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Development of chitosan-based nanoparticles through inter-polymeric complexation for oral drug delivery

Sougata Jana[a,*], Nirmal Maji[a], Amit Kumar Nayak[b], Kalyan Kumar Sen[a], Sanat Kumar Basu[a]

[a] Department of Pharmaceutics, Gupta College of Technological Sciences, Asansol 713301, West Bengal, India
[b] Department of Pharmaceutics, Seemanta Institute of Pharmaceutical Sciences, Mayurbhanj 757086, Odisha, India

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ABSTRACT

The possibility of inter-polymeric complexation of cationic chitosan and anionic egg albumin stabilized with PEG 400 to develop novel nanoparticles for oral delivery of alprazolam by heat coagulation method at pH 5.4 and 80 °C. Nine formulations were prepared by changing the concentration of chitosan, PEG 400 and heating time. The alprazolam entrapment efficiency of these nanoparticles was in the range of 68.12 ± 1.27 to 99.37 ± 4.86%. These nanoparticles were characterized by FTIR, DSC, P-XRD and FE-SEM analysis. Average particle diameter, poly-dispersity index and zeta potential of these nanoparticles were found 259.60 nm, 0.501, and −9.00 mV, respectively. The *in vitro* drug release from these alprazolam-loaded nanoparticles showed sustained drug release over a period of 24 h. In conclusion, these newly developed chitosan-egg albumin-PEG nanoparticles were found to be a promising vehicle for sustained release delivery of lipophilic drugs.

1. Introduction

Oral route is the most acceptable route for drug administration due to ease of administration, low cost of therapy, patient compliance, *etc.* (Malakar & Nayak, 2012; Malakar, Nayak, & Pal, 2012). However, the oral administration of conventional dosage forms are unable to provide desired prolonged action and target specificity. Novel drug delivery systems can ameliorate the demerit of conventional dosage forms by prolonging the drug release, improving the drug solubility, minimizing side-effects, retaining the drug activity, and target specificity (Pawar, Douroumis, & Boateng, 2012). It has been shown that the drug properties like solubility, absorption, bioavailability, as well as carrier properties such as residence time at a particular site and site specificity can be improved by decreasing particle size (Muller, Jacobs, & Kayser, 2001).

During last few decades, there has been a considerable research interest in the area of nanoparticle-based drug delivery systems as carriers for various small and large molecules (Malakar, Ghosh, Basu, & Nayak, 2012; Nayak & Dhara, 2010; Pal & Nayak, 2010). Nanoparticles are solid particles ranging in size, 1–1000 nm. The major goals in designing nanoparticles as delivery systems are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action at the therapeutically optimal rate and dose regimen (Nayak & Dhara, 2010). Among various types of nanoparticles, polymeric nanoparticles have received lots of attention due their stability and ease of surface modification specificity (Liu, Jiao, Wang, Zhou, & Zhang, 2008; Pawar et al., 2012). Nanoparticles prepared from biocompatible and biodegradable polymers are expected to be absorbed intact from the gastrointestinal tract after oral administration (Florence, Hillery, Hussain, & Jani, 1995). In the present study, we have attempted to develop polymeric nanoparticles for oral drug delivery utilizing chitosan, egg albumin and poly ethylene glycol (PEG).

Recent years, chitosan-based nanoparticles have been used as drug delivery carriers for sustained drug release (Pawar et al., 2012; Sobaktakin, Tabatabaie, & Ramazanov, 2011; Yang et al., 2008). Chitosan is a cationic biocompatible and biodegradable natural polysaccharide obtained by alkaline deacetylation of chitin (Jana, Sen, & Basu, 2011; Varshosaz, Tavakoli, Minayian, & Rahdari, 2009). It is composed of α-1, 4-linked 2-amino-2-deoxy-α-D-glucose (N-acetyl glucosamine). It is a FDA GRAS (Generally Recognized as Safe) material and has been widely utilized in many applications including drug delivery, tissue engineering and food technology (Jana, Saha, Nayak, Sen, & Basu, 2013). Chitosan have been used in the development of various pharmaceutical formulations (Paul & Sharma, 2000). Although chitosan is a very promising biopolymer for use as carrier material in drug delivery systems, it has a limited capacity for controlling drug release from oral dosage forms due to its fast dissolution in the stomach. To overcome this disadvantage,

* Corresponding author. Tel.: +91 9434896683.
E-mail address: janapharmacy@rediffmail.com (S. Jana).

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chemical modification of chitosan has been employed in the development of drug delivery systems (Ding, Huang, Li, & Lin, 2007; Pawar et al., 2012; Sobaktakin et al., 2011; Yang et al., 2008).

The egg albumin is widely used in various food applications. However, its use as excipient in the pharmaceutical formulations has recently received attention (Kulkarni, Soppimath, Aminabhavi, & Rudzinski, 2001; Kulkarni, Mangod, Mutalik, & Sa, 2011; Mora & Pato, 1993). With the exposure of heat, egg albumin aggregates or gels were built from the partially unfolded molecules with a significant amount of secondary structure. Egg albumin also behaves as anionic polymer above its isoelectric point (pH 4.8) (Yu, Hu, Pan, Yao, & Jiang, 2006). Therefore, anionic egg albumin and cationic chitosan might form polyelectrolyte polymeric complex, if they are processed with each other. In the previous literature, Yu et al., have formulated chitosan and ovalbumin nanogels using heat coagulation method for the use in cosmetic and pharmaceutical applications (Yu et al., 2006). However, this type of nanosystems made by heat coagulation method is not studied for as drug delivery matrices till date.

PEG is widely used as excipient in various pharmaceutical formulations (Hasnain & Nayak, 2012; Peppas, Keys, Torres-Lugo, & Lowman, 1999; Tajarobi, Abrahmse'n-Alami, & Larsson, 2011). It is considered as a non-biodegradable polymer and undergoes slow degradation by alcohol dehydrogenase, aldehyde dehydrogenase, cytochrome-P-450, *etc.* (Pawar et al., 2012). In this study, PEG was used as stabilizer to produce polymeric nanoparticles.

In the current work, alprazolam was used as model drug to evaluate the sustained drug release potential from various newly formulated chitosan-egg albumin-PEG nanoparticles prepared through heat coagulation method.

2. Materials and methods

2.1. Materials

Alprazolam was received as a gift sample from Drakt Pharmaceutical Pvt. Ltd., India. Chitosan was commercially purchased from Everest Edward, India. Egg albumin and poly ethylene glycol 400 (PEG 400) were purchased from Loba Chemie Pvt. Ltd., India. All chemicals, and reagents used were of analytical grade.

2.2. Preparation of chitosan-egg albumin-PEG nanoparticles containing alprazolam

Chitosan-egg albumin-PEG nanoparticles were prepared by heat coagulation method. Required amounts of chitosan were dissolved in 1% (v/v) acetic acid aqueous solutions under continuous stirring. Alprazolam (100 mg) was added into the chitosan solutions and mixed thoroughly using a homogenizer (Remi Motors, India). PEG 400 was added drop wise into the alprazolam containing chitosan solutions and stirred well using magnetic stirring for 30 min at room temperature. Then, required amounts of egg albumin solutions were prepared in distilled water under continuous stirring and the undiluted particles were removed by centrifugation at 2000 rpm to obtain particle free solution. After that chitosan-alprazolam mixtures were added drop wise into egg albumin solutions under continuous stirring of 250–300 rpm using magnetic stirring for 30 min. The resultant alprazolam-polymer (chitosan and egg albumin) solutions showed pH 4–4.3. The pHs of these solutions were adjusted to 5.4 with the help of 0.1 N sodium hydroxide. Then, these pH adjusted solutions were heated at 80 °C for few min depending upon the design of the formulation. The produced nanoparticles were collected by centrifugation and then lyophilized (Eyela FDU 1200) to obtain dried samples and store in a decicator.

The similar method was adopted for the preparation of nanoparticles containing alprazolam without using PEG 400. The formulation chart of different nanoparticles containing alprazolam is enlisted in Table 1.

2.3. Estimation of drug entrapment efficiency

Accurately weighed 10 mg of lyophilized nanoparticles containing alprazolam from each formulation batch were taken separately and kept suspended in 50 ml phosphate buffer, pH 7.4 for 48 h under continuous stirring and sonicated for 20 min using a sonicator (Frontline Sonicator, FS-600, Frontline Electronics and Machinery Pvt. Ltd., India) followed by centrifugation at 2000 rpm for 10 min. Then, the supernatant fraction was further filtered through Whatman® filter paper (No. 40). The drug (alprazolam) content in the filtrate was determined using a UV–VIS spectrophotometer (Shimadzu, Japan) by measuring absorbance at λ_{Max} of 221 nm. The drug entrapment efficiency of nanoparticles was calculated using this following formula:

$$\text{Drug entrapment efficiency, \%} = \frac{\text{Actual drug content in nanoparticles}}{\text{Theoretical drug content in nanoparticles}} \times 100$$

2.4. Particle size, polydispersity index and zeta potential determination

The naoparticles were dispersed into 10 ml phosphate buffer, pH 7.4 and sonicated for 5 min before size measurement. The obtained homogenious suspensions were examined for particle size, polydispersity index and zeta potential using a laser scattering particle size analyzer (MALVERN ZETASIZER, MAL500999, UK).

2.5. Fourier transform-infrared (FTIR) spectroscopy

Samples were reduced to powder and analyzed as KBr pellets by using a FTIR spectroscope (Perkin Elmer Spectrum RX I, USA). The pellet was placed in the sample holder. Spectral scanning was taken in the wavelength region between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1} with scan speed of 2 mm/s.

2.6. Differential scanning calorimetry (DSC)

The samples were heated to remove the moisture. Then, the samples (7 mg) were placed into a platinum crucible 40-μl aluminium pan in hermetically sealed condition, where ∞-alumina powder was used as a reference. Thermograms were recorded from 29.80 °C to 351.90 °C at the heating rate of 5 °C/min under a constant flow of an inert nitrogen gas atmosphere with the flow rate of 20 ml/min. These analyses were done using a Differential Scanning Calorimeter (Perkin Elmer® Instrument, Pyris Diamond, Japan).

2.7. Powder X-ray diffraction (P-XRD) analysis

Samples were exposed to CuKα radiation (40 kV × 20 mA) in a wide-angle X-ray diffractometer (Siemens D5000, Munich, Germany). The instrument was operated in the step-scan mode in increments of 0.050° 2θ. The angular range was 10° to 80° 2θ, and counts were accumulated for 1 s at each step.

2.8. Field emission-scanning electron microscopy (FE-SEM)

The lyophilized particles were spread onto metal stubs and platinum coating applied by using an ion-sputtering device. The

Table 1
The formulation chart for the preparation of different alprazolam-loaded nanoparticles and their drug entrapment efficiency.

Code	Alprazolam (mg)	Egg albumin (mg)	Chitosan (mg)	PEG 400 (ml)	Heating time (min)	Drug entrapment efficiency (%) (mean ± S.D.; $n = 3$)
F-1	100	80	80	1.5	30	90.48 ± 4.78
F-2	100	80	80	0.5	10	86.90 ± 3.43
F-3	100	80	20	1.5	10	83.22 ± 3.06
F-4	100	80	20	0.5	30	92.62 ± 4.08
F-5	100	80	20	1.5	30	83.58 ± 3.28
F-6	100	80	80	0.5	30	99.37 ± 4.86
F-7	100	80	80	1.5	10	68.12 ± 1.27
F-8	100	80	20	0.5	10	80.14 ± 2.64
F-9	100	80	80	–	30	84.70 ± 3.42

coated particles were then examined under FE-SEM (JSM 6701; JEOL, Japan).

2.9. In vitro release study

In vitro drug release from these prepared nanoparticles was evaluated using dialysis bag diffusion technique. Accurately weighed quantities of microparticles containing aceclofenac equivalent to 20 mg alprazolam were placed in one end of the dialysis bag (Cellophane membrane, molecular cut off 10000–12000 Da, Hi-Media, India) containing 5 ml of phosphate buffer, pH 7.4. After that, order side of the dialysis bag was tied and immersed in phosphate buffer (pH 7.4) contained in the USP type II dissolution apparatus (Veego VDA-6D, Veego Instruments Co-operation, India). The system was maintained at 37 ± 1 °C under 100 rpm speed. The dialysis bag acts as a donor compartment, and the vessel of dissolution apparatus acts as the receptor compartment. 5 ml of aliquots was collected at regular time intervals, and the same amount of fresh dissolution medium was replaced into dissolution vessel to maintain the sink condition throughout the experiment. The collected aliquots were filtered, and suitably diluted to determine the absorbance using a UV–VIS spectrophotometer (Thermo Spectronic UV-1, USA) by measuring absorbance at λ_{Max} of 221 nm.

2.10. Analysis of in vitro drug release kinetics and mechanism

In order to predict and correlate *the in vitro* drug release behaviour from these nanoparticles containing alprazolam, it is necessary to fit into a suitable mathematical model. The *in vitro* drug release data were evaluated kinetically using various important mathematical models like zero order, first order, Higuchi, and Korsmeyer–Peppas models (Malakar, Nayak, et al., 2012; Nayak & Pal, 2011; Pal & Nayak, 2011).

Zero-order model: $Q = kt + Q_0$; where Q represents the drug released amount in time t, and Q_0 is the start value of Q; k is the rate constant.

First-order model: $Q = Q_0 e^{kt}$; where Q represents the drug released amount in time t, and Q_0 is the start value of Q; k is the rate constant.

Higuchi model: $Q = kt^{0.5}$; where Q represents the drug released amount in time t, and k is the rate constant.

Hixson–Crowell model: $Q^{1/3} = kt + Q_0{}^{1/3}$; where Q represents the drug released amount in time t, and Q_0 is the start value of Q; k is the rate constant.

Korsmeyer–Peppas model: $Q = kt^n$; where Q represents the drug released amount in time t, k is the rate constant and n is the diffusional exponent, indicative of drug release mechanism.

Again, The Korsmeyer–Peppas model was employed in the *in vitro* drug release behaviour analysis of these formulations to distinguish between competing release mechanisms: Fickian release (diffusion-controlled release), non-Fickian release (anomalous transport), and case-II transport (relaxation-controlled release). When n is ≤0.43, it is Fickian release. The n value between 0.43 and 0.85 is defined as non-Fickian release. When, $n \geq 0.85$, it is case-II transport (Malakar, Nayak, et al., 2012; Pal & Nayak, 2011).

2.11. Statistical analysis

All measured data are expressed as mean ± standard deviation (S.D.). The simple statistical analyses were conducted using MedCalc software version 11.6.1.0.

3. Results and discussion

3.1. Preparation of chitosan-egg albumin-PEG nanoparticles containing alprazolam

The chitosan-egg albumin-PEG nanoparticles containing alprazolam were prepared based on heat coagulation method at pH 5.4 and 80 °C. Chitosan is a polycation having pK_a of about 6.3 and egg albumin is a polyampholyte above its isoelectric point, pH 4.8 (Doi & Kitabatake, 1997; Yu et al., 2006). Therefore, a polyelectrolyte complex could be formed in the form of nanoparticles due to electrostatic attraction of these two oppositely charged polymers.

3.2. Drug entrapment efficiency

Drug entrapment efficiency was expressed as the percentage of drug (alprazolam) entrapped in these prepared polymeric nanoparticles containing alprazolam compared to the initial amount of alprazolam included in the formulation. The drug entrapment efficiency of these nanoparticles was within the range, 68.12 ± 1.27 to 99.37 ± 4.86% (Table 1). Highest drug entrapment efficiency (99.37 ± 4.86%) was found in case of formulation F-6, prepared using 80 mg chitosan, 80 mg egg albumin, 0.5 ml PEG 400 and 30 min heating time. The drug entrapment efficiency in the formulation F-9 was found lower (84.70 ± 3.42%) when compared with the nanoparticles prepared using same excipients except PEG 400 and same conditions used for the preparation of formulation F-6. From the results of drug entrapment efficiency, it was evidenced that the increasing drug entrapment in these chitosan-egg albumin-PEG nanoparticles was found with the decrease of PEG 400 amount and the increment of chitosan amount in their formulation. The increasing drug entrapment was also found with the increment of heating time at the preparation of these nanoparticles.

3.3. Particle size, polydispersity index and zeta potential determination

Particle size distributions of the formulation F-6 and F-9 were presented in Fig. 1(a) and (b), respectively. The average particle diameter of the alprazolam-loaded chitosan-egg albumin nanoparticles (F-9) was within the range of 450.00 nm. When PEG 400 was used in the preparation of these alprazolam-loaded nanoparticles

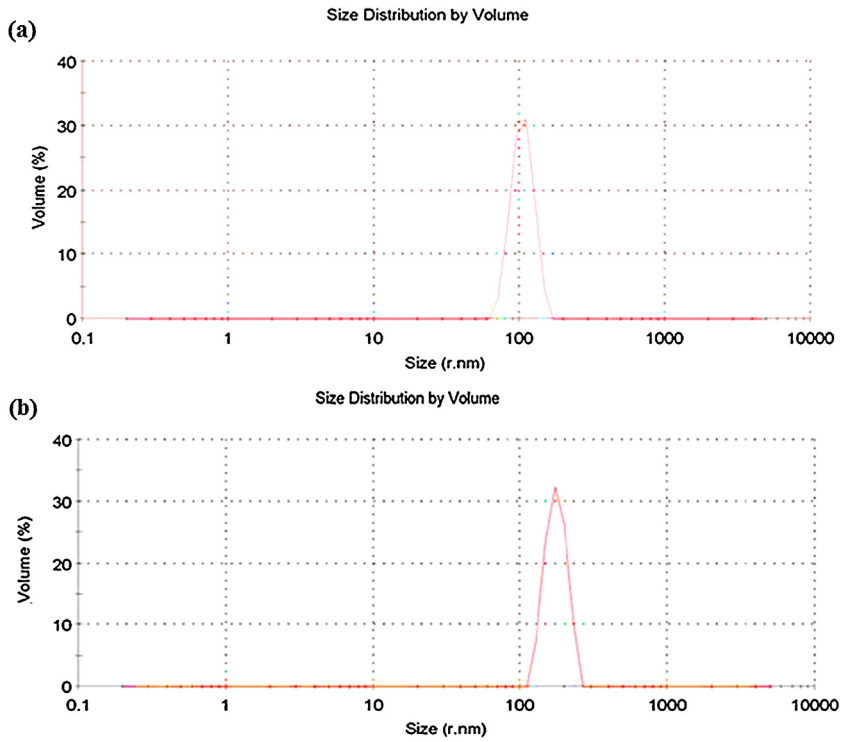

Fig. 1. (a) Particle size distributions of the alprazolam-loaded chitosan-egg albumin-PEG nanoparticles, formulation F-6; (b) particle size distributions of the alprazolam-loaded chitosan-egg albumin-PEG nanoparticles, formulation F-9.

(F-6), average particle diameter was found to decrease (259.60 nm). This phenomenon could be due to the steric barrier of PEG shell, which might avoid aggregation of internal phase droplets in preparation pathway.

Generally the polydispersity index is defined as the ratio of size deviation (or width of distribution) to mean particle diameter. A polydispersity index of 1 indicates large variation in particle size, zero means that the size variation is absent; whereas, polydispersity index $\leq$0.3 means particles are quite monodisperse. The polydispersity values for alprazolam-loaded chitosan-egg albumin nanoparticles (F-9) and alprazolam-loaded chitosan-egg albumin-PEG nanoparticles (F-6) were found 0.721 and 0.501, respectively. This indicates some differences in homogeneity in their size variation. However, the decreased polydispersity index value with the addition of PEG in the formulation of these alprazolam-loaded nanoparticles indicates an increase in homogeneity in particle size.

The measurement of the zeta potential allows predictions about the storage stability of various colloidal dispersions. In general, particle aggregation is occurred for charged particles with high zeta potential due to electric repulsion. The zeta potentials of alprazolam-loaded chitosan-egg albumin nanoparticles (F-9) and alprazolam-loaded chitosan-egg albumin-PEG nanoparticles (F-6) were found 7.88 mV and −9.00 mV, respectively. From these results, it was clearly evidenced that the zeta potential of these nanoparticles decreased with the addition of PEG 400 in their formulation. The PEG might coated the surface of the nanoparticle or the -OH part of glycol molecule reacted with the positively charged surfaces, which could result decrease in zeta potential of the surfaces.

3.4. FTIR spectroscopy analysis

FTIR spectrum of chitosan, alprazolam, egg albumin, alprazolam-loaded chitosan-egg albumin nanoparticles (F-9) and alprazolam-loaded chitosan-egg albumin-PEG nanoparticles (F-6) is shown in Fig. 2. The FTIR spectra of alprazolam showed principal characteristic peaks at 698.49 cm^{-1} due to C—Cl stretch, at 3053.12 cm^{-1} for aromatic C—H group stretch, at 1611.22 cm^{-1}, 1565.92 cm^{-1} due to phenyl ring, 1488.06 cm^{-1} for C—H stretching, 779.75 cm^{-1} for aromatic C—H bending. In case of FTIR spectra of chitosan, peaks at 3411.86 cm^{-1} for O—H stretch, 2972.72 cm^{-1} for aliphatic C—H stretch, 1650.00 cm^{-1} due to amide, 1560.37 cm^{-1} due to N—H bending vibration of secondary amine, 1076.08 cm^{-1} for C—O stretch of ether, 560.37 cm^{-1} for 1° N—H groups and bands at 1100–1000 cm^{-1} are due to the saccharide structure were observed. In addition, characteristic peaks at 1464.72 cm^{-1} and 1431.03 cm^{-1} were seen due to bending vibration of —CH_2 group and —OH group, respectively. The FTIR spectra of egg

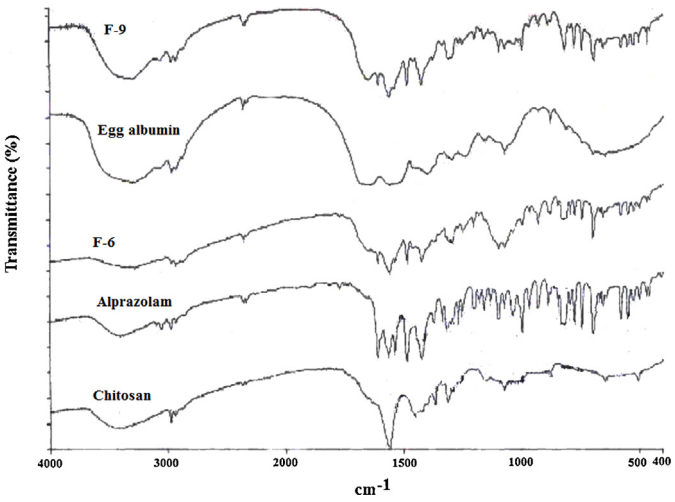

Fig. 2. FTIR spectrum of chitosan, alprazolam, egg albumin, alprazolam-loaded chitosan-egg albumin nanoparticles (F-9) and alprazolam-loaded chitosan-egg albumin-PEG nanoparticles (F-6).

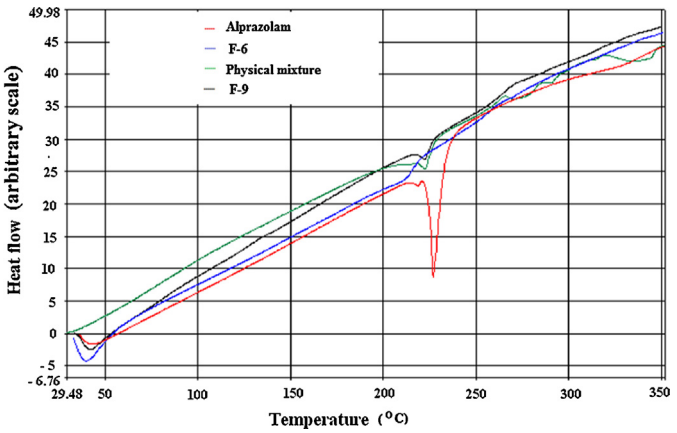

Fig. 3. DSC thermograms of alprazolam, alprazolam-loaded chitosan-egg albumin nanoparticles (F-9), alprazolam-loaded chitosan-egg albumin-PEG nanoparticles (F-6), and physical mixture of alprazolam, chitosan and egg albumin.

albumin showed characteristic peaks at 3300.78 cm^{-1} for N substituted amide, 2969.92 cm^{-1} for aliphatic C—H stretching, 1645.34 cm^{-1} due to amide band, 1560.63 cm^{-1} for N—H bending vibration, 1403.22 cm^{-1} for aliphatic C—H bending vibration and 1157.99 cm^{-1} due to C—N stretching vibrations. Characteristic peaks appeared in the FTIR spectra of alprazolam were also appeared clearly in the both FTIR spectrum of alprazolam-loaded chitosan-egg albumin nanoparticles (F-9) and alprazolam-loaded chitosan-egg albumin-PEG nanoparticles (F-6). A small but distinct change or shifting of the amide band was observed in these spectra. Both the spectrum exhibited the characteristic absorption bands, which were appeared in both egg albumin and chitosan spectrum with only intensity differences except the appearance of two additional peaks at 1080.88 cm^{-1} and 1028.23 cm^{-1}. These peaks were not present in either chitosan or egg albumin spectrum. This phenomenon indicates the interaction between anionic egg albumin and cationic chitosan.

3.5. DSC analysis

The DSC thermograms of alprazolam, alprazolam-loaded chitosan-egg albumin nanoparticles (F-9), alprazolam-loaded chitosan-egg albumin-PEG nanoparticles (F-6), and physical mixture of alprazolam, chitosan and egg albumin is presented in Fig. 3. The thermogram of alprazolam showed a sharp endothermic peak at 227 °C due to melting of the alprazolam. The onset of endothermic peak appears at 223 °C, while it ends at 232 °C. The thermogram of drug (alprazolam)-polymer (chitosan and egg albumin) physical mixture also showed a low endothermic peak below the endothermic peak of pure drug (alprazolam) at about 222 °C. However, the thermogram of alprazolam-loaded chitosan-egg albumin nanoparticles (F-9) showed also endothermic peak at the same position of the physical mixture. This decrease in melting temperature of alprazolam may be due to some short of its original form (*i.e.*, in crystalline form) in the alprazolam-loaded chitosan-egg albumin nanoparticles. In the thermogram of alprazolam-loaded chitosan-egg albumin-PEG nanoparticles (F-6), no sharp endothermic peak found at the same position or in the lower region, indicating that the drug was uniformly dispersed in an amorphous state in the nanoparticles and no incompatibility arises between the drug and polymers used.

3.6. P-XRD analysis

P-XRD patterns of alprazolam, alprazolam-loaded chitosan-egg albumin nanoparticles (F-9), and alprazolam-loaded chitosan-egg albumin-PEG nanoparticles (F-6) are presented in Fig. 4. P-XRD of alprazolam showed characteristic intense peaks between the 20° of 18.4° due to its crystalline nature. In the P-XRD pattern of alprazolam-loaded chitosan-egg albumin nanoparticles (F-9) and alprazolam-loaded chitosan-egg albumin-PEG nanoparticles (F-6), slightly less intense peaks were observed at 23.4° and 24°. The above result suggests that drug (alprazolam) was converted from crystalline to amorphous form in these alprazolam-loaded nanoparticles, which could be due to the effect of polymers or formulation process.

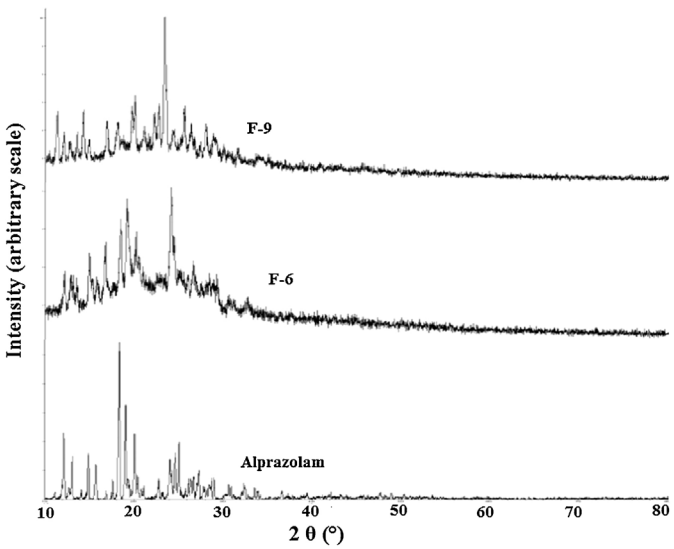

Fig. 4. P-XRD patterns of alprazolam, alprazolam-loaded chitosan-egg albumin nanoparticles (F-9), and alprazolam-loaded chitosan-egg albumin-PEG nanoparticles (F-6).

3.7. FE-SEM analysis

FE-SEM of alprazolam-loaded chitosan-egg albumin-PEG nanoparticles (F-6) was performed. In the FE-SEM photograph at 65000× magnification (Fig. 5), these particles were appeared densely packed to each other. Particles were mostly in the nanoscale range and spherical in shape.

3.8. In vitro drug release

In vitro drug release from these prepared alprazolam-loaded nanoparticles was evaluated using dialysis bag diffusion technique in phosphate buffer, pH 7.4. The cumulative percentage drug release from these nanoparticles was found sustained over a period of 24 h (Fig. 6). The *in vitro* drug release from all the formulation batches of nanoparticles showed an initial burst release of drugs, which might be attributed to the presence of free crystals and/or weakly bound drug on the surface of the nanoparticulates. The percentage drug released from alprazolam-loaded chitosan-egg albumin-PEG nanoparticles (F-1 to F-8) and chitosan-egg albumin nanoparticles (F-9) in 24 h was within the range of 51.33 ± 0.77 to 85.19 ± 1.85%. The drug release from these nanoparticles was mostly dependent on the amount of drug-load. The drug release was found faster from the nanoparticles of lower drug entrapment efficiency.

The *in vitro* drug release data from various alprazolam-loaded nanoparticles were evaluated kinetically using various important mathematical models like zero order, first order, Higuchi, and Korsmeyer–Peppas models. The R^2 values of these models were determined for evaluation of accuracy. The result of the curve fitting into various mathematical models is given in Table 2. When

Table 2
Results of curve fitting of the *in vitro* drug release profile of various alprazolam-loaded nanoparticles.

Code	Correlation coefficient (R^2)				Release exponent (n)
	Zero order	First order	Higuchi	Korsmeyer–Peppas	
F-1	0.526	0.927	0.944	0.923	0.62
F-2	0.684	0.978	0.992	0.961	0.59
F-3	0.812	0.983	0.981	0.972	0.73
F-4	0.920	0.979	0.958	0.995	0.63
F-5	0.759	0.962	0.984	0.963	0.56
F-6	0.921	0.991	0.954	0.992	0.67
F-7	0.788	0.759	0.991	0.966	0.66
F-8	0.574	0.926	0.951	0.920	0.83
F-9	0.746	0.923	0.990	0.976	0.60

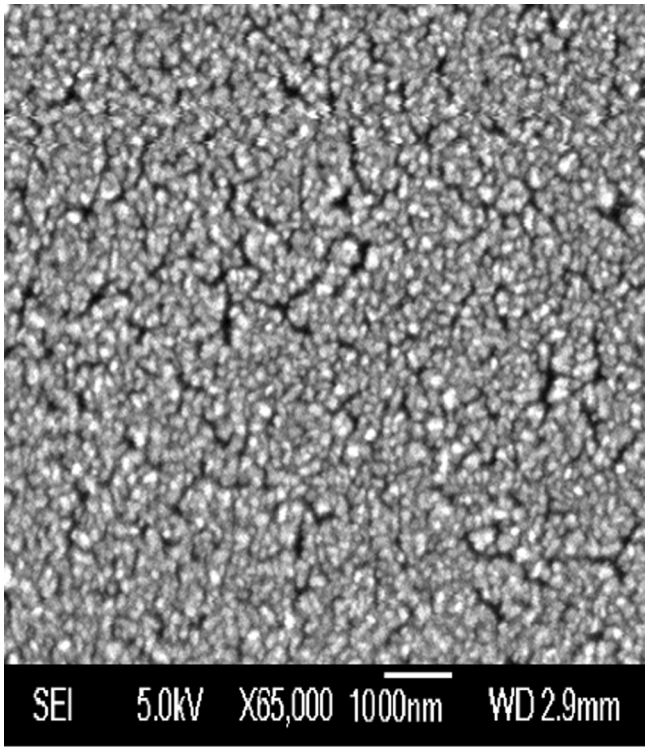

Fig. 5. FE-SEM image of alprazolam-loaded chitosan-egg albumin-PEG nanoparticles (F-6) at 65000× magnification.

the respective R^2 of these nanoparticles were compared, it was found that F-1, F-2, F-3, F-5, F-7, F-8 and F-9 followed the Higuchi model; whereas F-4 and F-6 followed the Korsmeyer–Peppas model over a period of 24 h. The value of release exponent (n) determined from *in vitro* alprazolam release data of various alprazolam-loaded nanoparticles ranged from 0.56 to 0.83, indicating anomalous (non-Fickian) diffusion mechanism for drug release. The anomalous diffusion mechanism of drug release demonstrates both diffusion controlled, and swelling controlled drug release.

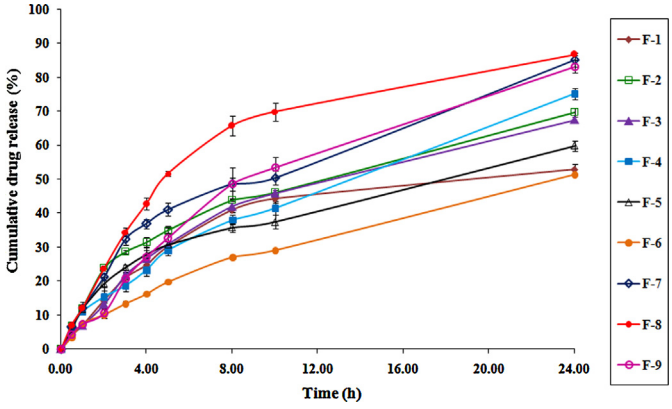

Fig. 6. The *in vitro* drug release from alprazolam-loaded nanoparticles in phosphate buffer, pH 7.4 (mean ± S.D.; $n = 3$).

4. Conclusion

Alprazolam-loaded chitosan-egg albumin-PEG nanoparticles were prepared using heat coagulation method by manipulating environmental conditions, such as pH, temperature, and egg albumin to chitosan ratio. The drug entrapment efficiency of these nanoparticles was within the range, 68.12 ± 1.27 to 99.37 ± 4.86%. Among them, highest drug entrapment efficiency (99.37 ± 4.86%) was found in case of formulation F-6, prepared using 80 mg chitosan, 80 mg egg albumin, 0.5 ml PEG 400 and 30 min heating time. The average particle diameter, polydispersity index and zeta potential of F-6 alprazolam-loaded chitosan-egg albumin-PEG nanoparticles were found 259.60 nm, 0.501, and −9.00 mV, respectively. The stability and physical condition of the loaded alprazolam within these nanoparticles were confirmed using FTIR, DSC and P-XRD analyses. The *in vitro* drug release in phosphate buffer, pH 7.4 of these prepared alprazolam-loaded nanoparticles showed sustained drug release over a period of 24 h. Overall, these results showed the promise of these newly developed chitosan-egg albumin-PEG nanoparticles for sustained release of lipophilic drugs.

References

Ding, P., Huang, K.-L., Li, G.-Y., & Lin, Y.-F. (2007). Preparation and properties of modified chitosan as potential matrix materials for drug sustained-release beads. *International Journal of Biological Macromolecules*, *41*, 125–131.

Doi, E., & Kitabatake, N. (1997). Structure and functionality of egg proteins. In S. Damodaran, & A. Paraf (Eds.), *Food proteins and their application* (Vol. 80) (pp. 325–340). New York: Marcel Dekker, Inc.

Florence, A. T., Hillery, A. M., Hussain, N., & Jani, P. U. (1995). Nanoparticles as carriers for oral peptide absorption – studies on particle uptake and fate. *Journal of Controlled Release*, *36*, 39–46.

Hasnain, M. S., & Nayak, A. K. (2012). Solubility and dissolution enhancement of ibuprofen by solid dispersion technique using PEG 6000-PVP K 30 combination carrier. *Chemistry: Bulgarian Journal of Science Education*, *21*, 118–132.

Jana, S., Saha, A., Nayak, A. K., Sen, K. K., & Basu, S. K. (2013). Aceclofena-loaded chitosan-tamarind seed polysaccharide interpenetrating polymeric network microparticles. *Colloids and Surfaces B: Biointerfaces*, *105*, 303–309.

Jana, S., Sen, K. K., & Basu, S. K. (2011). Chitosan derivatives and their application in pharmaceutical fields. *International Journal of Pharmaceutical Research*, *3*, 1–8.

Kulkarni, A. R., Soppimath, K. S., Aminabhavi, T. M., & Rudzinski, W. E. (2001). *In-vitro* release kinetics of cefadroxil loaded sodium alginate interpenetrating network beads. *European Journal of Pharmaceutics and Biopharmaceutics*, *51*, 127–133.

Kulkarni, R. V., Mangod, B. S., Mutalik, S., & Sa, B. (2011). Interpenetrating polymer network microcapsules of gellan gum and egg albumin entrapped with diltiazem-resin complex for controlled release application. *Carbohydrate Polymers*, *83*, 1001–1007.

Liu, Z., Jiao, Y., Wang, Y., Zhou, C., & Zhang, Z. (2008). Polysaccharide-based nanoparticles as drug delivery systems. *Advanced Drug Delivery Reviews*, *60*, 1650–1662.

Malakar, J., & Nayak, A. K. (2012). Formulation and statistical optimization of multiple-unit ibuprofen-loaded buoyant system using 2^3 factorial design. *Chemical Engineering Research and Design*, *90*, 1834–1846.

Malakar, J., Nayak, A. K., & Pal, D. (2012). Development of cloxacillin loaded multiple-unit alginate-based floating system by emulsion–gelation method. *International Journal of Biological Macromolecules*, *50*, 138–147.

Malakar, J., Ghosh, A., Basu, A., & Nayak, A. K. (2012). Nanotechnology: A promising carrier for intracellular drug delivery system. *International Research Journal of Pharmacy*, *3*, 36–40.

Mora, M., & Pato, J. (1993). Preparation of egg albumin microparticles for oral application. *Journal of Controlled Release*, *25*, 107–113.

Muller, R. H., Jacobs, C., & Kayser, O. (2001). Nanosuspensions as particulate drug formulation therapy: Rational for development and what we can expect for the future. *Advanced Drug Delivery Reviews*, *47*, 3–19.

Nayak, A. K., & Dhara, A. K. (2010). Nanotechnology in drug delivery applications: A review. *Archives of Applied Science Research*, *2*, 284–293.

Nayak, A. K., & Pal, D. (2011). Development of pH-sensitive tamarind seed polysaccharide-alginate composite beads for controlled diclofenac sodium delivery using response surface methodology. *International Journal of Biological Macromolecules*, *49*, 784–793.

Pal, D., & Nayak, A. K. (2011). Development, optimization, and anti-diabetic activity of gliclazide-loaded alginate-methyl cellulose mucoadhesive microcapsules. *AAPS PharmSciTech*, *12*, 1431–1441.

Pal, D., & Nayak, A. K. (2010). Nanotechnology for targeted delivery in cancer therapeutics. *International Journal of Pharmaceutical Sciences Review and Research*, *1*, 1–7.

Paul, W., & Sharma, C. P. (2000). *Chitosan, a drug carrier for the 21st century: A review*. Societa' Francaise des Sciences et Techniques Pharmaceutics. *Pharmaceutical Sciences*, *10*, 5–22.

Pawar, H., Douroumis, D., & Boateng, J. S. (2012). Preparation and optimization of PMMA-chitosan-PEG nanoparticles for oral drug delivery. *Colloidal Surfaces B: Biointerfaces*, *90*, 102–108.

Peppas, N. A., Keys, K. B., Torres-Lugo, M., & Lowman, A. M. (1999). Poly(ethylene glycol)-containing hydrogels in drug delivery. *Journal of Controlled Release*, *62*, 81–97.

Sobaktakin, M. R., Tabatabaie, R. M., & Ramazanov, M. A. (2011). Development and *in vitro* evaluation of thiolated chitosan-poly (methacrylic acid) nanoparticles as local mucoadhesive delivery systems. *International Journal of Biological Macromolecules*, *48*, 403–407.

Tajarobi, F., Abrahmse'n-Alami, S., & Larsson, A. (2011). Dissolution rate enhancement of parabens in PEG solid dispersions and its influence on the release from hydrophilic matrix tablets. *Journal of Pharmaceutical Sciences*, *100*, 275–283.

Varshosaz, J., Tavakoli, N., Minayian, M., & Rahdari, N. (2009). Applying the Taguchi design for optimized formulation of sustained release gliclazide chitosan beads: An *in vitro*/*in vivo* study. *AAPS PharmaSciTech*, *10*, 158–165.

Yang, X. D., Zhang, Q. Q., Wang, Y. S., Chen, H., Zhang, H. Z., Gao, F. P., et al. (2008). Self-aggregated nanoparticles from methoxy poly (ethylene glycol) modified chitosan: Synthesis, characterization, aggregation and methotrexate release *in vitro*. *Colloidal Surfaces B: Biointerfaces*, *61*, 125–131.

Yu, S., Hu, J., Pan, X., Yao, P., & Jiang, M. (2006). Stable and pH-sensitive nanogels prepared by self-assembly of chitosan and ovalbumin. *Langmuir*, *22*, 2754–2759.