



FORMULATION AND EVALUATION OF FLURBIPROFEN LOADED CHITOSAN MICROSPHERES BY DENATURATION METHOD

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ABSTRACT

The aim of this study was to formulate and evaluate flurbiprofen loaded chitosan microspheres by denaturation method for controlled drug release using glutaraldehyde as crosslinking agent. The prepared microspheres were characterized for their yield and drug loading, as well by Fourier transform infrared spectroscopy (FTIR), X-ray diffractometry and Scanning electron microscopy. The in vitro release studies were performed in pH 7.4, phosphate buffer. The prepared microspheres were free flowing and spherical in shape. The drug-loaded microspheres showed 85.64%-92.12% of entrapment and release was extended up to 12h. The infrared spectra showed stable character of flurbiprofen in the drug-loaded microspheres and revealed the absence of drug-polymer interactions. X-ray diffraction patterns showed that there was decrease in crystallinity of the drug. Scanning electron microscopy study revealed that the microspheres were spherical and porous in nature. It was found that the drug: polymer ratio, the stirring speed, the concentration of surfactant, and the amount of glutaraldehyde used for crosslinking were the most significant variables which influenced the size of the chitosan microspheres under the applied experimental condition.

KEY WORDS

Microspheres, Crosslinking agent, denaturation

INTRODUCTION:

Chitosan is a polysaccharide comprising copolymers of glucosamine and N-acetyl glucosamine. Being biodegradable and biocompatible, chitosan has been used in the formulation of particulate drug delivery systems to achieve controlled drug delivery [1, 2]. Chitosan microspheres have been prepared by chemical denaturation, [3, 4] ion-induced coagulation, [5, 6] and spray-drying methods. [7, 8] Of these methods, the most common method used to prepare chitosan microspheres is the denaturation method. Denaturation involves denaturation of chitosan present in the inner phase of water/oil (w/o) emulsion. Denaturation is usually carried out using glutaraldehyde with continuous stirring. Flurbiprofen, a, 2-(2-fluorobiphenyl-4-yl) propionic acid belongs to a group of non-steroidal anti-inflammatory drugs used for the treatment of mild

to moderate pain [9]. They impart their action by inhibiting the synthesis of prostaglandins involved in pain and inflammation [10]. There are many methods for microencapsulation and selection of method depends on hydrophilicity or hydrophobicity of the drug [11]. The present work was aimed to study the development and evaluation of microencapsulated flurbiprofen by denaturation technique using chitosan for controlled drug release [12]. Hence different batches of microspheres were prepared according to the working plan [13, 14]. The resultant microspheres were evaluated for percentage yield, entrapment efficiency, particle size and in vitro drug release [15]. The effect of process variables on microspheres was also studied

MATERIALS AND METHODS:

Materials

Flurbiprofen was obtained as a gift from M/s Natco Pharma Ltd., Hyderabad. Chitosan was obtained as a gift sample from Central Institute of Fisheries Technology, Cochin. All other reagents and solvents used were of pharmaceutical or analytical grade.

Methods:

Preparation of microspheres [16]

The microspheres were prepared by denaturation method using glutaraldehyde as crosslinking agent. Accurately weighed quantity of chitosan was dissolved in 1% (v/v) aqueous acetic acid. The drug was added to the polymer solution and mixed thoroughly. The dispersed phase was then added dropwise through a disposable syringe (10 ml) to the continuous phase consisting of 10% calcium chloride dihydrate containing different amounts of surfactant (span 80). Stirring was

continued at different speed using a 3-blade propeller stirrer. After 20 min of stirring, a measured quantity of aqueous glutaraldehyde (25% v/v) was added dropwise at regular intervals of 1, 2 and 3 hours respectively and continued for 1 hour after the final addition of glutaraldehyde. The preparation was centrifuged at 3000 rpm, the supernatant was decanted, and microspheres obtained as residue were washed 4 times with petroleum ether (60-80° C). After the final wash, microspheres were then air dried at room temperature, collected and stored in desiccators. Blank microsphere was prepared for comparison with the drug loaded microspheres.

Effect of process variables on microsphere properties

Chitosan microspheres were prepared at different stirring rates, surfactant concentrations (Span 80), crosslinking agent (glutaraldehyde) amount and with various drugs: polymer ratios as given in Table 1.

Table No. 1: Process variables in the preparation of microspheres

Process variables	Process variables
stirring rates (rpm)	500, 1500, 2500
surfactant concentrations (% w/v)	0.5, 1.0, 1.5
crosslinking agent amount (ml)	0.5, 1.0, 1.5
drug: polymer ratios	1:1, 1:2, 1:3, 1:4
Volume of processing medium (ml)	100, 150, 200

Table No. 2: Formulation design in the preparation of different batches of microspheres

Formulation code	Drug Polymer ratio	Stirring speed (rpm)	Span 80 Concentration (% v/v)	Glutaraldehyde Concentration (ml)	10% calcium chloride dihydrate (ml)
F1	1:1	500	0.5	0.5	100
F2	1:2	500	0.5	0.5	100
F3	1:3	500	0.5	0.5	100
F4	1:4	500	0.5	0.5	100
F5	1:2	1500	0.5	0.5	100
F6	1:2	1500	1.0	1.0	150
F7	1:2	1500	1.0	0.5	150
F8	1:2	1500	1.5	0.5	150
F9	1:2	1500	1.0	1.5	150
F10	1:2	2500	0.5	0.5	150
F11	1:2	2500	1.0	0.5	200
F12	1:2	2500	1.0	1.0	200
F13	1:2	2500	1.0	1.5	200
F14	1:2	1500	1.0	1.0	200
F15	1:2	1500	1.0	1.0	200
Blank Microsphere	0:2	1500	1.0	1.0	100

During the preparation of different batches of microspheres as given in Table 2, optimization was

carried out for different drug polymer ratios, stirring speed, surfactant concentration, crosslinking agent

amount and volume of processing medium. The effect of these process variables on yield, encapsulation efficiency and particle size were investigated.

Evaluation of Microspheres

Percentage Yield

The prepared floating microspheres were collected and weighed. The measured weight was divided by total amount of all non-volatile components, which were used for the preparation of microspheres. The % yield was calculated using following formula

Percentage yield = $\frac{\text{Weight of microspheres recovered}}{\text{Weight (drug + polymer)}} \times 100$

Drug entrapment efficiency

Weighed quantity of microspheres were crushed and suspended in ethanol to extract the drug from microspheres. After 24 hrs, the filtrate was assayed spectrophotometrically at 247 nm for drug content against ethanol as blank. Corresponding drug concentrations in the samples were calculated from the calibration plot using a regression equation derived from the standard graph. The % drug entrapment efficiency (DEE) was calculated by the equation.

% Encapsulation efficiency = $\frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$

Fourier Transform Infrared Spectroscopy (FTIR)

Drug-polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug and drug-loaded microspheres using FTIR

X-ray powder Diffractometry (X-RD)

X-ray powder diffractometry was carried out to investigate the effect of microencapsulation process on crystallinity of drug.

In-vitro Release Studies:

In-Vitro Dissolution study of pure drug

This was carried out by taking 100 mg pure drug, using USP basket dissolution apparatus basket type at temperature $37 \pm 0.5^\circ\text{C}$, 100 rpm and dissolution medium phosphate buffer 7.4pH (900 ml). 5 ml samples were withdrawn from the dissolution medium at 3 minutes intervals and equivalent volume of fresh dissolution medium was added. After suitable dilution, these samples were analyzed spectrophotometrically by UV visible spectrophotometer at 247 nm and using standard curve equation.

In vitro Dissolution Study of drug-loaded microspheres

In the present study, drug release was studied using a USP dissolution apparatus basket type using phosphate buffer solution (pH 7.4) as dissolution fluids (900 ml) maintained at $37 \pm 0.5^\circ\text{C}$ at 100 rpm. Accurately weighed samples of microspheres were added to dissolution medium. 5 ml of samples were withdrawn from the dissolution medium at 1 hr. intervals. The volume was replenished with the same amount of fresh dissolution fluid each time to maintain the sink condition. These samples were analyzed spectrophotometrically by UV visible spectrophotometer at 247 nm and using standard curve equation.

RESULTS & DISCUSSION

The microspheres formed were spherical in shape and having smooth or slightly rough in surface. The yield and entrapment efficiency of various formulations was given in Table 3.

Table No. 3: Percentage Yield and Encapsulation Efficiency of microspheres

Formulation code	Drug: polymer Ratio	% Yield	% Drug Entrapment
Blank microspheres	0:2	91	--
F5	1:2	84	90.1
F6	1:2	92	92.12
F7	1:2	90	91.21
F8	1:2	89	90.82
F9	1:2	86	85.64

Effect of process variables on Particle Size

It is observed from the Figure 1 that increasing the polymer ratio the mean particle size was increased. When the drug polymer ratio was increased from 1:1 to 1:2, larger particles were formed, because the viscosity

of the emulsion medium was increased with increasing amount of polymer. Due to increase in viscosity, larger emulsion droplets were formed which were difficult to break and hence they are precipitated as such leading to increase in mean particle size.

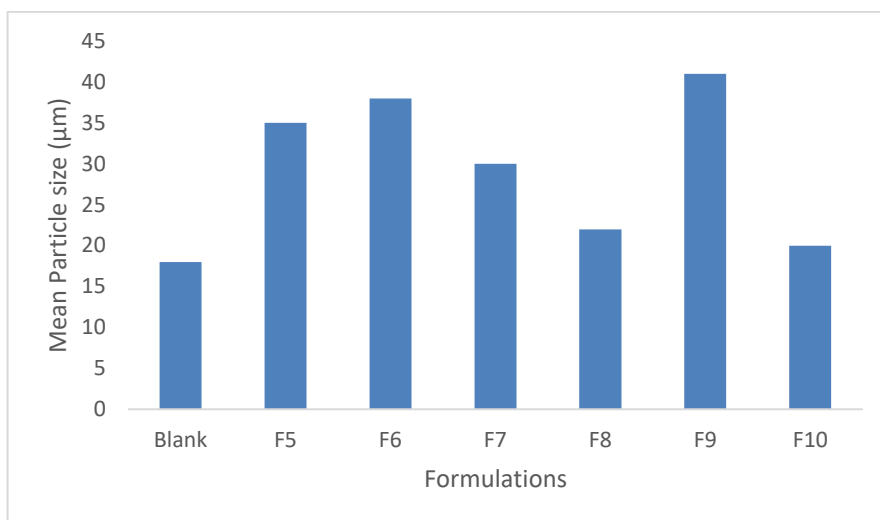


Fig. No. 1: Mean Particle Size Distribution in Various Formulations

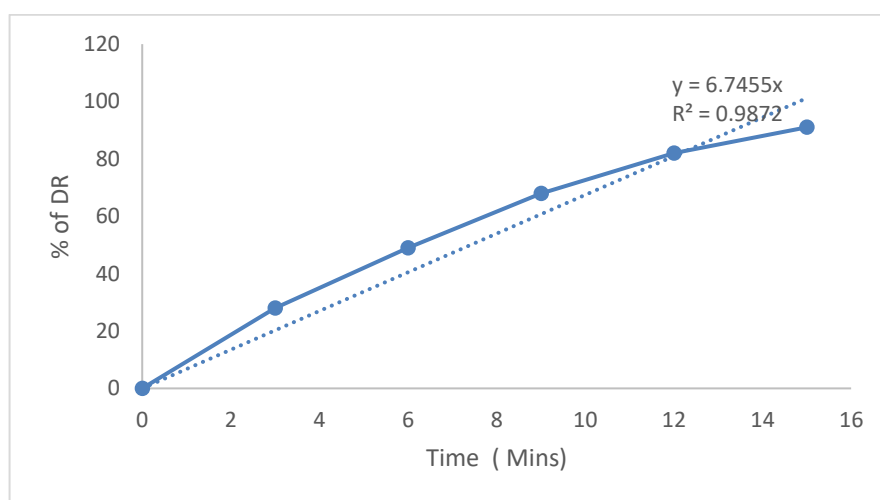


Fig. No. 2: In vitro release Profile of Pure Drug

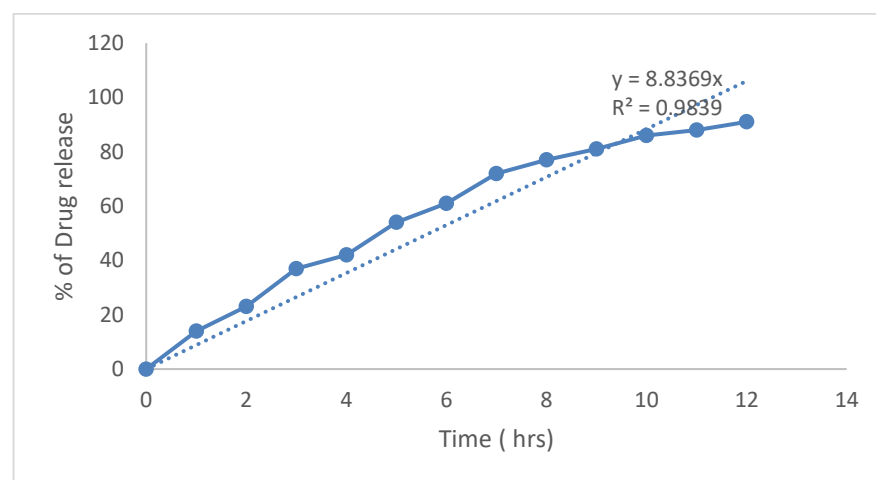


Fig. No. 3: In vitro release Profile of Optimized Formulation

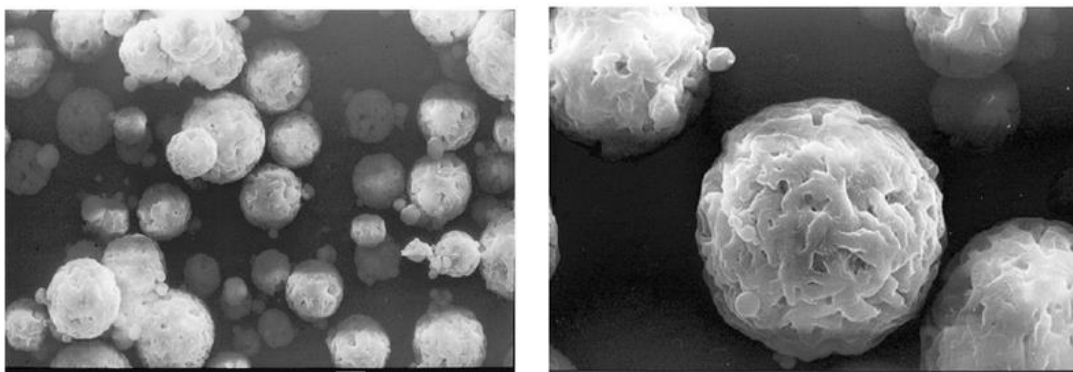


Fig. No. 4: Scanning electron photomicrograph of a) blank microspheres b) drug loaded microspheres

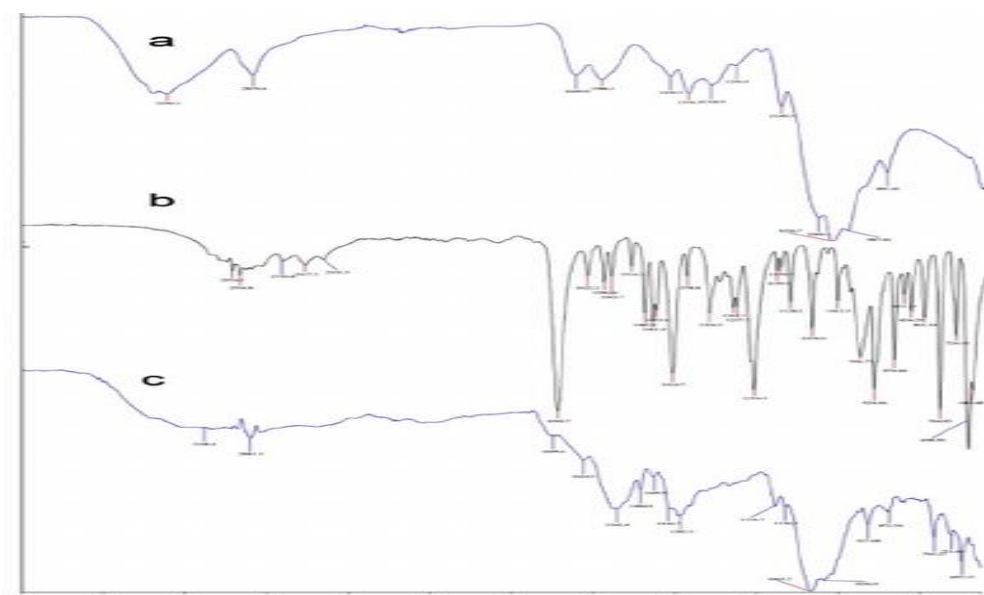


Fig. No. 5: Fourier transform infrared spectroscopy spectrums of (a) chitosan (b) flurbiprofen and (c) chitosan-flurbiprofen microspheres

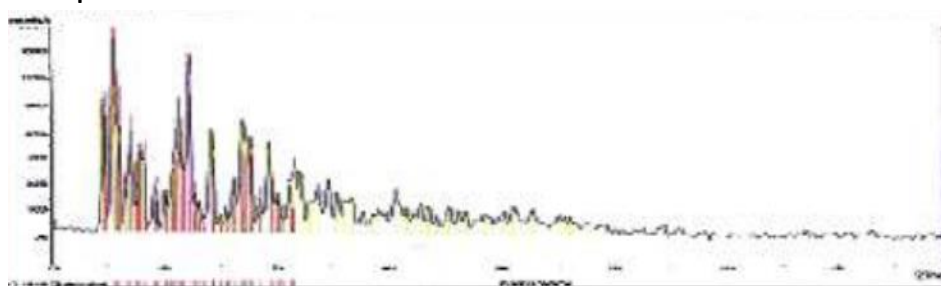


Fig. No. 6: XRD of pure drug

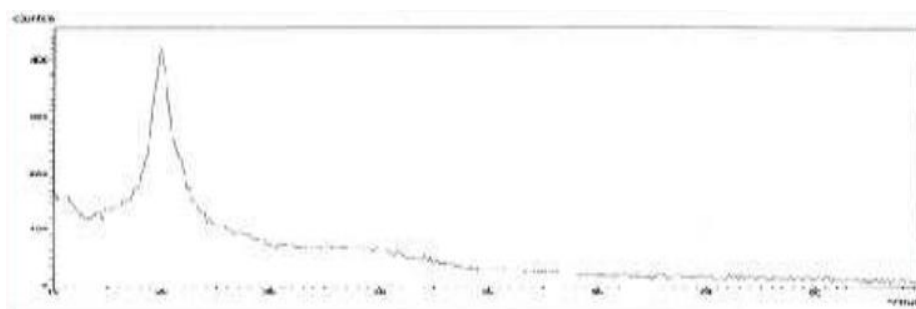


Fig. No. 7: XRD of polymer

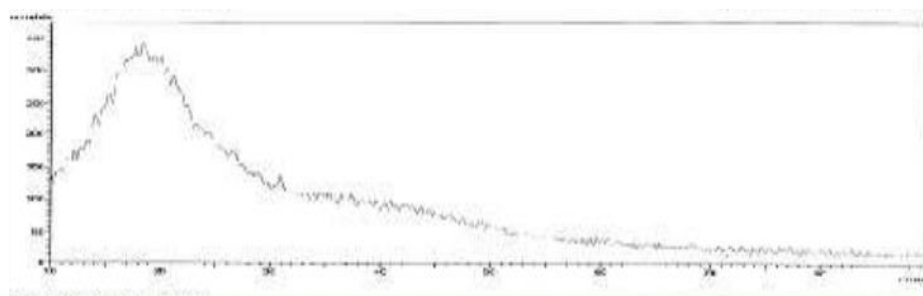


Fig. No. 8: XRD of blank microsphere

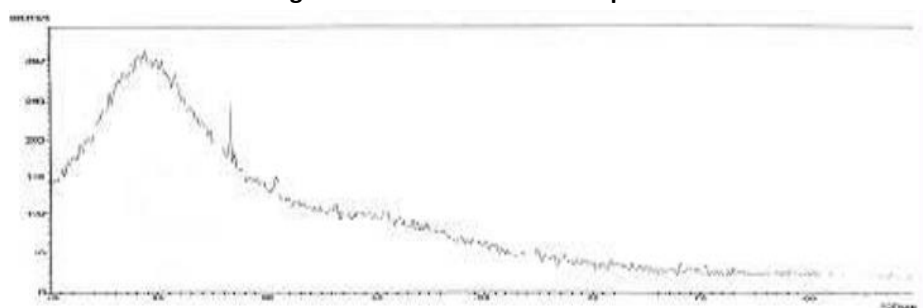


Fig. No. 9: XRD of drug loaded microspheres

It was observed from the investigation that when stirring speed was low (500 rpm), larger spherical microspheres were formed. This may be due to inadequate stirring speed which was not able to break the emulsion droplets. Stirring speed above 1500 rpm resulted in formation of spherical small microspheres, which may be due to higher degree of emulsification of the polymeric phase at a higher speed. Increasing the surfactant concentration, the mean particle size had diminished considerably. When medium concentration of span 80 was used, the particle size increases because low amount fails to prevent droplet coalescence in the oil medium. As the concentration increased to 1.5% the mean particle size was reduced. Further increasing the amount of crosslinking agent (glutaraldehyde) caused a slight increase in the particle size of the formulation. This could be due to adherence of excess crosslinking agents on the surface of the microspheres. It was observed that the particle size of blank microspheres was lower than the drug loaded microspheres. Hence it confirms that effective drug loading has been taken place in microspheres which increased the particle size of the microspheres.

Effect of various parameters on entrapment efficiency

The volume of processing medium significantly influences the entrapment efficiency of the formulations. As the volume of processing medium was increased from 100 ml to 150 ml and to 200 ml, the entrapment efficiency significantly decreased. As the

volume of processing medium was increased, the emulsion droplets probably moved freely in the medium, thus reducing the collision induced aggregation which could be the reason of high drug extraction into the processing medium resulting in lower entrapment efficiency. Effect of crosslinking agent amount on entrapment efficiency was also studied. It was found that on increasing the amount of the crosslinking agent, the drug entrapment efficiency was decreased

In vitro dissolution study

In vitro dissolution study of pure drug (Figure 6) and optimized formulation (F6) as shown in Figure 7, it was observed that pure drug was released at faster rate. About 91% drug was released within 15 minutes but when encapsulated in chitosan microspheres, 88% drug was released from the formulation in 12 hrs indicating controlled release of the drug from the optimized formulation.

Scanning Electron Microscopy (SEM)

The surface topography of the microspheres was investigated by SEM as per given in Figure 4. SEM analysis suggests that the microspheres were found to be spherical. Drug-loaded microspheres had rough surfaces due to higher concentration of drug in the microspheres as compared to the blank microspheres.

Fourier transform infrared spectroscopy

FTIR spectroscopy of flurbiprofen, chitosan and chitosan-FBP spheres were instructed to explain drug-

biopolymer interaction. FTIR spectrums of flurbiprofen, chitosan and CS-FP spheres were compared in Fig. 5. As seen from the Figure 5a, the characteristic sharp peaks of flurbiprofen at 1694.7, 1414.7 and 1216.1 cm^{-1} were due to C=O stretching, O-H bending and C-F stretching, respectively. The characteristic band of flurbiprofen due to the hydrogen bonds of the carboxyl group appeared in the range of the 3400-2400 cm^{-1} were seen from the Figure 5b at 3290.4 cm^{-1} , 1649.0 cm^{-1} , 1586.1 cm^{-1} and 1318.9 cm^{-1} which correspond to OH and NH stretching, amide I (C=O), amide II (NH₂) and amide III (C-N), respectively. Spectrum of chitosan-FBP spheres (Figure. 5c) compared with the other spectrums, there are some changes indicating the structural differences of chitosan after the encapsulation process. It is seen that the O-H and N-H stretching bands were shifted to lower wavenumbers at 3108.4 cm^{-1} due to H bonding system. Furthermore, peaks observed at 927.68 cm^{-1} , 765.27 cm^{-1} , 721.6 cm^{-1} and 697.15 cm^{-1} indicate the presence of the substitute aromatic rings of flurbiprofen. These changes greatly showed that flurbiprofen successfully encapsulated into chitosan particles.

X-ray Diffractometry (X-RD)

The X-ray diffraction patterns of pure drug, polymer, blank microspheres and drug loaded microspheres as stated in Figure 3 reveals that the intensity of the peaks for the pure drug was sharp. But when it was incorporated into the polymer matrix, the drug peaks showed a loss of sharpness probably due to decreased crystallinity of the drug.

CONCLUSION

Formulation and evaluation of flurbiprofen loaded chitosan microspheres for controlled release was found to be potential and effective in terms yield, encapsulation efficiency, particle size distribution and in vitro release characteristics. The investigation of optimum formulation showed controlled drug release and could, therefore, produce some benefits such as reduction in total dose, frequency of administration, and dose related systemic side effects in patients.

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