Original Article

FORMULATION AND CHARACTERISATION OF SUSTAINED RELEASE MICROBEADS LOADED WITH ZALTOPROFEN

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ABSTRACT

Objective: The main aim of the present investigation was to formulate and evaluate microbeads of zaltoprofen. Zaltoprofen, a BCS class II drug used in the treatment of rheumatoid arthritis. Zaltoprofen has a shorter half-life of 2.8 h, and it is administered at a dose of 80 mg thrice a day. By encapsulating the drug into microbeads, it is expected that the release of the drug would be prolonged and thereby, it reduces the frequency of administration and better patient compliance may be improved.

Methods: Gellan-chitosan and calcium chloride beads of zaltoprofen were prepared employing ionotropic gelation method using different concentrations of gellan, chitosan, calcium chloride and zaltoprofen. The microbeads were evaluated for its various Physico-chemical parameters such as particle size determination, drug entrapment efficiency, infrared spectroscopy study, differential scanning calorimetry, X-ray diffraction analysis, scanning electron microscopy, *in vitro* drug release study and *in vivo* oral bioavailability studies.

Results: The results suggested that the batch FG-II exhibited higher drug entrapment efficiency (72.42±0.013), a sustained drug release for a period of 24 h. The pharmacokinetic profile of the drug from microbeads exhibited an enhanced oral bioavailability (2.4 times higher than that of pure drug), lower elimination rate (1.14 times lesser for the drug in microbeads) with prolonged elimination half-life (2.561 times higher than pure zaltoprofen).

Conclusion: Zaltoprofen entrapped microbeads demonstrated as a better delivery system for the sustained release of drug and also to circumvent the drawbacks associated with conventional therapy.

Keywords: Zaltoprofen, Gellan, Chitosan, Ionotropic gelation, Oral bioavailability studies

INTRODUCTION

Oral drug delivery system is considered to be the most ideal route for the administration of drugs for therapeutic efficacy. However, conventional drug delivery systems have certain limitations of repetitive administration, poor oral bioavailability and poor patient compliance. Therefore, there is need for the modified release formulations, which could overcome the flaw associated with conventional therapy. Among the recent inventions, the sustained or modified release systems are gaining good attention recently. A sustained release drug delivery system is an ideal approach that provides a uniform concentration of drug to be available at the site of absorption. Because of this ideal property, the system tends to maintain adequate plasma concentrations in the therapeutic range [1]. In the last few decades, researchers have contributed to a higher extent in the development of novel drug delivery systems, which assures the targeting of drug to a site and to maintain the required concentration to exhibit better therapeutic effect [2]. In most of the circumstances, the optimal therapeutic response is observed when adequate blood levels are being maintained with minimal variation. Sustained-release formulations are gained importance in oral administration of drug and also maintenance of consistent blood levels. Among them, microbeads have received greater attention in these days owing to their unique property in designing and fabricating the formulation. Microbeads are particulate carriers in which the drug is dispersed in solution or crystalline form, which facilitates the sustained release profile of drug [3]. Micro-encapsulation technology facilitates an effective administration of drugs by enhancing their solubility profile, minimizing their side effects and improving therapeutic response [4]. Further, improved therapeutic efficacy coupled with reduced adverse effect is need of the hour in research activity [5]. The utility of natural biodegradable polymers in developing a sustained drug delivery still continues to be the interesting research area owing to their abundance natural availability, capability to undergo chemical modifications and compatibility [6-7]. Zaltoprofen, a COX inhibitor prescribed for the treatment of rheumatoid arthritis, has shorter half-life of 2.8 h [8]. Zaltoprofen is administered 80 mg thrice daily. By incorporating the drug into a microbeads drug delivery system, it would be expected to sustain the release of the drug, reduce the dosing frequency, improve the effectiveness in rheumatoid arthritis and may improve better patient compliance. Hence, the main purpose of this study was to formulate and evaluate zaltoprofen microbeads for the sustained release of the drug for the effective management of rheumatoid arthritis. Various characterization parameters such as particle size determination, drug entrapment efficiency, infrared spectroscopy study, differential scanning calorimetry, x-ray diffraction analysis, scanning electron microscopy, *in vitro* drug release study and *in vivo* oral bioavailability studies were systematically investigated.

MATERIALS AND METHODS

Materials

Zaltoprofen was purchased from Titan Chemicals, Shanghai, China. Gellan gum was procured form Sigma-Aldrich Ltd, (Mumbai, India) and chitosan was procured from Sigma-Aldrich Ltd, (Mumbai, India). All other chemicals used in the study were of pharmaceutical or analytical grade.

Preparation of microbeads containing zaltoprofen

The microbeads of zaltoprofen were prepared by ionotropic gelation technique. Firstly, gellan gum solution was prepared by dissolving gellan in deionized water and followed by heating it at 60 ° C. About 50 ml of the gellan gel from each batch was taken separately and a different concentration of drug was dispersed uniformly in the gel maintained at 40 ° C, with continuous stirring. The stirring was further continued until uniform dispersion of drug was achieved. The resultant homogenous slurry was dropped into a 50 ml solution containing different concentrations of calcium chloride and chitosan,

using a syringe (21G) under stirring condition. The stirring was continued for certain period of time to improve the mechanical strength of the beads and also to prevent aggregation. The formed beads were separated from the solution by filtration and dried at 40 °C. The gellan beads with the exception of chitosan were also prepared using aforesaid procedure [9]. The details of the composition of microbeads, particle size and drug encapsulation efficiency of different formulations were shown in table 1.

Particle size determination

The particle size of the beads was measured using a micrometer (Mitotuyo, Japan). The average diameter of 100 particles per batch was estimated [9].

Drug entrapment efficiency (DEE)

The drug content in the beads was estimated by digestion method, where a known quantity of zaltoprofen loaded beads (20 mg) was pulverized in a glass mortar with pestle and incubated in phosphate buffer ($_p$H 6.8) at room temperature for 1 h to extract the drug completely. The clear supernatant solution was assayed spectrophotometrically for drug content at the wavelength of 243 nm. Supernatant from the empty beads was taken as blank. All samples were analyzed triplicate [9].

Infrared spectroscopy study (FTIR)

Infrared spectra of zaltoprofen, blank gellan and chitosan microbeads and drug-loaded beads were performed using in KBr pellets using a FTIR spectrophotometer (Perkin-Elmer, Japan). The samples were scanned between in the range of 4000 to 400 cm^{-1}.

Differential scanning calorimetry analysis (DSC)

DSC analysis was carried out to observe the changes if any during the formulation of beads. DSC of zaltoprofen, blank gellan and chitosan microbeads and drug loaded beads were examined by using a thermal analyzer (TA instruments, Bangalore). The samples were heated from 50-200 ° at a heating rate of 10 ° C/min under constant purging of nitrogen at a flow rate of 50 ml/min respectively.

X-ray diffraction analysis (XRD)

X-ray powder diffraction analysis of zaltoprofen, drug-loaded beads were carried out using the X-ray diffractometer (Jeol JDX 8030 X-ray diffractometer Tokyo, Japan) using Ni-filtered, CuKα radiation, a voltage of 40 kV and a 25-mA current. The scanning rate employed was 1 °/min and scanned over 2θ range of the 10 to 80 °.

Scanning electron microscopy (SEM)

The surface morphology of the beads was examined using scanning electron microscope (Jeol, JSM, 35CF, Japan). The beads were mounted onto individual stab and then coated with carbon and gold (100 and 50 Å thickness respectively). The coated samples were then observed under scanning electron microscope operated at 7 kV.

***In vitro* drug release study**

In vitro drug release studies of zaltoprofen from beads were carried out using 900 ml of phosphate buffer ($_p$H 6.8) in a USP paddle-type dissolution apparatus (USP XXIII, Electrolab, Mumbai) at 50 rpm. A weighed quantity of beads equivalent to 50 mg of drug was used in each test. At predetermined time intervals a 5 ml of samples were withdrawn and same volume of fresh media was replaced. The amount of drug release was analyzed at 243 nm after proper dilution if required.

Release kinetics

Different kinetic equations (zero order, first order and Higuchi's equations) were applied to interpret the release rate of the drug from the matrix system. The best fit with higher correlation (r^2>0.98) was found with Higuchi's equation for all the formulations. Two factors, however, diminish the applicability of Higuchi's equation to matrix systems [10]. This model fails to allow for the influence of swelling of the matrix (upon hydration) and the gradual erosion of the matrix. Therefore, the dissolution data were also fitted to the well-known exponential Koresmeyer–Peppa's equation [11].

$$\frac{M_t}{M_\alpha} = kt^n \qquad \text{...... (2)}$$

M_t/M_α is the fraction of drug release at time 't', 'k' is the kinetic constant and 'n' is the release exponent (indicating release mechanism). In addition, for determination of the exponent 'n', one must use only the initial portion of the release cure (M_t/M_α<0.6) [11]. Ritger and Peppa's have defined the exponent 'n' as a function of the aspect ratio (2a/l) defined as the ratio of diameter (2a) to thickness (l). For tablets, depending on the aspect ratios, 'n' values between 0.43 and 0.5 indicates fickian (Case I) diffusion mediated release, non-fickian (anomalous) release, coupled diffusion and polymer matrix relaxation occurs if 0.5<n<0.89, purely matrix relaxation or erosion-mediated release occurs for n=1 (zero-order kinetics) and super case II type of release occurs for n>0.89 [12].

Bioavailability studies

Albino rats (male) weighing approximately 200±50 g was used for the *in vivo* studies [13]. A randomized, two treatment, two-period, two sequences, single-dose cross over bioavailability study for the suspension of zaltoprofen and zaltoprofen microbeads was carried out in 6 healthy albino wister rats to prove the safety and efficacy of the formulations. The protocol of the study was approved by the institutional animal ethics committee (JSSCP/IAEC/M. PHARM/PH. CEUTICS/04/2014-15 dated 10/10/2014). The animals were divided into three groups of six animals each. Prior to the study the animals were kept for fasting. Group I served as control, group II pure drug in suspension form at a dose of 6 mg and group III received microbeads containing drug of 6 mg. 0.5 ml of blood was withdrawn from the marginal ear vein of the animals at the predetermined time intervals of 0, 1, 2, 4, 8, 12 and 24 h using sterilized disposable syringes. The blood sample were collected in a ria vial containing the anticoagulant (0.3 ml of 11% sodium citrate) were centrifuged at 4000 rpm for 4 min to separate plasma. The plasma samples were deproteinized using an equal volume of 10% perchloric acid and vortoxed for 2 min. It was further centrifuged at 4000 rpm for 4 min to separate the plasma and stored at-20 ° C until further analysis. A reproducible analytical technique was developed for the estimation of the drug in the plasma samples. The HPLC system consisted of a stationary phase, Lichrospher C18 (250×4.6 mm i.d., 5µ), the mobile phase consisted of acetonitrile: ammonium acetate buffer ($_p$H 3) with a ratio (60:40), with a flow rate of 1 ml/min, using a sample volume of 20 µl. The internal standard used was nevirapine. The detection was carried out at 260 nm using Shimadzu HPLC: Model LC-2010 A-HT autosampler. Various pharmacokinetic parameters such as C_{max}, T_{max}, K_{el}, AUC_{o-t} and $AUC_{o-\alpha}$ were estimated using PK1 and PK2 solutions.

Statistical analysis

Statistical analysis was performed using SPSS version 13.0. The pharmacokinetic parameters like C_{max}, T_{max}, K_{el}, AUC_{o-t} and $AUC_{o-\alpha}$ of both the formulations are presented in mean±SD One-way ANOVA (analysis of variance) was employed in the statistical analysis of the determined parameters in this study. Statistical significance was defined at p<0.05.

RESULTS AND DISCUSSION

Particle size determination

The microbeads prepared using reinforced method was found to be smooth and free-flowing. The average particle size of the different batches of formulation was varied from 610±1.23 to 823±2.21 µm. This suggested that the quantity of gellan increases, the sizes of the beads also increases proportionally due to the increase in the micro-viscosity of the polymer.

Drug entrapment efficiency (DEE)

The DEE was found to be in the range of 42.89±1.8% to 72.42±0.9%. These results indicated that the DEE of beads increases with an increase in the concentration of the polymer. This could be explained by the fact that the ionized state of polymer concentration enables intense cross-linking results in higher encapsulation efficiency of the drug. Formulations containing chitosan exhibited higher encapsulation efficiency than the formulations not containing chitosan.

Table 1: Composition of zaltoprofen loaded microbeads, bead size and drug entrapment efficiency

| Code of formulation	Drug (mg)	Gellan gum (mg)	Chitosan (mg)	Calcium chloride (%w/v)	Bead size (µm)*	Drug entrapment efficiency** (DEE) %
FG–I	50	50	50	3	728±2.12	65.62±1.3
FG–II	50	100	50	3	781±3.23	72.42±0.9
FG–III	100	50	50	5	702±3.21	60.14±1.4
FG–IV	50	50	50	5	640±2.77	62.41±0.8
FG–V	50	100	50	5	649±3.64	70.22±1.5
FG–VI	100	50	50	3	823±2.21	60.32±1.3
FG–VII	50	50	-	3	616±1.23	48.32±1.5
FG-VIII	50	100	-	3	631±2.62	56.21±0.9
FG-IX	100	50	-	3	724±3.72	42.8±1.8

*(mean±SD, n=100), **(mean±SD, n=3)

Infrared spectroscopy study (FTIR)

The infrared spectra of zaltoprofen, blank gellan and chitosan microbeads and drug-loaded beads are shown in fig. 1-3. These spectra's revealed that no shifting of peaks was observed, indicating the stability of the drug during the encapsulation process. The principle peaks of zaltoprofen-OH at 3336 cm^{-1}, Ar=H at 3057.27 cm^{-1}, CH$_2$ at 2896.21 cm^{-1}

and C=O at 1703.2 cm^{-1} and C-O at 1280 cm^{-1}. After interpretation through the above spectra with the drug-loaded microbeads, it was confirmed that there was no major shifting as well as any loss of functional peaks. From the spectra, it was concluded that the polymer gellan gum and chitosan is found to be compatible in entrapping the zaltoprofen. The decrease in the peak intensity of the formulation may be attributed to the fine dispersion of drug in the polymer matrix.
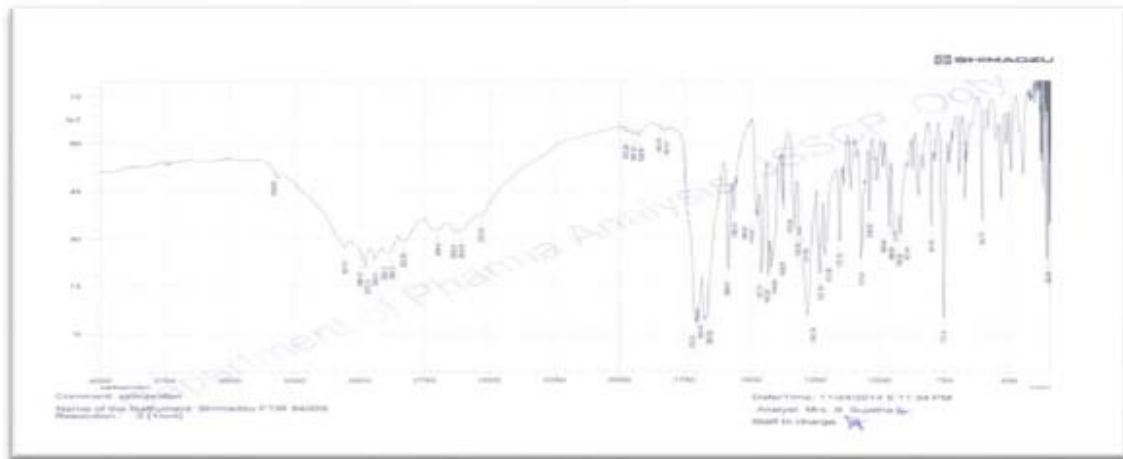
Fig. 1: Fourier transform infra red spectroscopy of zaltoprofen
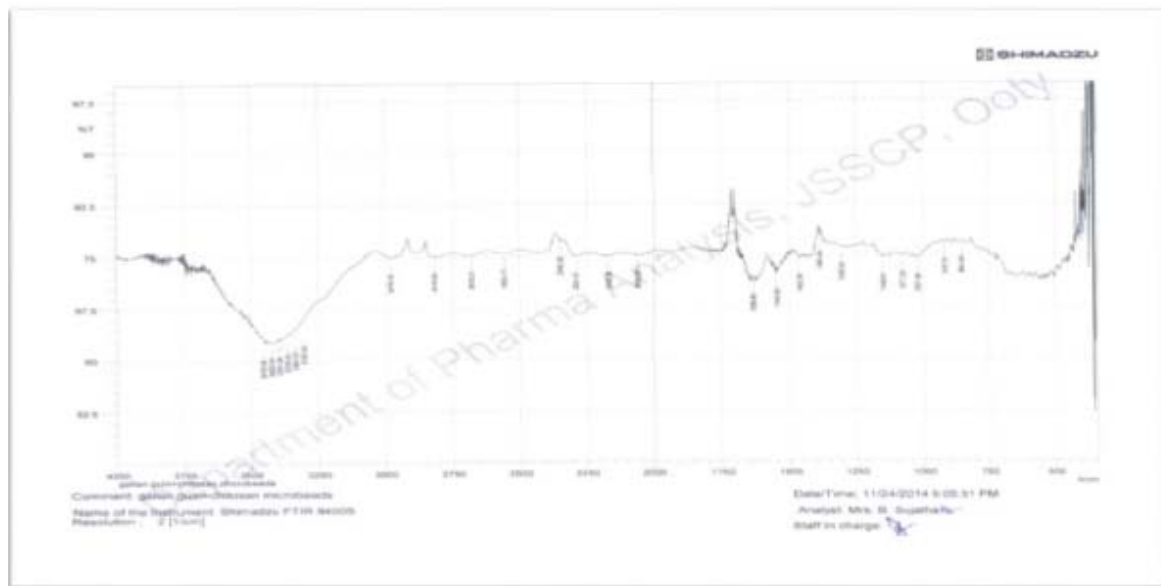
**Fig. 2: Fourier transform infra red spectroscopy of blank microbeads**
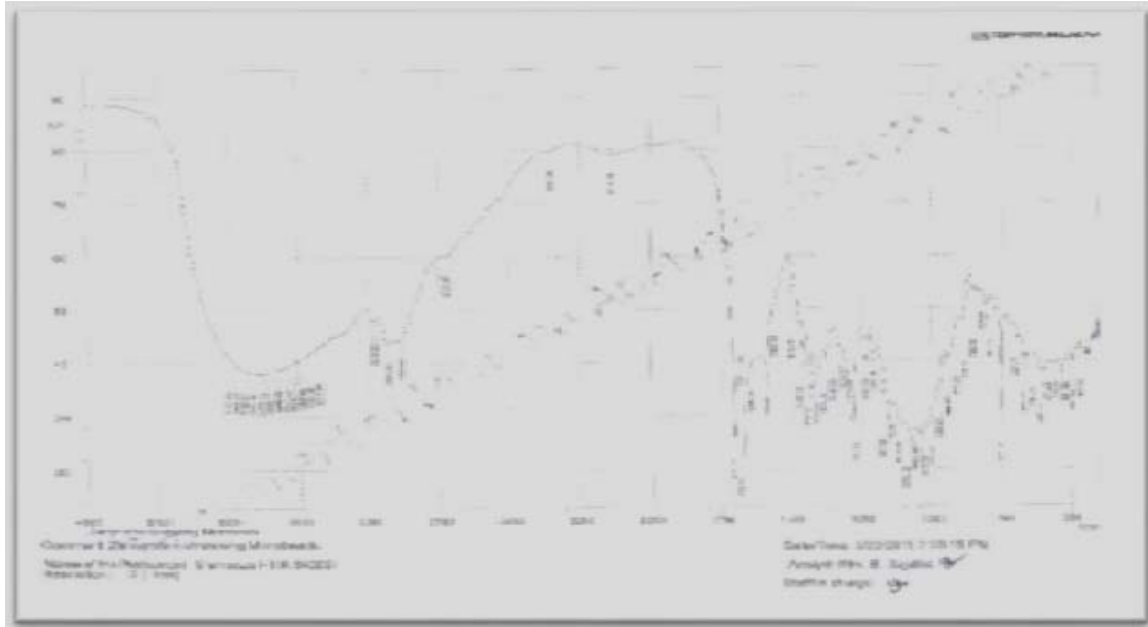
Fig. 3: Fourier transform infrared spectroscopy of zaltoprofen loaded microbeads

Differential scanning calorimetry analysis (DSC)

Fig. 4-6 illustrates the comparative DSC thermogram of zaltoprofen, gellan-chitosan beads, zaltoprofen loaded gellan-chitosan beads. The DSC thermogram of zaltoprofen showed a sharp endothermic peak corresponding to the melting point at 139.19 °C. The sharp endothermic peak of drug in microbeads has not appeared indicating that the drug was dispersed in an amorphous state in the polymer.
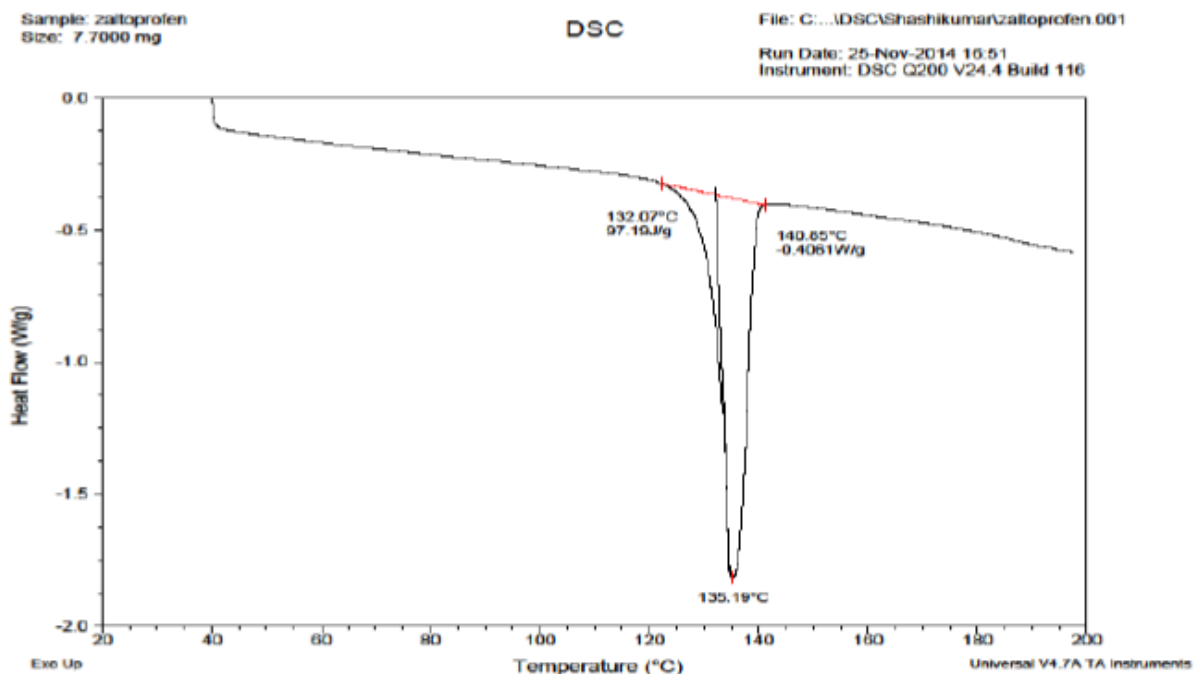
Fig. 4: Differential scanning calorimetry of zaltoprofen

X-ray diffraction analysis (XRD)

The X-ray powder diffraction patterns of drug and drug-loaded microbeads are compared. The X-ray powder diffraction pattern of pure drug showed its own crystal peaks between 2θ of 15 ° and 28 °. It was observed that the characteristic peaks of drug were found to be absent in the drug entrapped gellan-chitosan beads indicates the probable decrease in the crystallinity of zaltoprofen. The undefined, broad, diffused peaks with the low intensities for drug entrapped beads revealed that the conversion of drug from crystalline to amorphous form in the formulation fig. 7-8.
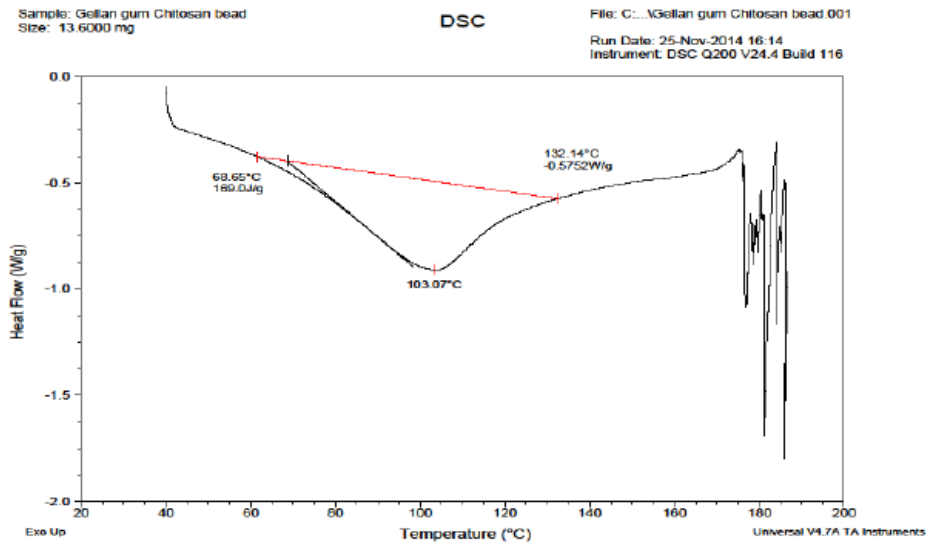
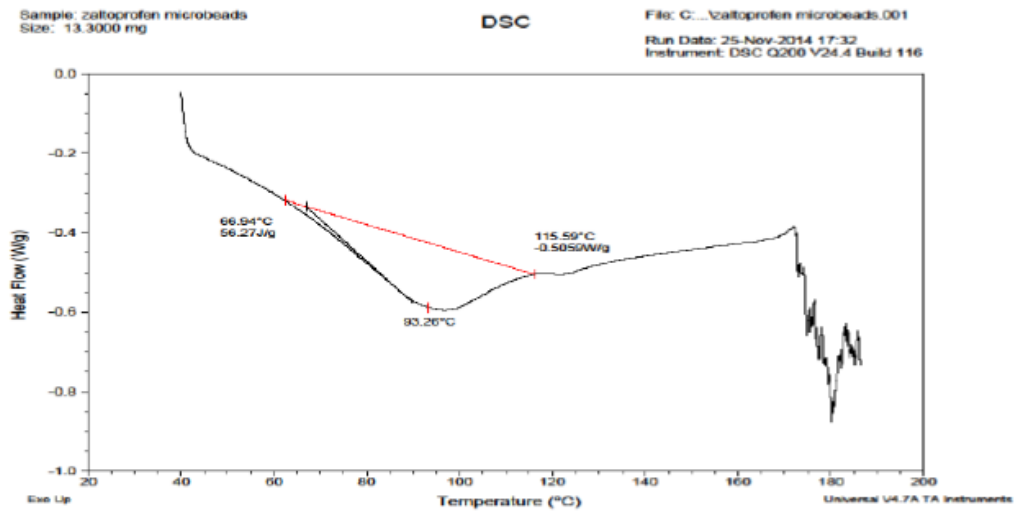**Fig. 5: Differential scanning calorimetry of blank microbeads**

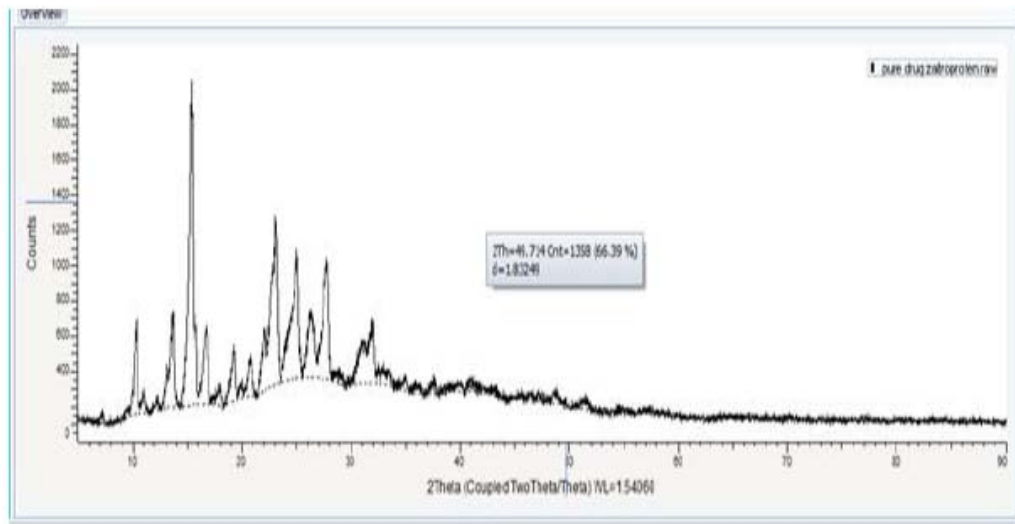**Fig. 6: Differential scanning calorimetry of zaltoprofen loaded microbeads**

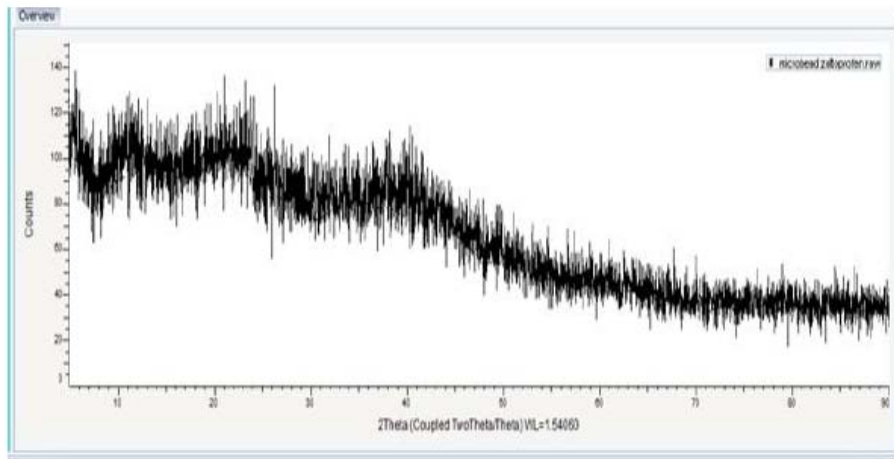**Fig. 7: X-ray diffraction analysis of zaltoprofen**

Fig. 8: X-ray diffraction analysis of zaltoprofen loaded microbeads

Scanning electron microscopy (SEM)

The SEM of the drug-loaded beads was found to be irregular in shape having smooth and dense surface with inward dent and shrinkage due to collapse of the wall during dehydration. The fibrous network was also observed on the surface of the beads as shown in fig. 9-10.
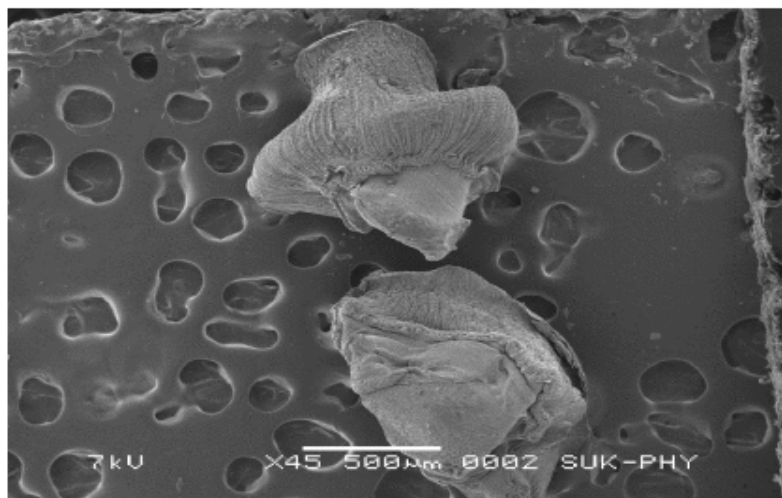

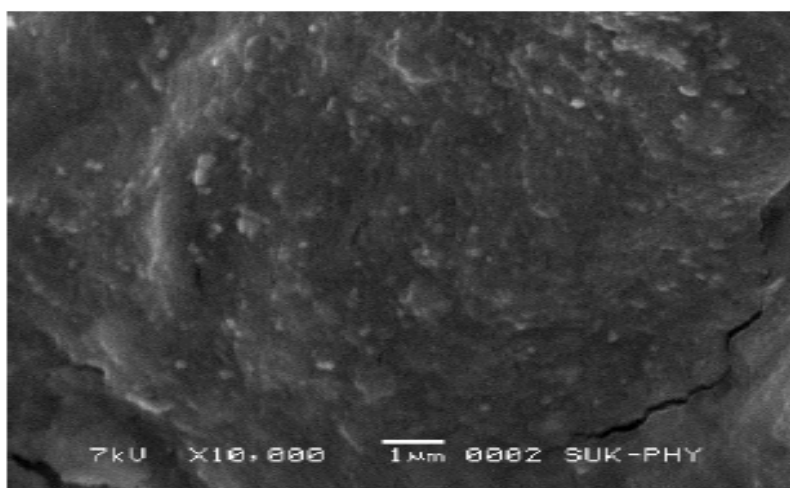**Fig. 9: Scanning electron microscopy (SEM) of zaltoprofen microbeads**

Fig. 10: Scanning electron microscopy (SEM) of zaltoprofen microbeads with dense surface

In vitro drug release study

The *in vitro* release profile of drug-loaded beads was depicted in fig. 11. From the results it can be observed that the drug release from the beads significantly decreased with an increase in the concentration of the polymer. This could be explained as an increase in the concentration of polymer substantially increases the density of the matrix and diffusional path in which allows the drug molecules have to traverse. A biphasic pattern of drug release was observed with initial burst effect followed by the sustained release due to the process of gelation as a result of cross-linking, which is a characteristic feature of matrix diffusion. It was observed that the initial burst effect of drug was substantially decreased in case of beads coated with chitosan over non-coated beads. The fact could be due to the coating of chitosan exhibited better incorporation efficiency and offered a thick coating over the beads. This could be resulted in the decrease burst effect of drug from the beads.
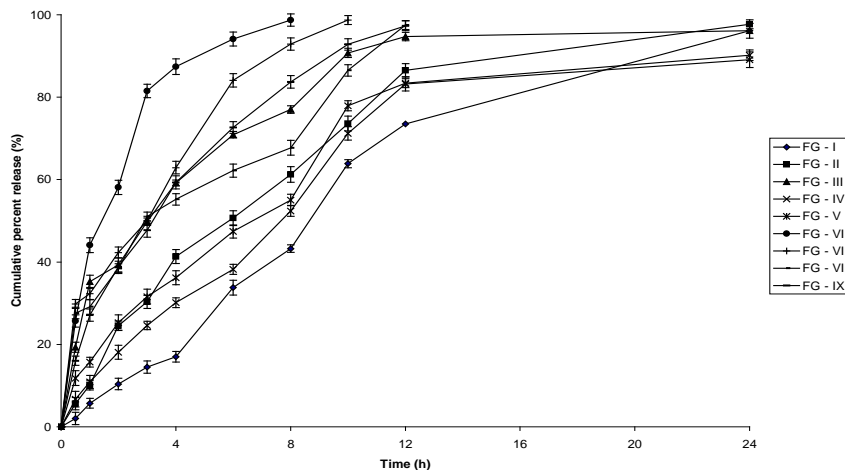
**Fig. 11: *In vitro* release profiles of zaltoprofen from microbeads (mean±SD, n=3)**

Release kinetics

In order to elucidate the mechanism of drug release, the data were fitted into various models such as zero order, first order, Higuchi, Koresmeyar and Peppa's. The data are shown in table 2. The examination of the coefficient of correlation (r²) values indicated that the prepared beads followed first-order kinetics with non-fickian diffusion mechanism of drug release.

Table 2: Regression coefficient (r²) of zaltoprofen microbeads data from studied matrices according to different kinetic models, diffusion exponent (n) of peppa's model

Code of formulation	Zero-order (r²)	First-order (r²)	Higuchi (r²)	Peppa's	
				n	(r²)
FG-I	0.779	0.972	0.981	0.610	0.903
FG–II	0.9234	0.9668	0.9278	0.6558	0.9894
FG–III	0.861	0.943	0.901	0.601	0.978
FG–IV	0.881	0.970	0.963	0.606	0.939
FG–V	0.856	0.951	0.946	0.689	0.952
FG–VI	0.762	0.974	0.947	0.699	0.967
FG–VII	0.888	0.989	0.988	0.634	0.973
FG-VIII	0.872	0.946	0.956	0.702	0.931
FG-IX	0.886	0.976	0.986	0.721	0.985
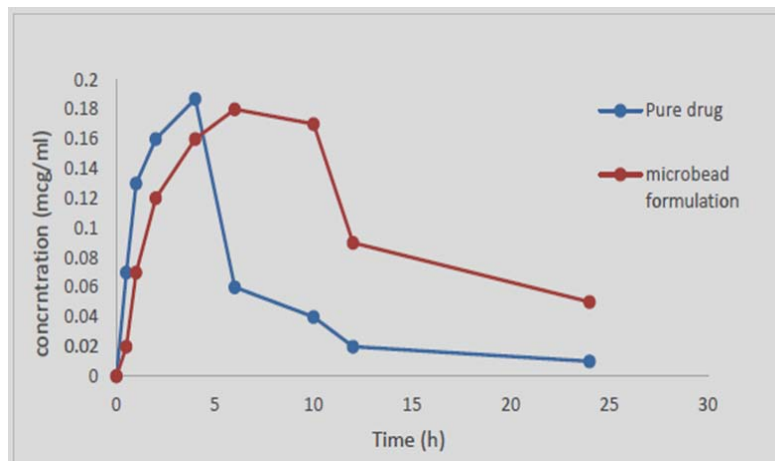
Fig. 12: Mean plasma concentration of pure drug and zaltoprofen microbeads (mean±SD, n=6)

Table 3: Pharmacokinetic profile of pure drug and drug loaded microbeads (mean±SD, n=6)

Pharmacokinetic parameters	Type of formulations	
	Pure drug	Drug loaded microbeads
C_{max} (ng/ml)	0.187±0.045	0.18±0.043
T_{max} (h)	4.0±0.5	6±0.9 ‡
AUC_{o-t} (ng. h/ml)	1.246±0.04	2.542±0.22 ‡
$t_{½}$ (h)	2.8±0.07	7.17±0.08‡
K_{el} (h^{-1})	0.086±0.003	0.0757±0.005†
$AUC_{0-\alpha}$ (ng. h/ml)	1.349±0.015	3.22±0.016‡

‡ Significantly higher than pure drug (p<0.05), † Significantly lower than pure drug (p<0.05)

Bioavailability studies

The relative bioavailability of the developed microbeads of zaltoprofen (FG–II) was compared with the drug in suspension form. The drug-loaded microbeads produced a plasma concentration-time profile typical of the prolonged dissolution characteristic of a sustained release as evident from fig. 12 and table 3. The microbeads formulation demonstrated a longer time to reach a peak concentration than the pure drug in suspension form and appeared to be more consistent in overall performance. This was indicated by lower variation in plasma concentrations, longer time to peak plasma concentration. There was a significant difference in the extent of absorption as assessed by measurements of AUC_{o-t}. However, $AUC_{o-\alpha}$ value for the drug-loaded microbeads was 1.25 times higher than that of pure drug. The drug-loaded microbeads offered more efficient and sustained drug delivery, which would maintain plasma zaltoprofen levels better. This was also evident by the lower elimination rate (K_{el}) (1.32 times lesser for drug in microbeads). The pharmacokinetic parameters of the two different formulations of zaltoprofen were compared statistically by one-way ANOVA (analysis of variance) using SPSS version 13.0. The pharmacokinetic parameters such as C_{max}, T_{max}, K_{el}, AUC_{o-t} and $AUC_{o-\alpha}$ of the pure drug and drug-loaded microbeads were found to be significantly different (p<0.05) by one-way ANOVA.

CONCLUSION

Zaltoprofen microbeads were successfully prepared using ionotropic gelation method with moderate entrapment efficiency. A change in the drug crystallinity during the formulation was revealed by DSC and XRD study. FTIR spectral study revealed no chemical change in the microbeads formulation. Zaltoprofen microbeads exhibited enhanced oral bioavailability as compared to pure drug. The present investigation demonstrated microbeads as a potential drug delivery system for improving the oral bioavailability of zaltoprofen.

AUTHORS CONTRIBUTIONS

First author conceived the idea, the second author carried out the research work under the supervision of first and third author. First author drafted the manuscript with the help of third author.

CONFLICTS OF INTERESTS

Authors declare no conflicts of interest

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