After that, the bead was erected vertically so that the lateral diameter was feasible to measure at three different positions. The mean values of the lateral (Dl) and the frontal (Df) diameters were calculated. The parameter in the present work named as sphericity of the beads was defined as the ratio between the mean values of the lateral and the frontal diameter. The values closer to 1 indicated more spherical beads.

The morphological properties of bead surface and the internal structure characteristics of beads were evaluated using scanning electron microscope Supra^{TM} 35 VP, Zeiss, Germany.

Statistical analysis

Statistical analysis was performed using SPSS software program (Version 12.0.1, SPSS Inc., Chicago, 2004). The one-way ANOVA followed by the LSD test for post hoc comparisons was applied for evaluation of the influences of hardening time, concentration and temperature of calcium chloride solution and drying conditions on bead's size and sphericity. Games-Howell test was used instead of the LSD test when homoscedasticity of variances was not proven. The significance level was set at 0.05 for all the tests.

References

[1] Bruneton J.
Algae polysaccharides.
In: Pharmacognosy, phytochemistry medicinal plants.
2nd ed. New York: Lavosier Publishing, Inc., 1999: 47–52.

[2] Østberg T, Lund EM, Graffner C.
Calcium alginate matrices for oral multiple unit administration: IV. Release characteristics in different media.
Int J Pharm. 1994; 112: 241–248.
[doi:10.1016/0378-5173(94)90360-3]

[3] Bodmeier R, Wang J.
Microencapsulation of drugs with aqueous colloidal polymer dispersions.
J Pharm Sci. 1993; 82: 191–194.
[doi:10.1002/jps.2600820215]

[4] Smrdel P, Bogataj M, Podlogar F, Planinšek O, Zajc N, Mazaj M, Kaučič V, Mrhar M.
Characterisation of calcium alginate beads containing structurally similar drugs.
Drug Dev Ind Pharm. 2006; 32: 623–633.
[doi:10.1080/03639040600599863]

[5] Tang Y D, Venkatraman S S, Boey Y C, Wang L W.
Sustained release of hydrophobic and hydrophilic drugs from a floating dosage form.
Int J Pharm. 2007; 336: 159–165.
[doi:10.1016/j.ijpharm.2006.11.060]

[6] Østberg T, Vesterhus L, Graffner C.
Calcium alginate matrices for oral multiple unit administration: II. Effect of process and formulation factors on matrix properties.
Int J Pharm. 1993; 97: 183–193.
[doi:10.1016/0378-5173(93)90138-6]

[7] Acartürk F, Takka S.
Calcium alginate microparticles for oral administration: II. Effect of formulation factors on drug release and drug entrapment efficiency.
J Microencapsul. 1999; 16: 291–301.
[doi:10.1080/026520499289022]

[8] El-Kamel A H, Al-Gohary O M N, Hosny E A.
Alginate-diltiazem hydrocloride beads: optimization of formulation factors, in vitro and in vivo bioavailability.
J Microencapsul. 2003; 20: 211–225.
[doi:10.1080/02652040210162568]

[9] Pongjanyakul T, Puttipipatkhachorn S.
Xanthan-alginate composite gel beads: Molecular interactions and in vitro characterization.
Int J Pharm. 2007; 331: 61–71.
[doi:10.1016/j.ijpharm.2006.09.011]

[10] Puttipipatkhachorn S, Pongjanyakul T, Priprem A.
Molecular interactions in alginate beads reinforced with sodium starch glycolate or magnesium silicate, and their physical characteristics.
Int J Pharm. 2005; 293: 51–62.
[doi:10.1016/j.ijpharm.2004.12.006]

[11] Gal A, Nussinovitch A.
Hydrocolloid carriers with filler inclusion for diltiazem hydrochloride release.
J Pharm Sci. 2007; 96: 168–178.
[doi:10.1002/jps.20775]

[12] Smrdel P, Bogataj M, Zega A, Planinšek O, Mrhar A.
Shape optimization and characterization of polysaccharide beads prepared by ionotropic gelation.
J Microencapsul. 2008; 25: 90–105.
[doi:10.1080/02652040701776109]

[13] Fereira Almeida P, Almeida A J.
Cross-linked alginate-gelatine beads: a new matrix for controlled release of pindolol.
J Controlled Release. 2004; 97: 431–439.
[doi:10.1016/j.jconrel.2004.03.015]

[14] González-Rodríguez M L, Holgado M A, Sánchez-Lafuente C, Rabasco A M, Fini A.
Alginate/chitosan particulate systems for sodium diclofenac release.
Int J Pharm. 2002; 232: 225–234.
[doi:10.1016/S0378-5173(01)00915-2]

[15] Takka S, Acartürk F.
Calcium alginate microparticles for oral administration: III. The effect of crosslink agents and various additive polymers on drug release and drug entrapment efficiency.
Pharmazie. 1999; 54: 137–139.

[16] George M, Abraham T E.
pH sensitive alginate-guar gum hydrogel for the controlled delivery of protein drugs.
Int J Pharm. 2007; 335: 123–129.
[doi:10.1016/j.ijpharm.2006.11.009]

[17] Rousseau I, Le Cerf D, Picton L, Argillier J F, Muller G.
Entrapment and release of sodium polystyrene sulfonate (SPS) from calcium alginate gel beads.
Eur Polym J. 2004; 40: 2709–2715.
[doi:10.1016/j.eurpolymj.2004.07.022]

[18] Sriamornsak P, Nunthanid J.
Calcium alginate gel beads for controlled release drug delivery: II. Effect of formulation and processing variables on drug release.
J Microencapsul. 1999; 16: 303–313.
[doi:10.1080/026520499289031]

[19] Bajpai S K, Sharma S.
Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca2+ and Ba2+ ions.
React Funct Polym. 2004; 59: 129–140.
[doi:10.1016/j.reactfunctpolym.2004.01.002]

[20] Aslani P, Kennedy R A.
Studies on diffusion in alginate gels. I. Effect of cross-linking with calcium or zinc ions on diffusion of acetaminophen.
J Controlled Release. 1996; 42: 75–82.
[doi:10.1016/0168-3659(96)01369-7]

[21] Fundueanu G, Nastruzzi C, Carpov A, Desbrieres J, Rinaudo M.
Physico-chemical characterization of Ca-alginate microparticles produced with different methods.
Biomaterials. 1999; 20: 1427–1435.
[doi:10.1016/S0142-9612(99)00050-2]

[22] Arshia S, Manna P K, Paranjothy K L K, Manjula M.
Entrapment of andrographolide in cross-linked alginate pellets: I. Formulation and evaluation of associated release kinetics.
Pak J Pharm Sci. 2007; 20: 1–9.

[23] Talukder R, Fassihi R.
Gastroretentive Delivery systems: hollow beads.
Drug Dev Ind Pharm. 2004; 30: 405–412.
[doi:10.1081/DDC-120030935]

[24] Sriamornsak P.
Investigation of pectin as a carrier for oral delivery of proteins using calcium pectinate gel beads.
Int J Pharm. 1998; 169: 213–220.
[doi:10.1016/S0378-5173(98)00129-X]

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