Studies in the Development of Nateglinide Loaded Calcium Alginate and Chitosan Coated Calcium Alginate Beads

Sanat Kumar Basu[a] and Adhiyaman Rajendran*[,b]

[a] Division of Pharmaceutics, Department of Pharmaceutical Technology, Jadavpur University; Kolkata 700032, India: and [b] School of Pharmacy and Health Sciences, International Medical University; Kuala Lumpur 57000, Malaysia.

Received November 12, 2007; accepted May 6, 2008; published online May 15, 2008

Nateglinide loaded alginate-chitosan beads were prepared by ionic gelation method for controlling the drug release by using various combinations of chitosan and Ca^{2+} as cation and alginate as anion. IR spectrometry, scanning electron microscopy, differential scanning calorimetry and X-ray powder diffractometry were used to investigate the physicochemical characteristics of the drug in the bead formulations. The calcium content in beads was determined by atomic absorption spectroscopy. The swelling ability of the beads in different media (pH 1.2, 4.5, 6.8) has been found to be dependent on the presence of polyelectrolyte complex of the beads and the pH of the media. The ability to release the Nateglinide was examined as a function of chitosan and calcium chloride content in the gelation medium. It is evident that the rate of drug release and its kinetics could be controlled by changing the chitosan and the calcium chloride concentrations. Calcium alginate beads released more than 95% of drug with in 8 h; whereas coated beads sustained the drug release and released only 75—80% of drug. The drug release mechanism analyzed indicates that the release follows either "anomalous transport" or "case-II transport".

Key words alginate-chitosan bead; Nateglinide; swelling; characterization; sustained release

The drug delivery systems using biodegradable natural polymeric particles are becoming a clinical reality with many benefits for the patient. The versatility of polymeric materials allows the fabrication of the delivery device with desirable degree of swelling, and drug release. Alginate is a natural biopolymer which forms a hydrogel in the presence of divalent cations like Ca^{2+}.[1,2] The inert environment within the biopolymer network of alginates allows for the entrapment of a wide range of bioactive substances, cells and drug molecules, with minor interactions between them.[3]

The use of calcium-alginate beads in controlled drug delivery technology for the gastro-intestinal administration of proteins[4] and other drug molecules[5—7] are well known. Much attention has been received in recent years regarding the use of chitosan-alginate polyelectrolyte complex in controlled drug delivery.[8—11] The use of chitosan has been repeated in the literature is either for coating alginate beads in order to alter the diffusion rate of the encapsulated substances[12] or as an additive for the bulk modification of the beads structure.[13,14] Nateglinide is an antidiabetic drug used for the treatment of Type-II diabetes mellitus and has a short half-life of 1.5 h, and the usual oral dosage regimen is 60—240 mg taken 3 times a day. Thus Nateglinide is a suitable candidate for oral sustained release drug delivery. Keeping these in view, the present study has been undertaken. The investigation was phased out in the following manner. Firstly the effect of calcium ion and chitosan in the coagulation fluid on drug release characteristics and its mechanism was considered. Then the physical state of the drug in the beads and the swelling behavior of the bead formulations were undertaken.

Experimental

Materials The following materials were obtained from the indicated suppliers and used as received: sodium alginate (low viscosity; viscosity of 2% solution 25 °C, ≈250 cps from SNAP Natural and alginate products limited, Ranipet, India), chitosan (85% degree of deacetylation, molecular weight more than 10^3 kDa from India sea foods, Cochin, India). Calcium chloride dihydrate (Qualigens, Mumbai, India), di-sodium hydrogen phosphate anhydrous, potassium di-hydrogen phosphate, sodium acetate, potassium chloride, sodium hydroxide, dichloromethane, ethanol (99%), hydrochloric acid and acetic acid glacial (Merck, Mumbai, India). Nateglinide was of pharmaceutical grade (Alembic chemical works Ltd., Vadodara, India).

Preparation of Nateglinide Loaded Calcium Alginate Beads (CAB) In this method the Nateglinide loaded calcium alginate beads were prepared using varying concentration of calcium chloride in the gelation medium [2% (w/v), 5% (w/v)] while maintaining the drug : polymer ratio as 1 : 3 and 1 : 4 separately for varying concentration of calcium chloride on each occasion. Required amount of Nateglinide dissolved in dichloromethane was slowly dispersed in sodium alginate solution with constant stirring for 3 h, maintaining the drug : polymer ratio as 1 : 3 and 1 : 4 as mentioned above and the same was added dropwise into the gelation medium using a 5 ml hypodermic syringe having a 21 gauge needle under constant stirring at room temperature (25 °C) with the development of beads of appropriate dimensions. The beads formed were cured in the gelation medium for 4 h and then taken out, washed with distilled water twice and then dried at 30 °C in a dust free chamber till they attained constant weight.

Preparation of Nateglinide Loaded Alginate-Chitosan Beads (ACB-I) In this method the drug loaded alginate-chitosan beads were prepared using varying concentration of calcium chloride [2% (w/v), 5% (w/v)] and varying concentration of chitosan [0.5% (w/v), 0.75% (w/v); chitosan solutions were prepared with 3% v/v glacial acetic acid in distilled water] in the gelation medium while maintaining the drug : polymer ratio as 1 : 3 and 1 : 4 separately for varying concentration of calcium chloride and chitosan on each occasion. The gelation medium was prepared by mixing equal proportions of calcium chloride and chitosan solutions and the pH of the medium was adjusted to 4.5±0.1 with 0.1 N sodium hydroxide. The gelation medium was prepared 2 h before use. Required amount of Nateglinide dissolved in dichloromethane was slowly dispersed in sodium alginate solution with constant stirring for 3 h, maintaining the drug : polymer ratio as 1 : 3 and 1 : 4 as mentioned above and the same was added dropwise into the gelation medium using a 5 ml hypodermic syringe having a 21 gauge needle under constant stirring at room temperature (25 °C) with the development of beads of appropriate dimensions. The beads formed were cured in the gelation medium for 4 h and then taken out, washed with distilled water twice and then dried at 30 °C in a dust free chamber till they attained constant weight.

Preparation of Nateglinide Loaded Alginate-Chitosan Beads (ACB-II) In this method the technique utilized is modified from the method described under CAB by changing the time of curing from 4 to 2 h. The gelation medium used in this method is remaining the same but in this method instead of adding fine dispersion of Nateglinide in sodium alginate into the gelation medium (as in ACB-I), the drug loaded calcium alginate beads

* To whom correspondence should be addressed. e-mail: genomic2002@rediffmail.com

(CAB) were put into the gelation medium directly with constant stirring and then the beads were cured for 4 h at room temperature (25 °C). Finally, the beads were taken out, followed by washing with distilled water twice. The beads were dried at 30 °C in a dust free chamber till they attained constant weight.

Preparation of Nateglinide Loaded Multilayered Beads (MB) The method, described above to prepare the Nateglinide loaded alginate-chitosan beads (ACB-II) with 0.75% chitosan was repeated and the beads so obtained were immediately put in the sodium alginate solution (0.1% w/v) for 5 min and then transferred into calcium chloride solution (1% w/v) and cured for 30 min at room temperature (25 °C). Finally, the beads were taken out, followed by washing with distilled water twice and then allowed to dry at 30 °C in a dust free chamber till they attained constant weight.

Scanning Electron Microscopic Studies The surface morphology and appearance of randomly selected beads of CAB, ACB-I and II and multilayer beads were examined by a scanning electron microscopy (JEOL JSM-5200, Japan) operating between 5—24 kV. The specimens were mounted on a metal stub (with double side adhesive tape) and coated under vacuum with gold in nitrogen atmosphere prior to observation. The micrographs are shown in Fig. 1.

Particle Size Determination Bead size of all formulations was determined by optical microscopy. At least 100 beads were analyzed for each preparation and the mean particle size was calculated.

Determination of Drug Loading (%) Accurately weighed amount (10 mg) of drug-loaded beads were pulverized using mortor and pestle and incubated in 10 ml 0.02 M phosphate buffer (pH 6.8) at room temperature for 24 h for complete digestion and the drug was extracted with ethanol (99% v/v). The solution was filtered through a filter disc (particle retention: 11 μm) and then the filtrate was assayed spectrophotometrically for drug content at 210 nm. The same method was utilized to prepare the blank. All the experiments were performed in triplicate and then the percent of drug loading and incorporation efficiency was calculated using the following formula:

$$\text{experimental drug loading in \% (EL)} = L/L_0 \times 100$$

Where L is the actual drug content in the weighed quantity of the beads and L_0 is the weighed quantity of beads.

Swelling Behavior Studies The swelling properties of the calcium alginate, the alginate-chitosan and alginate-chitosan multilayer beads were determined in buffer solutions having pH 1.2, 4.5 and pH 6.8 at predetermined time intervals. Samples of beads of known weight (10 mg) were placed in a petridish containing 20 ml of swelling medium and allowed to swell at room temperature. The swollen beads were periodically (every 1 h) removed and weighed. The wet weight of the swollen beads was determined by blotting them with filter paper to remove moisture adhering on the surface, immediately followed by weighing on an electronic balance. The swelling ratio of the beads was calculated from the formula given below.[15]

$$\text{swelling ratio} = W_t/W_0$$

Were W_t is the weight of the beads at the defined time and W_0 is the initial weight of the beads. All the experiments were performed in triplicate.

Determination of Calcium Content Calcium content of calcium alginate and alginate chitosan beads of Nateglinide was determined by adopting the procedure given in the literature.[16]

Beads (100 mg) accurately weighed were dissolved in a few milliliters of concentrated nitric acid by boiling. The samples were made up to 10 ml with 1% v/v nitric acid and the calcium content was determined by atomic absorption spectroscopy (Perkin Elmer Model No-Analysis-200).

***In Vitro* Drug Release Studies** The drug release tests of the calcium alginate beads (CAB), alginate-chitosan beads (ACB-I and II) and multilayer beads were carried out using USP dissolution rate test apparatus types II (Electro Lab model TDT-08L) for 8 h with a stirring speed of 100 rpm using the temperature of 37±0.5 °C. The beads were accurately weighed equivalent to 20 mg of Nateglinide and filled in a hard gelatin capsule. The capsules were placed in the dissolution medium containing 600 ml of acetate buffer pH 6.8 B.P. containing 0.3% (w/v) of sodium dodecyl sulphate (SDS) to maintain the sink condition for the drug. Then 5 ml of the dissolution medium was sampled at predetermined time intervals, and fresh dissolution medium was simultaneously replaced in the apparatus on each occasion to keep the volume constant. The sample was filtered through filter disc (particle retention: 11 μm) during each withdrawal and analyzed for drug content at 210 nm on a spectrophotometer (160-UV–visible Shimadzu spectrophotometer). The results are shown as the graphical plots in Figs. 4 and 5. In addition, the release kinetics of Nateglinide from the beads was also evaluated using different models *viz.* Zero order, First order, Higuchi matrix, Peppas–Korsmeyer and Hixon–Crowell. Kinetic assessment of release data was carried out with a program PCP Disso v 2.08.

FT-Infrared Spectroscopic Analysis FT-IR spectroscopic studies of Nateglinide, sodium alginate, chitosan, and physical mixture of Nateglinide with sodium alginate (PM5), Nateglinide with sodium alginate and chitosan (PM6) and, Nateglinide loaded calcium alginate (C0) and alginate chitosan beads-II (C4) was analysed by adapting the procedure given below.

Individual beads were crushed in a mortar and pestle. The crushed material was mixed with potassium bromide (Merck IR spectroscopy grade) in 1 : 100 proportions and dried at 40 °C. The mixture was compressed to a 12 mm semitransparent disk by applying a pressure of 10 tons for 2 min. The FT-IR spectra over the wavelength range 4000 to 400 cm^{-1} were recorded using a FT-IR spectrometer (PERKIN ELMER 1600 series-FTIR).

Differential Scanning Calorimetric Analysis Differential scanning calorimetry (DSC) analysis was undertaken to characterize the changes if any observed during preparation of beads. DSC of pure drug, polymers like sodium alginate, chitosan, and physical mixtures PM5 and PM6, and bead samples C0 and C4 was carried out using a thermal analysis system (METTLER TA 4000 System). Calibration with standard (Indium) was undertaken prior to subjecting the samples for study (between 30—400 °C), which were heated at 10 °C/min in an aluminum pan under a nitrogen atmosphere while using an empty pan as the reference in this instrument. The instrument automatically calculated onsets of melting points and enthalpy of fusion.

X-Ray Powder Diffraction Analysis X-ray powder diffraction (XRD) analysis of the samples of Nateglinide, sodium alginate, chitosan, and physical mixtures PM5 and PM6, and bead samples C0 and C4 was carried out using the X-ray diffractometer (Rich Seifert Model 3000 P) at 30 kV, 15 mA over a range of 10—100 2θ, using Cu$K\alpha$ radiation wavelength 1.5405 Å. In the technique the cavity of the metal sample holder of X-ray diffractometer was filled with ground sample powder and then smoothed out with a spatula.

Results and Discussion

Mean Particle Size and Morphological Characteristics of the Beads Mean particle size of Nateglinide loaded bead formulations (CAB, ACB-I, II and multilayer beads) was varying 1.383±0.021 to 1.491±0.007 μm (Tables 1b, 2b). The multilayer beads showed an increase in particle size, probably due to extra coating with sodium alginate solution.

Scanning electron micrographs of Nateglinide loaded CAB, ACB-II and multilayer beads are presented in Fig. 1. The shape of the drug loaded alginate-chitosan beads was spherical (Fig. 1b) and the size of the beads was found to be very slightly higher with the increase in concentration of chitosan in the gelation medium suggesting the formation of a chitosan layer. The increase in size of the beads is quite obvious with increased in concentration of chitosan in the gelation medium.[6,17]

Drug Loading Efficiency The variation in the concentrations of chitosan, calcium chloride and drug to polymer ratio had less effect on the loading and incorporation efficiency of Nateglinide in the beads prepared. The variation in the concentrations of chitosan had an effect on the loading of Nateglinide in chitosan-alginate beads. In the absence of chitosan, entrapment of drug was slightly decreased. This may be due to insufficient cross-linking and large pore size permitting the drug to diffuse out during and after gelation.[12] Addition of 0.5—0.75% of chitosan in the gelation medium resulted in an increase in the entrapment. Increase in the concentration of calcium chloride decrease in drug loading; this may be attributed to increases in the porosity of the beads.[18] The drug loading was increased with increase in the drug to polymer ratio (Tables 1b, 2b).

Swelling Ratio of Nateglinide Loaded Calcium Alginate and Alginate-Chitosan (ACB-I, II), Multilayer Beads

Table 1a. Formulation Design for the Preparation of Nateglinide Loaded Calcium Alginate, Alginate-Chitosan Beads (ACB-I, II) and Multilayer Beads

Formulation code (drug polymer ratio 1 : 3/1 : 4)	Polymer concentration	Gelation medium		Post gelation treatment subsequently in the following solutions			
	SA (%, w/v)	$CaCl_2$ (%, w/v)	CH (%, w/v)	CH (%, w/v)	$CaCl_2$ (%, w/v)	SA (%, w/v)	$CaCl_2$ (%, w/v)
C0/C0a	5	2	—	—	—	—	—
C1/C1a	5	2	0.5	—	—	—	—
C2/C2a	5	2	0.75	—	—	—	—
C3/C3a	5	2	—	0.5	1	—	—
C4/C4a	5	2	—	0.75	1	—	—
C5/C5a	5	2	—	0.75	1	0.1	1

SA, sodium alginate, CH, chitosan.

Table 1b. Mean Particle Size, Drug Loading and Entrapment Efficiency of Nateglinide Loaded Calcium Alginate, Alginate-Chitosan Beads (ACB-I, II) and Multilayer Beads Prepared with a Gelation Medium Containing 2% (w/v) Calcium Chloride (Mean±S.D.; $n=3$)

Formulation code	Mean particle size (mm)±S.D.		Drug loading (%)±S.D.		Incorporation efficiency (%)±S.D.	
	1 : 3	1 : 4	1 : 3	1 : 4	1 : 3	1 : 4
C0/C0a	1.383±0.021	1.396±0.011	23.85±0.80	18.84±0.46	95.42±2.62	94.30±1.84
C1/C1a	1.376±0.039	1.420±0.011	24.18±0.52	19.45±0.31	96.73±1.72	97.28±1.26
C2/C2a	1.422±0.015	1.437±0.012	24.54±0.24	19.74±0.15	98.14±0.96	98.73±0.61
C3/C3a	1.416±0.011	1.431±0.010	23.40±0.63	18.90±0.37	93.62±2.08	94.50±1.54
C4/C4a	1.435±0.017	1.454±0.012	23.75±0.72	19.12±0.33	95.02±2.36	95.88±1.37
C5/C5a	1.447±0.015	1.461±0.012	23.73±0.36	19.10±0.24	94.92±1.46	95.58±0.98

Table 2a. Formulation Design for the Preparation of Nateglinide Loaded Calcium Alginate, Alginate-Chitosan Beads (ACB-I, II) and Multilayer Beads

Formulation code (drug polymer ratio 1 : 3/1 : 4)	Polymer concentration	Gelation medium		Post gelation treatment subsequently in the following solutions			
	SA (%, w/v)	$CaCl_2$ (%, w/v)	CH (%, w/v)	CH (%, w/v)	$CaCl_2$ (%, w/v)	SA (%, w/v)	$CaCl_2$ (%, w/v)
FC0/FC0a	5	5	—	—	—	—	—
FC1/FC1a	5	5	0.5	—	—	—	—
FC2/FC2a	5	5	0.75	—	—	—	—
FC3/FC3a	5	5	—	0.5	1	—	—
FC4/FC4a	5	5	—	0.75	1	—	—
FC5/FC5a	5	5	—	0.75	1	0.1	1

SA, sodium alginate, CH, chitosan.

Table 2b. Mean Particle Size, Drug Loading and Entrapment Efficiency of Nateglinide Loaded Calcium Alginate, Alginate-Chitosan Beads (ACB-I, II) and Multilayer Beads Prepared with a Gelation Medium Containing 5% (w/v) Calcium Chloride (Mean±S.D.; $n=3$)

Formulation code	Mean particle size (mm)±S.D.		Drug loading (%)±S.D.		Incorporation efficiency (%)±S.D.	
	1 : 3	1 : 4	1 : 3	1 : 4	1 : 3	1 : 4
FC0/FC0a	1.385±0.101	1.406±0.008	23.13±1.10	18.64±0.62	92.52±3.62	93.20±2.54
FC1/FC1a	1.415±0.130	1.423±0.007	24.10±0.49	19.44±0.32	96.42±1.61	97.23±1.64
FC2/FC2a	1.446±0.128	1.446±0.010	24.48±0.32	19.72±0.19	97.93±1.06	98.61±0.95
FC3/FC3a	1.430±0.011	1.442±0.008	23.20±0.78	18.24±0.49	92.81±2.56	91.21±2.01
FC4/FC4a	1.459±0.011	1.481±0.007	23.46±0.54	18.77±0.53	93.82±1.77	92.60±2.35
FC5/FC5a	1.491±0.007	1.450±0.013	23.48±0.56	18.74±0.76	93.92±1.85	93.70±3.11

The different types of beads of Nateglinide showed poor swelling without any sign of disintegration at pH 1.2 and at pH 4.5 during 8 h. However the swelling ratio of the beads at pH 4.5 was considerably greater than that at pH 1.2.

Figures 2 and 3 illustrates the swelling ratio of Nateglinide loaded calcium alginate and alginate-chitosan beads, multilayer beads at pH 1.2, 4.5 and at pH 6.8. It was observed that the chitosan treatment of the calcium alginate beads showed a swelling increment at low pH values of 1.2 and 4.5. Apart from the hydration of hydrophilic groups of chitosan treated alginate beads, another important factor that influences their swelling behavior at acidic pH is the protonization of the amino groups of chitosan while creates a repulsive force causes the swelling of the chitosan membrane. Thus, algi-

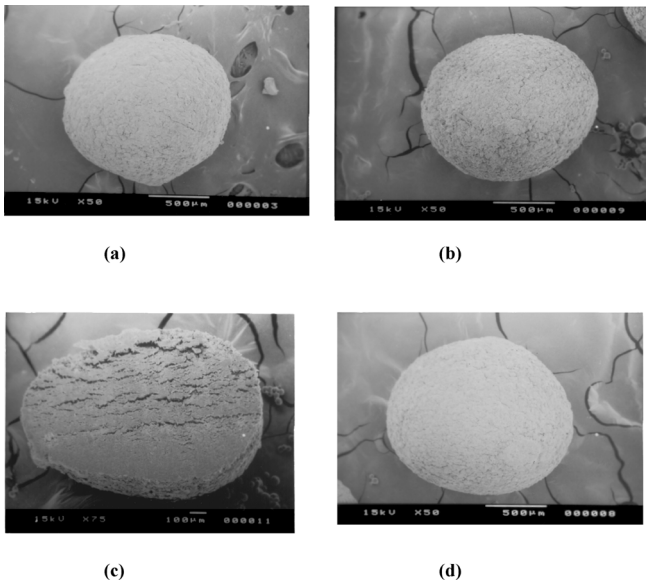

Fig. 1. Scanning Electron Micrographs of; (a) Nateglinide Loaded Calcium Alginate Bead (C0); (b) Nateglinide Loaded Alginate-Chitosan Bead (C4); (c) Cross Section of a Nateglinide Loaded Alginate-Chitosan Bead (ACB-II); (d) Nateglinide Loaded Alginate-Chitosan Multilayer Bead (MB)

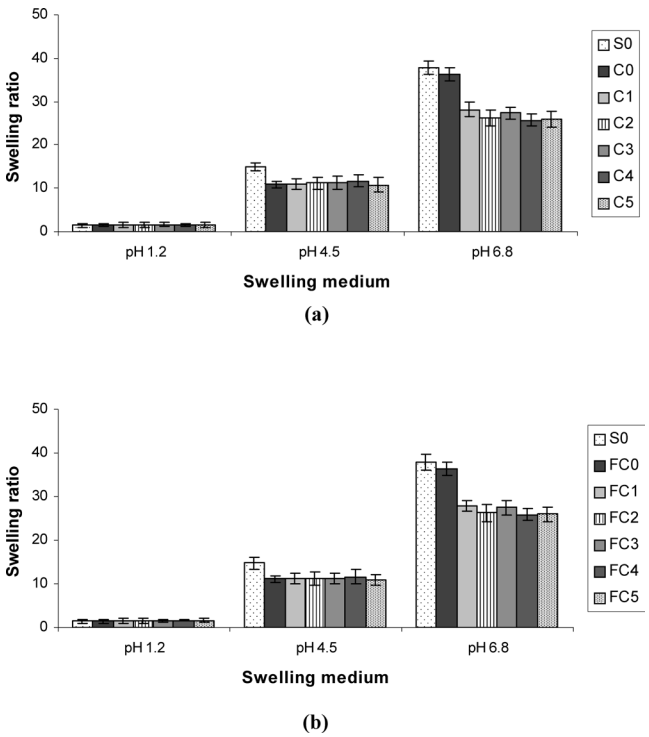

Fig. 2. Histogram Showing the Swelling Ratio *versus* Different Swelling Medium Relationship of Nateglinide Loaded Calcium Alginate, Alginate-Chitosan Beads (ACB-I, II) and Multilayer Beads Prepared with 1 : 3 Drug to Polymer Ratio Using (a) 2% (w/v) Calcium Chloride; (b) 5% (w/v) Calcium Chloride (Mean±S.D.; $n=3$)

nate-chitosan beads swell more than the calcium alginate beads at lower pH of 1.2 and 4.5.[19]

The swelling ratio of CAB, ACB-I, II and multilayer beads were found to be high in phosphate buffer pH 6.8 than the swelling ratio of the beads in other pH values of 1.2 and 4.5 (Figs. 2, 3). However the swelling ratio of calcium alginate beads of Nateglinide in phosphate buffer pH 6.8 was higher

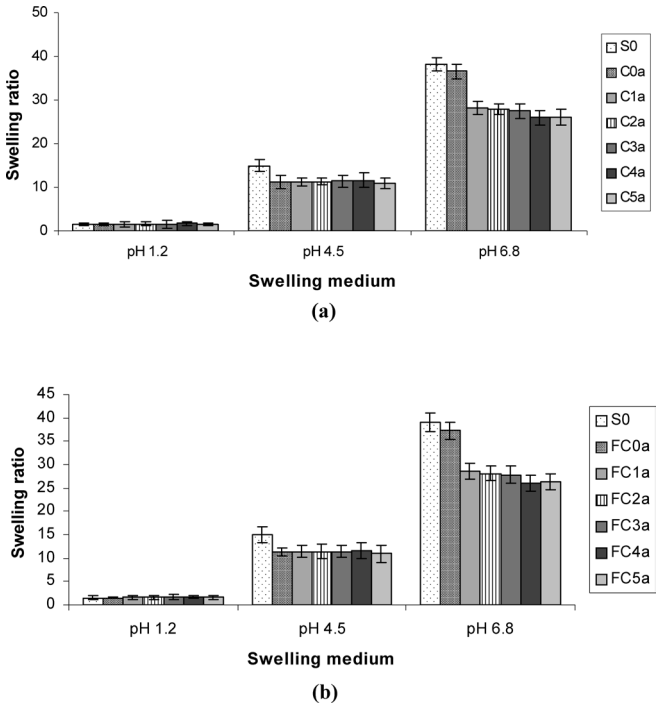

Fig. 3. Histogram Showing the Swelling Ratio *versus* Different Swelling Medium Relationship of Nateglinide Loaded Calcium Alginate, Alginate-Chitosan Beads (ACB-I, II) and Multilayer Beads Prepared with 1 : 4 Drug to Polymer Ratio Using (a) 2% (w/v) Calcium Chloride; (b) 5% (w/v) Calcium Chloride (Mean±S.D.; $n=3$)

than the alginate-chitosan beads and after 7 h the beads were started to disintegrate slowly. Thus it can be said that in the initial phase of the swelling process the Ca^{2+} ions present in polymannuronate units are exchanged with Na^{+} ions present in the buffer solution, which ultimately causes the chain relaxation and enhances the gel swelling. In the later stage of swelling process, the Ca^{2+} ions which are binding with $-COO^{-}$ group of the polyglucuronate units and thus form the tight egg-box structure also start to exchange with Na^{+} ions of the buffer medium because polyglucuronate sequences have a strong auto-cooperative binding of Ca^{2+} ions.[20]

Increase in the concentration of calcium chloride in the gelation medium resulted in an increase in the swelling ratio of the Nateglinide loaded calcium alginate and alginate-chitosan beads this may be attributed to increase in the porosity of the beads.[9]

Calcium Content of Nateglinide Loaded Calcium Alginate, Alginate-Chitosan Beads Calcium ion contents of the blank beads and Nateglinide loaded beads are shown in Table 3. It was observed that increase in polymer concentration in drug : polymer ratio caused an increase in the amount of calcium, which may be attributed to availability of larger binding sites in the polymer.[17] It was observed that amount of calcium decreased in formulation (C4, FC4) when compared with the formulation (C0). This indicates that alginate formed a complex both with calcium ions and chitosan.[21]

***In Vitro* Drug Release Studies of Nateglinide Loaded Beads** Comparing the Nateglinide release from drug loaded calcium alginate, alginate-chitosan beads and multilayer beads of Nateglinide; one should expect that the chitosan treatment would lessen the release profile of the drug.

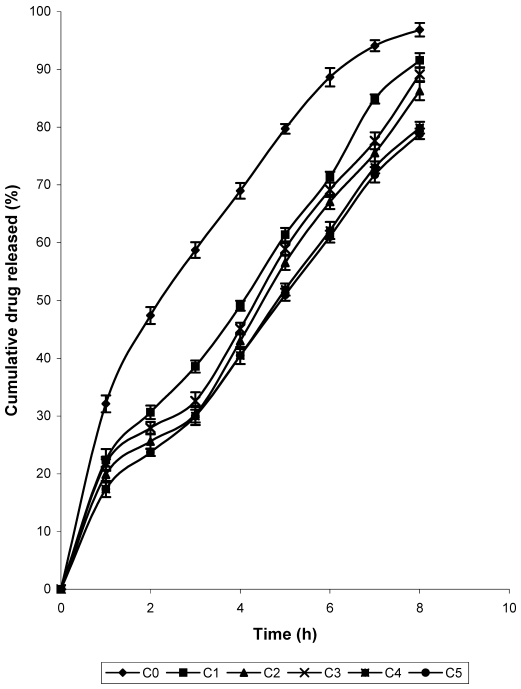

Fig. 4a. Cumulative % Release of Nateglinide at pH 6.8 Phosphate Buffer Containing 0.3% SLS, from Nateglinide Loaded Calcium Alginate, Alginate-Chitosan Beads (ACB-I, II) and Multilayer Beads Prepared with 1 : 3 Drug to Polymer Ratio Using 2% (w/v) Calcium Chloride (Mean±S.D.; $n=3$)

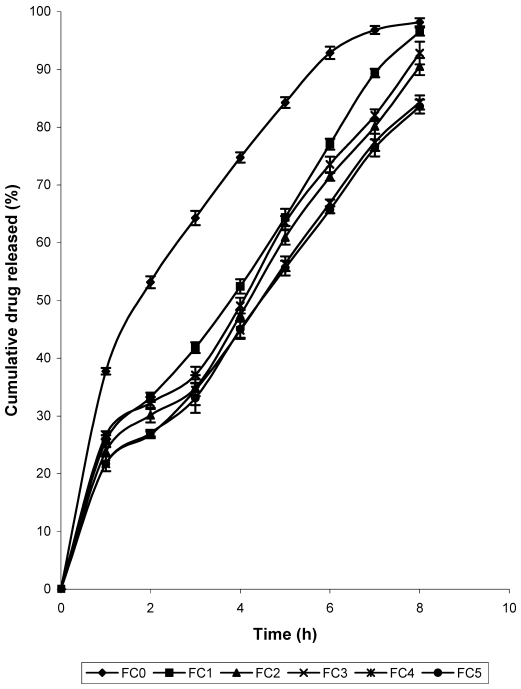

Fig. 4b. Cumulative % Release of Nateglinide at pH 6.8 Phosphate Buffer Containing 0.3% SLS, from Calcium Alginate, Alginate Chitosan Beads (ACB-I, II) and Multilayer Beads Prepared with 1 : 3 Drug to Polymer Ratio Using 5% (w/v) Calcium Chloride (Mean±S.D.; $n=3$)

Apart from the effect of chitosan concentration, the other factors such as calcium chloride concentration and drug to polymer ratio also influence the drug release. Beads prepared with 5% (w/v) calcium chloride showed increases in the release rate than the beads prepared with 2% (w/v) calcium chloride; this maybe due to increase in the porosity and swelling ratio of the beads with increase in the concentration of the calcium chloride. Increase in the drug : polymer ratio decreases the drug release because of higher polymer content with proportionately less drug at this specific condition so that the drug to polymer ratio was changed and thus release was reduced.

Table 3. Calcium (Ca^{2+}) Contents of the Bead Formulations of Nateglinide

Formulations	Ca^{++} content (mg/l)
SA0	361.338
SA4	303.563
C0	281.720
C4	257.880
FC0	310.483
FC4	272.422

SA0: blank calcium alginate beads; SA4: blank alginate-chitosan beads; C0: Nateglinide loaded calcium alginate beads prepared with 1 : 3 drug : polymer ratio using 2% calcium chloride; C4: Nateglinide loaded alginate-chitosan beads prepared with 1 : 3 drug : polymer ratio using 2% calcium chloride; FC0 Nateglinide loaded calcium alginate beads prepared using 5% calcium chloride; FC4: Nateglinide loaded alginate-chitosan beads prepared with 1 : 3 drug : polymer ratio using 5% calcium chloride.

Figures 4 and 5 illustrate the cumulative percent release of Nateglinide from calcium alginate beads, alginate chitosan beads and multilayer beads at pH 6.8 during 8 h. It was observed in the Nateglinide loaded calcium alginate beads (C0) prepared using 1 : 3 drug to polymer ratio with 2% (w/v) calcium chloride showed 96.86±1.16% of drug release with complete disintegration of the beads during 8 h at pH 6.8. The same bead formulation coated with 0.75% chitosan (C4) showed 79.91±1.02% of drug release, further treatment with alginate, the resultant multilayer bead formulation (C5) the drug release was slightly reduced to 78.84±0.91%. This may be due to the initial burst release that was considerably reduced with the incorporation of chitosan at drug loaded calcium alginate bead formulations. The probable reason for such behavior may be due to the electrostatic interaction between carboxyl groups of the alginate and the amino group of chitosan that reduced the swelling and erosion of the beads at pH 6.8.

Increase in the drug : polymer ratio decreases the drug release because higher polymer content with proportionately less drug, so that the drug–polymer ratio was changed and thus release was reduced. It was observed in the calcium alginate beads prepared using 1 : 3 drug to polymer ratio with 2% (w/v) calcium chloride showed 96.86±1.16% drug release with complete disintegration of the beads during 8 h at pH 6.8 and the drug release was reduced to 92.36±1.41% with increase in the drug to polymer ratio to 1 : 4. This may be attributed to an increase in the density of the polymer matrix, thereby retarding the burst effect to some extent.[22]

The drug loaded formulations both uncoated and chitosan coated C0 and C4 respectively were further utilized to characterize the physical state of the drug in the formulations and changes if any occurred during the experimental process.

Release Kinetics of Nateglinide Loaded Beads Drug release from a swellable matrix primarily depends on the degree of gelation, hydration, chain relaxation, and erosion of the polymer and follows the classical power law expression[23] as given below:

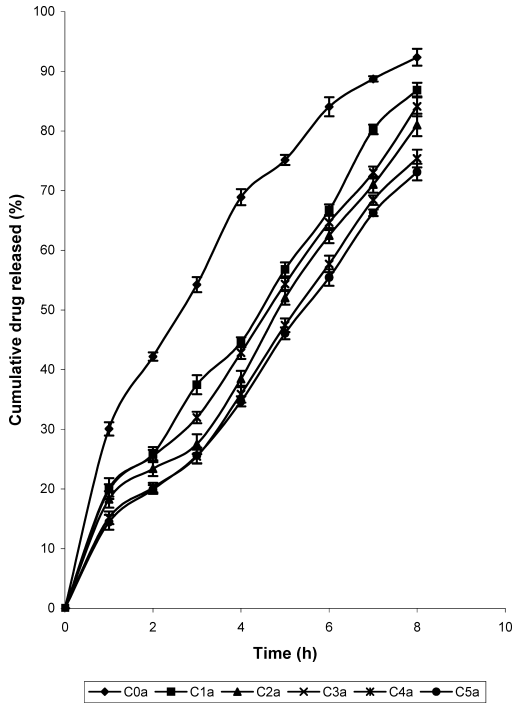

Fig. 5a. Cumulative % Release of Nateglinide at pH 6.8 Phosphate Buffer Containing 0.3% SLS, from Nateglinide Loaded Calcium Alginate, Alginate-Chitosan Beads (ACB-I, II) and Multilayer Beads Prepared with 1 : 4 Drug to Polymer Ratio Using 2% (w/v) Calcium Chloride (Mean±S.D.; $n=3$)

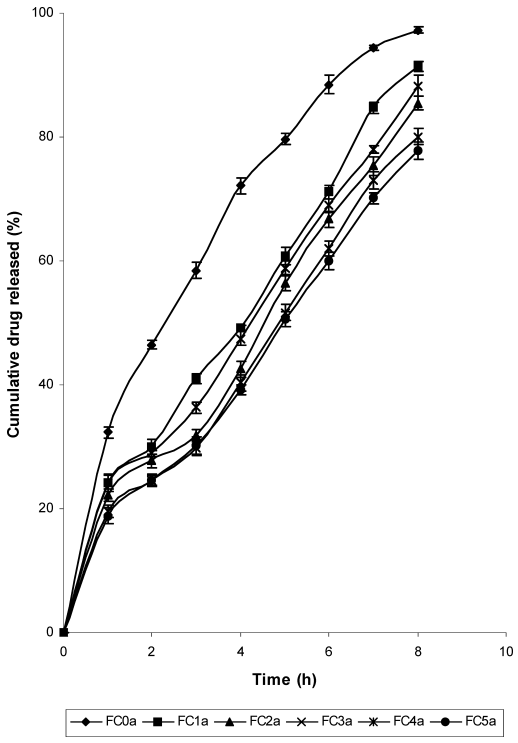

Fig. 5b. Cumulative % Release of Nateglinide at pH 6.8 Phosphate Buffer Containing 0.3% SLS, from Nateglinide Loaded Calcium Alginate, Alginate-Chitosan Beads (ACB-I, II) and Multilayer Beads Prepared with 1 : 4 Drug to Polymer Ratio Using 5% (w/v) Calcium Chloride (Mean±S.D.; $n=3$)

$$M_t/M_\infty = Kt^n$$

where M_t and M_∞ are, respectively, the amount of drug released at time t and at infinite time, K represents a constant incorporating structural and geometrical characteristics of the dosage forms, n denotes the diffusion exponent indicative of the mechanism of drug release. Values of n ranging from 0.45 to 0.5 indicate Fickian or diffusion controlled release; values of n ranging from 0.5 to 0.89 indicate non-Fickian or anomalous release, and values of n ranging from 0.89 to 1 indicate case II or zero order.

The release data of Nateglinide obtained from various calcium alginate beads were found to fit in the classical power law expression, and the values of n were found to be around 0.6. This indicates that drug release from alginate beads followed non-Fickian kinetics due to rapid swelling and erosion of the beads. The drug release data from alginate-chitosan beads also fitted well in the power law expression, and the values of n decreased below 1. The formation of polyelectrolyte complex membrane reduced the initial swelling and erosion of the beads and shifted the drug release mechanism toward anomalous diffusion indicating that drug was diffusing out through the beads with simultaneous polymer relaxation. Because the release of the drug from alginate-chitosan beads exhibited a small time lag in initial release, the release data were fitted in the modified power law expression, and excellent linearity was noted. However the drug release mechanism was that of anomalous diffusion.

FT-IR Spectroscopic Analysis FT-IR of Nateglinide shows the principle peaks at the wave numbers of 1213—1386 cm^{-1} justifying the presence of carboxyl, carboxylate groups, and carbonyl at 1646 cm^{-1}, C–H stretching between at 2857—3030 cm^{-1}, C=O vibration at 1723 and NH stretching appeared at 3296 cm^{-1}.

In the FT-IR spectrum of sodium alginate powder various distinct peaks of alginate are evident of hydroxyl group appeared at 3428 cm^{-1}, carbonyl at 1623 cm^{-1}, and carboxyl and carboxylate groups appeared between 1000 to 1400 cm^{-1}. The absorption band around 2933, 1623, 1419 and 1031 cm^{-1} corresponds to the stretching of –CH, COO–, –CH and C–O–C, respectively. In the FT-IR spectrum of drug loaded alginate-chitosan bead formulation (C4) the band 2876 cm^{-1} corresponding to chitosan pure was shifted to 2932 cm^{-1} indicates the confirmation of complex formation between chitosan and alginate.

The principle peaks corresponding to Nateglinide was appeared with less intensity in the bead formulations. The decreases in peak intensity may attribute to a fine dispersion of the drug in the polymers and to increasing drug to polymer ratios.[24]

Differential Scanning Calorimetric Studies Figure 6 illustrates the comparative DSC thermogram of Nateglinide, sodium alginate, chitosan, physical mixtures (PM5, PM6) and Nateglinide loaded bead formulations (C0, C4).

A sharp endothermic peak corresponding to the melting of crystalline Nateglinide was found at 138.7 °C and the melting endotherm of Nateglinide in physical mixtures PM5 and PM6 appeared at 139.6 °C and at 138.2 °C respectively. In bead formulation C0 the melting endotherm of Nateglinide appeared at 138.2 °C and in C4 appeared at 138.3 °C. The melting endotherm corresponding to interaction of alginate

Table 4a. Correlation Coefficients of Different Kinetic Models for Nateglinide Loaded Calcium Alginate and Alginate-Chitosan Beads Prepared with 1 : 3 Drug to Polymer Ratio Using 2% (w/v) and 5% (w/v) Calcium Chloride (Drug Release Studied at pH 6.8)

Formulation code	Kinetic models					
	Zero-order [% released $=k$.time] r^2	First-order [log(fraction unreleased) $=(k/2.303)$.time] r^2	Higuchi [% released $=k$ (time^0.5)] r^2	Hixon–Crowell [(fraction unreleased) ^(1/3)$=1-k$.time] r^2	Peppas–Korsmeyer [% released$=k$ (time^n)] r^2	n
C0	0.9060	0.9708	0.9974	0.9953	0.9974	0.5473
C1	0.9868	0.9422	0.9628	0.9777	0.9863	0.7056
C2	0.9909	0.9515	0.9457	0.9779	0.9713	0.7490
C3	0.9882	0.9433	0.9523	0.9756	0.9710	0.7124
C4	0.9934	0.9682	0.9469	0.9853	0.9820	0.7739
C5	0.9930	0.9708	0.9494	0.9829	0.9868	0.7641
FC0	0.8541	0.9732	0.9968	0.9939	0.9968	0.4786
FC1	0.9827	0.9065	0.9669	0.9663	0.9824	0.6693
FC2	0.9840	0.9445	0.9599	0.9776	0.9708	0.6767
FC3	0.9776	0.9369	0.9678	0.9757	0.9729	0.6356
FC4	0.9867	0.9670	0.9595	0.9861	0.9760	0.6972
FC5	0.9858	0.9671	0.9594	0.9858	0.9736	0.6902

Table 4b. Correlation Coefficients of Different Kinetic Models for Nateglinide Loaded Calcium Alginate and Alginate-Chitosan Beads Prepared with 1 : 4 Drug to Polymer Ratio Using 2% (w/v) and 5% (w/v) Calcium Chloride (Drug Release Studied at pH 6.8)

Formulation code	Kinetic models					
	Zero-order r^2	First-order r^2	Higuchi r^2	Hixon–Crowell r^2	Peppas–Korsmeyer r^2	n
C0a	0.9132	0.9934	0.9957	0.9943	0.9958	0.5656
C1a	0.9906	0.9550	0.9558	0.9811	0.9850	0.7418
C2a	0.9910	0.9590	0.9388	0.9790	0.9667	0.7605
C3a	0.9905	0.9586	0.9532	0.9819	0.9787	0.7275
C4a	0.9944	0.9663	0.9320	0.9818	0.9761	0.8211
C5a	0.9951	0.9702	0.9345	0.9842	0.9808	0.8224
FC0a	0.9057	0.9720	0.9972	0.9968	0.9972	0.5503
FC1a	0.9828	0.9443	0.9663	0.9784	0.9817	0.6717
FC2a	0.9853	0.9564	0.9533	0.9797	0.9652	0.6850
FC3a	0.9821	0.9546	0.9651	0.9819	0.9735	0.6590
FC4a	0.9895	0.9664	0.9489	0.9835	0.9710	0.7215
FC5a	0.9892	0.9723	0.9536	0.9766	0.9867	0.7146

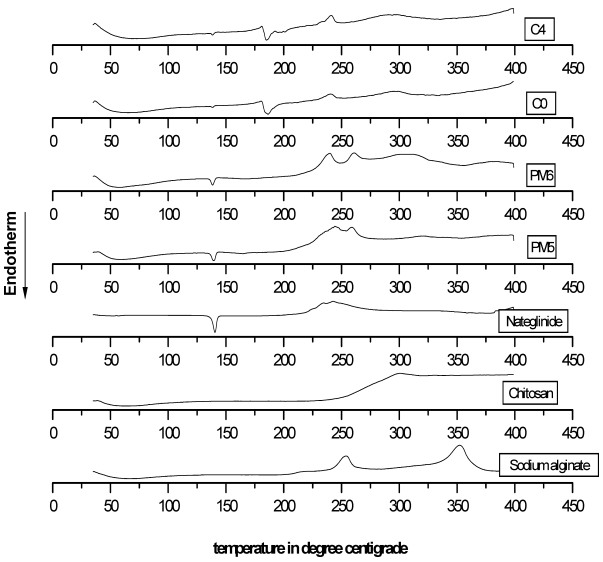

Fig. 6. Comparative DSC Thermogram of Sodium Alginate; Chitosan; Nateglinide; Physical Mixture of Nateglinide with Sodium Alginate (PM5); Physical Mixture of Nateglinide with Sodium Alginate and Chitosan (PM6); Nateglinide Loaded Alginate Beads (C0); Nateglinide Loaded Alginate Beads Coated with Chitosan (C4)

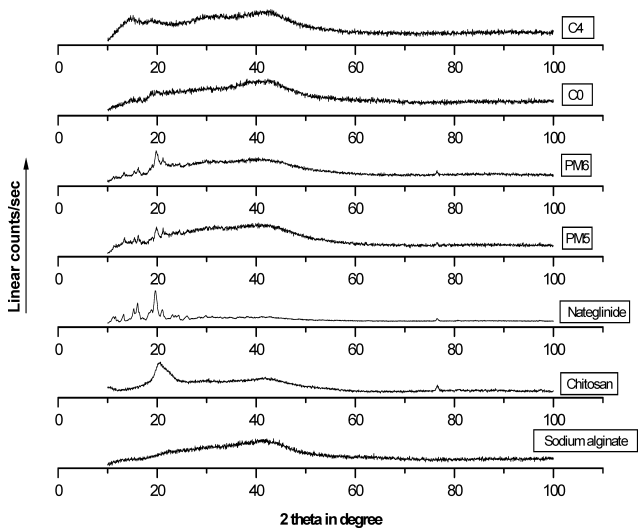

Fig. 7. Comparative X-Ray Powder Diffraction Pattern of Sodium Alginate; Chitosan; Nateglinide; Physical Mixture of Nateglinide with Sodium Alginate (PM5); Physical Mixture of Nateglinide with Sodium Alginate and Chitosan (PM6); Nateglinide Loaded Alginate Beads (C0); Nateglinide Loaded Alginate Beads Coated with Chitosan (C4)

with calcium ion was observed at 186.1 °C and 184.6 °C in Nateglinide loaded bead formulations C0 and C4 respectively. It was observed that no variation in melting endotherm but significantly varied in peak intensity; this may be attributable to a homogeneous dispersion of the drug in the polymers and also may be due to increase in drug to polymer ratio.[24)]

X-Ray Powder Diffraction Analysis Figure 7 illustrates the comparative X-ray powder diffraction (XRD) pattern of Nateglinide, sodium alginate, chitosan, physical mixtures (PM5, PM6) and Nateglinide loaded bead formulations (C0, C4). The X-ray diffractometry profile of pure Nateglinide showed the diffractogram of crystalline product, and the XRD profile of sodium alginate indicated the presence of a completely amorphous material, and chitosan showed few crystalline peaks with very low intensity. The XRD pattern of the physical mixtures (PM5, PM6) showed no difference in XRD pattern, but difference was observed in XRD patterns of bead formulations of Nateglinide when compared with that of physical mixtures. This was possibly due to fact thus a decreasing in the degree of crystalinity of the drug that might have occurred and were well dispersed in the polymer matrix.[25,26)]

Conclusions

In conclusion, this study shows that the drug loading, swelling ability and release characteristics of Nateglinide loaded calcium alginate, alginate-chitosan beads (ACB I, II) and multilayer beads is dependent on the presence of the polyelectrolyte complex between alginate and chitosan; calcium chloride concentration in the gelation medium, drug to polymer ratio, and the pH of dissolution medium. The FT-IR studies revealed that no drug polymer interaction occurred during the preparation of the formulations. Differential scanning calorimetric and XRD studies conformed qualitatively the physical state of the drug Nateglinide in the beads, but the XRD studies revealed that the crystalline peaks of the drug Nateglinide were significantly disappeared in the bead formulations this indicated that the drug well dispersed in the polymer matrix at molecular level. *In vitro* release study revealed that the drug release of alginate beads could be reduced considerably by treating the drug-loaded alginate beads with chitosan and further treatment with alginate coating of alginate-chitosan beads prolonged the release of Nateglinide to a little extent. Analysis of the release profiles showed that the drug release mechanisms were either "anomalous transport" or "case-II transport".

References

1) Ouwerx C., Velings N., Mestdagh M. M., Axelos M. A., *Polym. Gels Netw.*, **6**, 393—408 (1998).
2) Bajpai S. K., Sharma S., *React. Funct. Polym.*, **59**, 129—140 (2004).
3) Griffith L. G., *Acta Mater.*, **48**, 263—277 (2000).
4) Rasmussen M. R., Snabe T., Pedersen L. H., *J. Controlled Release*, **91**, 395—405 (2003).
5) Bodmeier R., Paeratakul O., *J. Pharm. Sci.*, **78**, 964—967 (1989).
6) Sezer A. D., Akbuga J., *J. Microencapsul.*, **16**, 687—696 (1999).
7) Fernández-Hervás M. J., Holgado M. A., Fini A., Fell J. T., *Int. J. Pharm.*, **163**, 23—34 (1998).
8) Lee K. Y., Park W. H., Ha W. S., *J. Appl. Polym. Sci.*, **63**, 425—432 (1996).
9) Gåserød O., Smidsrød O., Skjåk-Bræk G., *Biomaterials*, **19**, 1815—1825 (1998).
10) Becherán-Marón L., Peniche C., Argüelles-Monal W., *Int. J. Biol. Macromol.*, **34**, 127—133 (2004).
11) González-Rodríguez M. L., Holgado M. A., Sánchez-Lafuente C., Rabasco A. M., Fini A., *Int. J. Pharm.*, **232**, 225—234 (2002).
12) Anal A. K., Stevens W. F., *Int. J. Pharm.*, **290**, 45—54 (2005).
13) Lin Y. H., Liang H. F., Chung C. K., Chen M. C., Sung H. W., *Biomaterials*, **26**, 2105—2113 (2005).
14) Gotoh T., Matsushima K., Kikuchi K. I., *Chemosphere*, **55**, 135—140 (2004).
15) Shu X. Z., Zhu K. J., *Eur. J. Pharm. Biopharm.*, **53**, 193—201 (2002).
16) Takka S., Omer H., Acartürk F., *Eur. J. Pharm.*, **6**, 241—246 (1998).
17) Anal A. K., Bhopatkar D., Tokura S., Tamura H., Stevens W. F., *Drug Dev. Ind. Pharm.*, **29**, 713—724 (2003).
18) Martinsen A., Skjask-Brñk G., Smidsrod O., *Biotechnol. Bioeng.*, **33**, 79—89 (1989).
19) George P., Nikolaos B., *Int. J. Pharm.*, **323**, 34—42 (2006).
20) Smidsrod O., Skjask-Brñk G., *Trends Biotechnol.*, **8**, 71—78 (1990).
21) Takka S., Acarturk F., *J. Microencapsul.*, **16**, 275—290 (1999).
22) Sah H. K., Toddywala R., Chien Y. W., *J. Controlled Release*, **30**, 201—211 (1994).
23) Ritger P. L., Peppas N., *J. Controlled Release*, **5**, 37—42 (1987).
24) Rosario P., Marinella F., Giovanni P., *AAPS PharmSciTech*, **3**, 1—11 (2002).
25) Yang M.-S., Cui F.-D., You B.-G., Fan Y.-L., Wang L., Yue P., Yang H., *Int. J. Pharm.*, **259**, 103—113 (2003).
26) Rosario P., Daniela S., Maria Angela V., Flavio F., Giovanni P., *Drug Dev. Ind. Pharm.*, **30**, 277—288 (2004).