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ORIGINAL PAPER

Preparation and characterization of nifedipine-loaded cellulose acetate butyrate based microspheres and their controlled release behavior

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Abstract Eudragit L100/cellulose acetate butyrate blend microspheres were prepared by solvent evaporation technique using poly(vinyl alcohol) as an emulsifying agent nifedipine (NFD) was successfully loaded into these microspheres. The effect of experimental variables such as ratio of blend ratio on NFD encapsulation efficiency, release rate, size, and morphology of the microspheres has been investigated. Scanning electron micrographs indicated the formation of spherical microspheres. Mean particle size of the microspheres has been measured by the laser light scattering technique ranged between 100 and 120 μm. NFD was successfully encapsulated up to 80% in the polymeric matrices. In vitro dissolution experiments performed in pH 7.4 buffer medium indicated a controlled release of NFD from the blend microspheres up to 12 h.

Keywords Eudragit L100 · Cellulose acetate butyrate · Controlled release

Introduction

Evolution of pharmaceutical technology has lead to the development of newer methods of drug administration as well as the design and application of controlled release (CR) formulations for the effective targeting of certain drugs to the site of action. In particular, the use of polymeric systems provides a way to develop CR dosage formulations to achieve the desired therapeutic results to target site as well

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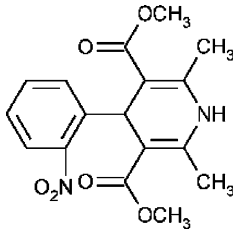
as optimization of CR of the drug to obtain the maximum dose regimen with minimum of side effects [1]. The release of a drug from a polymeric matrix occurs due to transport of solute molecules (drug) to the medium that surrounds the system by a molecular diffusion phenomenon through walls of the polymeric microspheres.

Microparticulate drug delivery systems are continuously investigated to study the CR of orally administered drugs as compared to the single-unit-dosage forms. Advantages of microspheres for the oral delivery of drugs over the conventional dosage forms have been reported by Abu-Izza et al. [2]. A uniform distribution of multiunit dosage form along the gastrointestinal tract (GIT) could result in a more reproducible drug absorption with a reduced risk of local irritations than the use of single-unit-dosage forms [3]. These particles protect the liable compounds (e.g., proteins and peptides) from degradation in the GIT [4]. Several techniques have been described in the literature for the preparation of microspheres, including solvent evaporation [5], phase separation [6], spray-drying [7], and in situ polymerization [8]. Of these, the solvent evaporation method is widely used because of its good reproducibility and versatility to offer the desired properties to the microspheres. This method involves an emulsification step followed by the removal of solvent via extraction and evaporation.

Solvent evaporation is commercially viable method as well as, the formulations can be designed in requirement form with desired properties such as encapsulation efficiency, particle size, and release pattern. Cellulose acetate butyrate and RL100 (RL) polymers are being used for the enteric coating of tablets and for preparing controlled-release formulations. RL is copolymers of poly(ethylacrylate, methyl-methacrylate and chlorotrimethyl-ammonioethyl methacrylate), containing an amount of quaternary ammonium groups ranging between 4.5% and 6.8%, RL, respectively. These matrixes are insoluble in water at physiological pH values and capable of swelling, so they are good for the dispersion of active compounds. As one group of the essential materials for the progress in drug delivery systems, intelligent polymers are illustrated. Depending on the change in temperature, light, pH, glucose concentration in blood and the like, the drug release ability of intelligent polymers developed up to now changes [9–13].

Nifedipine (NFD) (Scheme 1) is a prototype 1,4-dihydropyridine calcium channel blocker, which can be used to treat hypertension [14]. In the present work, we aimed to prepare biodegradable blend microspheres consisting of Eudragit L100 and cellulose acetate butyrate by taking different amounts of EL100 as well as cellulose acetate butyrate in the matrix. Previous studies [15–17] describe the preparation of ethylcellulose, hydroxypropylmethylcellulose-loaded NFD, oral formulations; whereas, our system overcome in terms of biodegradability and pH. Eudragit is a well-accepted polymer for coatings as well formulations. NFD was loaded into EL100/cellulose acetate butyrate blend microspheres using poly(vinyl alcohol) as a stabilizer cum emulsifier to produce drug-loaded microspheres of uniform size. The microspheres were characterized by scanning electron microscope and particle size analyzer for estimating their shapes and sizes, respectively. The dissolution experiments were performed to study the drug release characteristics of the microspheres.

Scheme 1 Chemical structure of nifedipine



Experimental

Materials

Eudragit L100 and cellulose acetate butyrate were gift samples from Evonik Ltd. Poly(vinyl alcohol) and Tween 80 were purchased from Aldrich Chemical Co. Milwaukee, USA. Nifedipine was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India.

Preparation of NFD-loaded Eudragit L100/cellulose acetate butyrate particles

Five hundred micrograms of Eudragit L100 (6.0–50.6% methacrylic acid units on dry substance (DS). Acid value: 300–330 mg KOH per g DS) was dissolved in 10 mL of methanol and 500 mg of cellulose acetate butyrate was dissolved in 50 mL of dichloromethane. Both the solutions were mixed well under stirring.

To this polymer solution, different amounts of NFD solution in acetone was added and homogenized at 11,000 rpm for 1 min to form a primary emulsion. After forming primary emulsion, the total solution was poured in to a beaker containing 100 mL of 1% poly(vinyl alcohol) and stirred at 600 rpm for 3 h to evaporate the solvent. After 3 h, the particles are filtered and washed with water and dried at RT for overnight. Different variations of drug loading blend ratio particle are prepared. The methanol and dichloromethane are miscible but, when drug-loaded solution in acetone poured in to this solution, drug has been precipitated. To avoid this precipitation, the total solution was homogenized immediately to form primary emulsion. Primary is dispersing phase, and PVA solution is continuous phase. The composition of disperse and continuous phases in this primary emulsion is 5:100. Due to unstability of NFD to light, sample preparation was carried in dark light in hood to avoid the photo degradation.

Scanning electron microscopy

Scanning electron microscopy (SEM) images of the microspheres were recorded using a Hitachi S520 scanning electron microscope (Japan) at the required magnification. A working distance of 33.5 mm was maintained and the acceleration voltage used was 15 kV with the secondary electron image (SEI) as a detector. Samples were gold coated with thickness in the range of 10–15 nm.

Particle size analysis

Particle size of the microspheres was measured by using a particle size analyzer (Mastersizer 2000, Malvern Instruments, UK). About 500 mg of microspheres were transferred to the dry sample holder and stirred vigorously to avoid the agglomeration of particles during measurements. For measurement of sizes of different formulations/batches, the sample holder was cleaned by vacuum. The particle size was also measured using an optical microscopy.

Estimation of drug loading and encapsulation efficiency

Specific amount of dry microspheres were vigorously stirred in a beaker containing 10 mL of dichloromethane to extract the drug from the microspheres. A 10 mL of 7.4 pH phosphate buffer containing 0.02% Tween-80 was added to the above solution to make the drug soluble and dichloromethane was evaporated with a gentle heating and continuous shaking. The aqueous solution was then filtered and assayed by UV spectrophotometer (model Anthelie, Secomam, Dumont, France) at the fixed λ_{max} value of 238 nm. The results of % NFD loading and encapsulation efficiency were calculated using Eqs 1 and 2. These results are compiled in Table 1.

$$\%\ \text{Drug loading} = \left(\frac{\text{wt of drug in microspheres}}{\text{wt of microspheres}}\right) \times 100 \quad (1)$$

$$\%\ \text{Encapsulation efficiency} = \left(\frac{\text{Actual loading}}{\text{Theoretical loading}}\right) \times 100. \quad (2)$$

In vitro release

In vitro release studies have been carried out by performing the dissolution experiments using a tablet dissolution tester (LabIndia, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at 37 °C under 100 rpm rotor speed. Drug release from the microspheres was studied in an intestinal (7.4 pH phosphate buffer) fluid. At regular intervals of time, sample aliquots were withdrawn and analyzed by UV spectrophotometer (Model Anthelie, Secomam, Dumont, France) at the fixed λ_{max} value of 238 nm.

Table 1 Results of encapsulation efficiency an mean particle size of different formulations

Formulation code	% EL100 (wt/wt)	% Cellulose acetate butyrate (wt/wt)	NFD (wt%)	Encapsulation efficiency (%)	Mean particle size (μm)
EL100/CAB1	10	90	5	65.3	92 ± 6
EL100/CAB-2	10	90	10	66.6	98 ± 2
EL100/CAB-3	10	90	15	70.5	101 ± 5
EL100/CAB-4	20	80	10	60.2	96 ± 3
EL100/CAB-5	30	70	10	62.1	112 ± 6
EL100/CAB-6	00	100	10	53.5	125 ± 8

Results and discussion

Scanning electron microscopy

SEM images of single microspheres (EL100/CAB1) taken at 500× magnifications are shown in Fig. 1. Microspheres are spherical without forming agglomeration and their surfaces are slightly rough. However, polymeric debris seen around some particles could be due to the method of particle production (i.e., simultaneous particle production and formation of the blend matrix).

Laser particle size analyzer

Results of mean particle size with standard errors are presented in Table 1, while the size distribution curve for a typical formulation containing 10% NFD-loaded EL100 particles was displayed in Fig. 2. It is obvious that size distribution is narrow and volume mean diameter of the microspheres is found to be 120 μm. Particle size of different formulations containing different amount of drug, different amount of cellulose acetate butyrate are presented in Table 1.

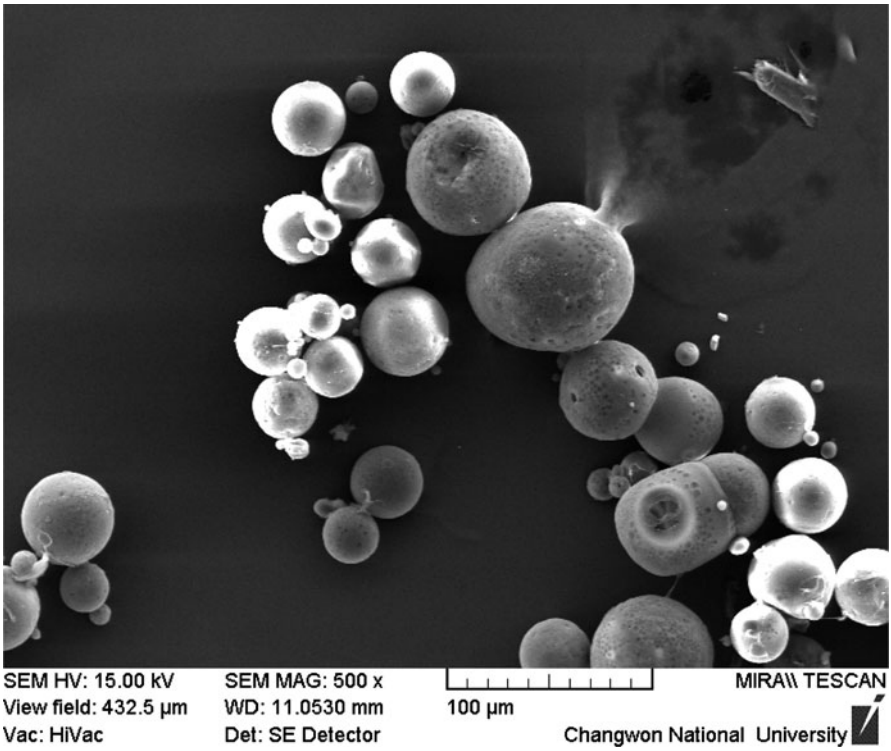


Fig. 1 SEM photograph of EL100/CAB1 microspheres

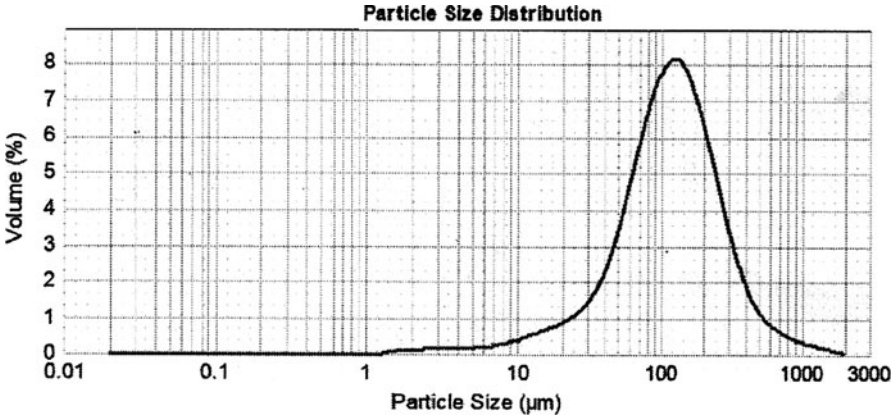


Fig. 2 Particle size distribution curve of EL100/CAB microspheres

For all the formulations, with increasing amount of drug in the microspheres, particle size also increased. For formulations containing 10% EL100 and microspheres loaded with different amounts of drug, particle size has increased from 92 to 101 μm; a similar trend was also observed for all other formulations (see Table 1). This is attributed to the fact that drug molecules might have occupied the free volume spaces within the matrix, thereby hindering the inward shrinkage of the polymer matrix.

Encapsulation efficiency

Three different concentrations of NFD, i.e., 5, 10 and 15 wt% were loaded during preparation of the microspheres. Results of % encapsulation efficiency included in Table 1 show increasing trends with increasing drug loading. Encapsulation efficiency of 53.5% was observed for pristine CAB microspheres, but for the remaining formulations, it ranged from 62% to 70%. Such smaller values are due to a lesser soluble drug in the polymer solution, thus incorporating a lesser amount of NFD into microspheres. Notice that % encapsulation efficiency increased with increasing amount of EL100 in the blend microspheres. For microspheres containing 10, 20, and 30 wt% EL100 and 10 wt% NFD encapsulation efficiencies were 65.3, 66.6 and 70.5, respectively.

Drug release kinetics

Drug release kinetics was analyzed by plotting the cumulative release data versus time by fitting these data to exponential equation of the type [14, 18].

$$\left(\frac{M_t}{M_\infty}\right) = kt^n \tag{3}$$

Here M_t/M_∞ represents the fractional drug release at time t, k is a constant characteristic of the drug-polymer system and n is an empirical parameter

Table 2 Release kinetics parameters of different formulations

Formulation code	$k \times 10^3$	n	Correlation[a] coefficient, r
EL100/CAB-1	0.2979	0.3713	0.9828
EL100/CAB-2	0.4641	0.4476	0.9715
EL100/CAB-3	0.6211	0.5509	0.9780
EL100/CAB-4	0.0491	0.3851	0.9704
EL100/CAB-5	0.9501	0.8081	0.9538
EL100/CAB-6	0.0893	0.3857	0.9552

[a] The r values were calculated at 95% confidence limit

characterizing the release mechanism. Using the least squares procedure, we have estimated the values of n and k for all the nine formulations and these values are given in Table 2. If $n = 0.5$, the drug diffuses and releases out of the polymer matrix following a Fickian diffusion. For $n > 0.5$, an anomalous or non-Fickian type drug diffusion occurs. If $n = 1$, a completely non-Fickian or Case II release kinetics is operative. The intermediary values ranging between 0.5 and 1.0 are attributed to anomalous-type diffusive transport [14].

The values of k and n have shown a dependence on the extent of crosslinking, % drug loading, and EL100 content of the matrix. Values of n for beads prepared by varying the amount of EL100 in the blend microspheres of 10, 20, and 30 wt% and keeping NFD (10%) are ranged from 0.3713 to 0.8081 leading to a shift of transport from Fickian to the anomalous type. The NFD-loaded particles exhibited n values ranging from 0.3713 to 0.5509 (see Table 2), indicating the shift from erosion-type release to a swelling-controlled, non-Fickian type mechanism. This may be due to the reduction in the regions of low microviscosity and closure of microcavities in the swollen state of the polymer. Similar findings have been observed elsewhere, wherein the effect of different polymer ratios on dissolution kinetics was studied.

Effect of Eudragit L100 content

The effect of EL100 content was studied at a constant loading of 10 wt% NFD. It was found that CAB produced almost 100% cumulative drug release in about 10 h, whereas EL100-CAB blend microspheres produced up to 90% cumulative release in 12 h. Release of EL100-CAB microspheres prepared with different amounts of EL100 are displayed in Fig. 3. Notice that during the dissolution experiments, microspheres have systematically swollen with the increasing amount of EL100. As the amount of EL100 increases, the hydrophibicity slightly decreases because EL100 is not fully hydrophilic. While comparing to CAB and EL1000, the hydrophobicity is more for CAB rather than for EL100. Therefore, the EL100/CAB-6 shows higher release than other formulations. The total hydrophobicity of the formulation depends on the blend ratio. As the amount of EL100 increases, the cumulative release has increased due to larger swelling of the EL100 chains than CAB. This is due to the fact that as the amount of EL100 increases in the blend

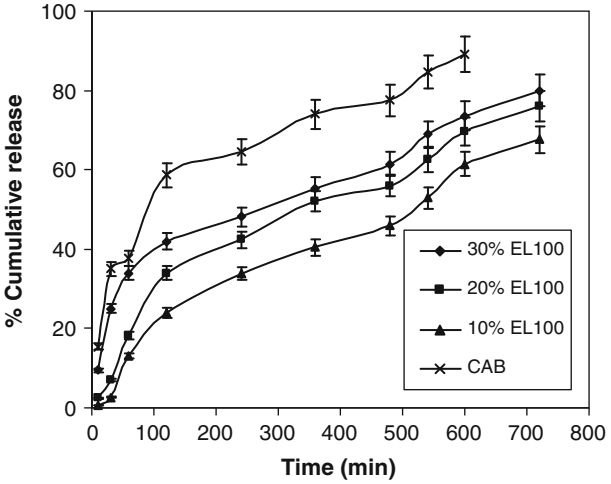


Fig. 3 % Cumulative release of NFD through EL100/CAB microspheres containing different amount of NFD at pH 7.4

matrix the hydrophobicity of the blend decreases. Thus, a regaining-type response of the polymeric chains is possible due to the stresses induced by the surrounding solvent media during dissolution, resulting in a decrease of chain dimension (radius of gyration) of the polymer; this will further decrease molecular volume of the hydrated polymer due to increased swelling of EL100 component of the blend matrix, thereby reducing the free volume space of the blend matrix. CAB is produced almost 100% release where as blend particles shows only 90% release, this is due to the independence of pH property of CAB, where as EL 100 is dependent on the pH of the media, however, having lesser encapsulation for CAB. Notice that the nature of release profiles remains almost identical in all the formulations containing different amounts of EL100, indicating a linear relationship with the drug release profiles.

Effect of drug loading

Figure 4 shows the release profiles of NFD-loaded EL100-CAB blend microspheres at different amount of drug loadings. Release data showed that formulations containing the highest amount of drug (15 wt%) displayed the fast and higher release rates than those formulations containing small amount of NFD. A prolonged release was observed for the formulation containing a lower amount of NFD. In other words, with decreasing amount of drug in the matrix, a shift from anomalous-type release to Case II is observed. Notice that the release rate becomes quite slower at the lower amount of drug in the matrix, due to the availability of more free void spaces through which a lesser number of drug molecules will transport. For all the NFD-loaded formulations, the complete release of NFD was not observed even after 600 min, but release rates have occurred around 700 min.

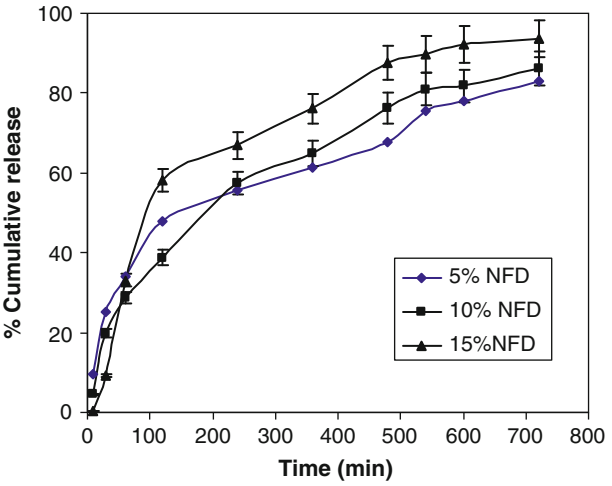


Fig. 4 % Cumulative release of NFD through EL100/CAB microspheres containing different amount of NFD at pH 7.4

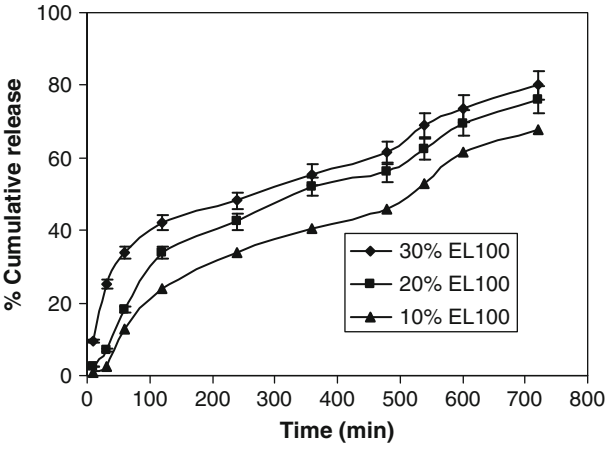


Fig. 5 % Cumulative release of NFD through EL100/CAB microspheres at pH 1.2

Effect of pH

To investigate the effect of pH and ionic strength of the external medium on the swelling of microspheres, we have measured the percentage cumulative release in both pH 1.2 and 7.4 media. Cumulative release data presented in Figs. 3 and 5 indicate that by increasing the pH from 1.2 to 7.4, a considerable increase in the cumulative release is observed for all microspheres. At higher pH (above the p*K*a = 6.8 of the microspheres). At higher pH (above the p*K*a = 6.8) of the microspheres, the swelling will be increased which makes the formulations to

release fast. However, cumulative release of the microspheres at higher pH depends upon the extent of hydrodynamic free volume, polymer chain relaxation, and availability of hydrophilic functional groups (–COO as in case of ionized polymer) for water to form hydrogen bonds.

The release data (shown in Figs. 3 and 5) obtained in pH 7.4 and 1.2 at the fixed amount of drug (10% NFD). The percentage cumulative release is quite fast and large in pH 7.4 media, whereas the release rate is quite slow in pH 1.2 media. Note that cumulative release of EL100/CAB microspheres in 1.2 pH media is almost half of the cumulative release observed in 7.4 pH media, which is due to lesser swelling of the microspheres in 1.2 pH media.

Conclusions

Eudragit L100/CAB blend microspheres were prepared using solvent evaporation technique. The prepared samples were characterized by SEM. SEM micrographs show the smooth and spherical morphology of the microspheres. The drug release studies of the microspheres have shown that with an increasing amount of EL100 in the microspheres, increase in the percentage of cumulative release. This effect is correlated with the release rates of the drug though the microspheres containing different amount of EL100 at different pHs. The microspheres could be retained in the gastric environment for more than 12 h, which would help to improve the bioavailability of NFD.

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References

1. Garcia MO, Blanco D, Martin JA, Teijon JM (2000) 5-Fluorouracil trapping in poly(2-hydroxyethyl methacrylate) hydrogels: in vitro drug delivery studies. Eur Polym J 36:111–122
2. Abu-Izza K, Tambrallo L, Lu DR (1997) In vivo evaluation of zidovudine (AZT)-loaded ethylcellulose microspheres after oral administration in beagle dogs. J Pharm Sci 86:554–559
3. Galeone M, Nizzola L, Cacioli D, Mosie G (1981) Delivery mechanism from sustained release pellets. Curr Ther Res 29:217–234
4. O'Hagan DT, Jeffery H, Roberts MJ, McGee JP, Davis SS (1991) Controlled release microparticles for vaccine development. Vaccine 9(10):768–771
5. Herrmann J, Bodmier R (1995) The effect of particle microstructure on the somatostatin release from poly(lactide) microspheres prepared by a W/O/W solvent evaporation method. J Control Release 36:3–71
6. Sveinsson SJ, Kristmundsdottir T, Ingvarsdottir K (1993) The effect of tabletting on the release characteristics of naproxen and ibuprofen microcapsules. Int J Pharm 92:29–34
7. Bodmeier R, Chen H (1988) Preparation of biodegradable poly(±)lactide microparticles using a spray-drying technique. J Pharm Pharmacol 40:754–757
8. Benita S (1996) Microencapsulation: methods and industrial applications. Marcel Dekker, New York
9. Yoshida R, Kaneko Y, Sakai K, Okano T, Kim SW (1994) Positive thermosensitive pulsatile drug release using negative thermosensitive hydrogels. J Control Res 32:97–102
10. Aoyagi T, Miyata F, Nagase Y (1994) Preparation of cross-linked aliphatic polyester and application to thermo-responsive material. J Control Res 32:87–96

11. Krishna Rao KSV, Naidu BVK, Subha MCS, Aminabhavi TM (2006) Novel chitosan-based pH-sensitive interpenetrating network microgels for the controlled release of cefadroxil. Carbohy Polym 66:333–344
12. Serres A, Baudys M, Kim SW (1996) Temperature and pH-sensitive polymers for human calcitonin delivery. Pharm Res 13:196–201
13. Obaidat AA, Park K (1996) Characterization of glucose dependent gel–sol phase transition of the polymeric glucose–concanavalin A hydrogel system. Pharm Res 13:989–995
14. Guyot M, Fawaz F (1998) Nifedipine loaded-polymeric microspheres: preparation and physical characteristics. Int J Pharm 175:61–74
15. Jeong YI, Sun HS, Shim YH, Kim C, Park SH, Choi KC, Cho CS (2004) Nifedipine encapsulated core-shell type nanoparticles based on poly(benzyl L-glutamate)/poly(ethylene glycol) diblock copolymers. J Microencapsul 21:445–453
16. Xun LD, Oh KJ, Hoon OD, Seok LW, Ja HM, Yang KJ, Seo CJ, Soo JWJ, Soon YC, Gon CH (2009) Development of nifedipine-loaded coated gelatin microcapsule as a long acting oral delivery. Arch Pharm Res 32:127–132
17. Guyot M, Fawaz F (1998) Nifedipine loaded-polymeric microspheres: preparation and physical characteristics. Int J Pharm 175:61–74
18. Ritger PL, Peppas NA (1987) A simple equation for description of solute release. II. Fickian and anomalous release from swellable devices. J Control Release 5:37–42