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Preparation and evaluation of oral controlled release mucoadhesive microspheres of Ketorolac tromethamine

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Abstract

Recently, lot of emphasis is being laid on oral controlled release multiple unit particulate (MUP) dosage forms, for their significant and potential benefits. Ketorolac tromethamine (KTM) is a potent non-narcotic analgesic and anti-inflamatory drug administered orally in multiple divided doses (10 mg four times a day) for the management of mild to moderate post-operative pain. KTM's short biological half-life demands frequent administration of the drug leading to poor patient compliance and inadequate pain management. Hence, the present investigation was undertaken to develop and evaluate oral controlled release mucoadhesive microspheres by ionotropic gelation method using natural and biodegradable polymers such as sodium carboxy methyl cellulose (Na CMC) and sodium alginate (SA). The influence of various formulation factors on the drug entrapment efficiency, in vitro drug release, micromeritic properties, and mucoadhesion ability was investigated. Scanning electron micrographs of alginate beads loaded with drug exhibited rough surface morphology and sizes were found to be in the range of 842 to 1265 µm. Among all the formulations, the drug loaded microspheres of formulation CA6 showed the highest drug release retarding effect over a period of 8 hours. The drug-polymer compatibility studies and solid state properties were investigated by Fourier transform infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC) X- Ray diffraction techniques (X-RD).

Keywords: Microspheres, Ketorolac tromethamine, controlled release, Ionotropic gelation technique, FTIR.

Introduction

Single-unit CR delivery systems or monolithic systems, generally may suffer from certain disadvantages such as unintentional disintegration of the formulation due to technological deficiency or abnormal or unusual gastric physiology leading to drastic changes in some patients.[1] Multi-particulate drug delivery systems contain a multiplicity of small individual units, each capable of exhibiting required characteristics. These systems, consists of thousands of spherical particles with diameter of 0.05-2.00mm.[2] MUPs offer all the advantages of a single unit formulations yet devoid of the dangers of alteration in a drug release profile and formulation behavior due to unit to unit variation, change in gastro-luminal pH and enzyme population. Such benefits can lead to increased bioavailability, less risk of systemic toxicity, reduced risk of local irritation, and predictable gastric emptying. MUPs.[3] These drug delivery systems, because of the smaller particle size, are able to pass through the gastrointestinal (GI) tract easily, leading to less inter- and intra-subject variability and dispersed more uniformly along the GI tract and result in more uniform drug absorption. The significant increase in the surface area of the drug loaded microspheres will enhance the exposure of the drug to the absorption site thus increasing the over all absorption of drug.[4] Moreover, MUPs can exhibit better in vivo performance than a single-unit system, as they show less erratic gastrointestinal transit times and are more sparsely scattered over the intestinal tract, thus providing greater uniformity of drug absorption, reduced potential for mucosal irritation and provide more reproducible drug release.[5] With these systems, even the safety profile the drug could be improved, mainly because the release characteristics are built into each sub unit.[6] These subunits units can either be filled into a sachet and encapsulated or compressed into a tablet.

Numerous hydrophilic polymers, and in particular, polysaccharides, as well as their derivatives, have been employed in the formulation of modified-release dosage forms.[7] Recently, alginate beads containing several substances have been prepared by the gelation of alginate with calcium cations and the behaviour of the release of these substances has been investigated.[8]

Alginic acid is a linear block polysaccharide copolymer made of β-D-mannuronic acid (M) and -L-guluronic acid (G) residues joined by 1,4 glycosidic linkages,and derived from sea weed.

The aqueous alginate solutions could form firm gels in presence of di- and tri-valent metal ions by a cooperative process involving consecutive guluronic residues in the G blocks of the alginate chain. The gelation or crosslinking is due to the stacking of the glucuronic acid blocks of alginate chains.[9]

Sodium carboxy methyl cellulose (SCMC) is a water swellable semisynthtic polymer belonging to the group of cellulose that could be used as a drug carrier.[10] It is used to make co-acervation with gelatin for the production of microcapsules.[11]

The SR formulations of non-steroidal anti-inflammatory drug (NSAID) have been proved to minimize the side effects.[12] Ketorolac Tromethamine (KTM) is a well known non-steroidal anti-inflammatory drug with potent analgesic activity prescribed for short term management of mild to moderate post-operative pain The half life of KTM ranges from 4-6 h.[13] When administered as the conventional formulations such as tablets or capsules, it causes gastro intestinal complications including irritation, ulcer, bleeding and perforation.[14] KTM is a relatively more favorable therapeutic agent for the management of moderate to severe pain.[15] The present study , therefore, undertaken to formulate and evaluate controlled release mucoadhesive microspheres of drug using sodium alginate, SCMC.

Materials and Methods

Materials

Sodium alginate and sodium carboxy methylcellulose were obtained from SD fine chemicals, Mumbai, India, KTM was obtained as a gift sample from Dr Reddy labs, Hyderabad, India. All the other chemicals used, were of analytical grade.

Preparation of NaCMC-NaAlg spheres

The ionic gelation method was employed for the preparation of microspheres followed by cross-linking with calcium chloride and aluminium chloride as cross linking agents. Solutions of sodium carboxymethyl cellulose (SCMC) and sodium alginate (total polymer concentration 4 % w/v) were prepared homogeneously using a magnetic stirrer.[16] Accurately weighed quantity of KTM was dissolved in the above solution and 20 ml of the solution was extruded into aqueous solution containing aluminium chloride ($AlCl_3$) and calcium chloride ($CaCl_2$) using 25 ml hypodermic syringe through a needle (number 23) under constant stirring. After incubating for additional 15 minutes in counter ion solution, they were removed, washed and dried at 40ºC for 10 hrs and stored for further use. Different formulations were prepared and shown in table 1

Percentage practical yield

It is the quantity of quantity of beads obtained as a function of loaded drug and the polymer used. The yield of microspheres was determined by comparing the whole weight of microspheres formed against the combined weight of the copolymer and drug.

$$\% \text{ Practical yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total Wt of drug and polymer used}} \times 100$$

Evaluation of the Microspheres

Morphological and Micromeritic properties:

Shape and size analysis of the prepared microspheres were performed by scanning electron microscopy (SEM) and optical microscopy. Optical micrometer was calibrated using stage micrometer and slides of dilute suspension of microspheres were prepared in liquid paraffin and were examined.[17] For SEM, samples of microspheres were mounted on metal stubs, gold coated under vacuum and examined in JEOL JSM-840 SEM Japan.

Swelling behavior

This property was studied by measuring the percentage water uptake by the microspheres. A known weight (50 mg) of microspheres was placed in a glass vial containing 100 ml of phosphate buffer (pH 7.4) and 0.1 N HCl (pH 1.2). They were removed from their respective swelling media periodically, blotted with filter paper and their change in weights were measured. Finally, the weight of the swollen microspheres was recorded after a period of 6 hours, and the swelling ratio (SR) was then calculated.18]

Determination of Encapsulation Efficiency

The drug entrapment efficiency of the beads was calculated from the ratio of actual to theoretical drug content. 100 mg of the microspheres were accurately weighed and crushed and this powder was added to 500 ml of pH 7.4 buffer and kept aside for about 24 hours with occasional shaking. The debris of the beads formed after disintegration was taken out and removed by filtering through Whatman filter paper (No. 40). The absorbance of the filtrate was measured using a UV Vis spectrophotometer (Lab India) at 322 nm.[19]

In vitro drug release studies

The in vitro dissolution study was carried out using dissolution rate test apparatus USP at 50 rpm. The dissolution medium consisted of 900ml simulated gastric fluid (pH 1.2) for first 2hrs followed by simulated intestinal fluid (pH 7.2) from 2 to 8 hrs. The samples were withdrawn at predetermined intervals and analyzed for drug content.[20] by UV-Visible Spectrophotometer at 322 nm. Three dissolution runs were conducted for each batch and the averaged results were taken.

Mucoadhesion testing

Test for mucoadhesion was carried out as per in vitro wash-off method.[21] Freshly excised pieces of goat intestinal mucosa (1 cm_1 cm, procured from a slaughter house) was mounted on a glass slide (7.5 cm_2.5 cm) using thread. Approximately, 100 beads were evenly spread out on each piece of mucosa and then hung from the arm of the tablet disintegration test apparatus. The tissue specimen was given a regular up and down movement in a vessel containing 900 ml of 0.1 N HCl (pH 1.2) and phosphate

buffer (pH 7.4) maintained at 37_0.5 C. The adherence of beads was regularly observed. The beads that remained adhered to the mucosa were counted at regular intervals for up to 10 h.

Fourier Transform infrared Spectroscope

FTIR spectra of the drug, polymer and drug loaded beads were taken by KBr pellet method and compared to assess drug excipient compatibility.[21]

Differential scanning calorimetry (DSC)

DSC determines the physical state of the drug. Any changes in the solid state would alter the drug release profile from the microspheres. DSC thermograms were obtained by taking about 2 mg sample was placing in pierced aluminium pans and heated at a scanning rate of 10ºC per minute from 50 to 250 ºC. The instrument was calibrated with an indium standard.[22]

X-ray diffraction technique (XRD)

The determination of physical state of the drug in the drug delivery system is essential due to the probability of change in solid state of the drug during the process, and such changes may in turn impact the drug release properties. The solid state properties of the drug are studied by X-ray powder diffraction technique (XRD).[23]

Results and Discussion

Size, Shape and percentage yield

The keterolac tromethamine loaded microspheres of NaCMC and SA and were prepared by ionotropic gelation method using aluminum chloride and calcium chloride as cross linking agents.
The polymer sod. alginate was used to control the release rate and NaCMC as a mucoadhesive polymer.
The obtained micro-beads were spherical in shape and freely flowing. The surface morphology was examined by scanning electron microscopy studies (SEM) and presented in Figure 1A and B respectively. The prepared beads are spherical, having rough and dense surface along with surface foldings and visible microscopic cracks. A similar kind of morphology was noted previously for beads made of alginates when the solid drug was microencapsulated.[24] The mean particle sizes of the obtained alginate beads are shown Table 2. Spherical microspheres were smaller in size at equal ratios of the polymers SA:SCMC and larger, when one of the polymer ratio is reduced. This could mean that the ratio of the two polymers has critical values for controlling the particle size of the micro spheres.[25] Finally, the mean particle size was increased from 954 um to1265 um when the sodium alginate polymer concentration was increased from 1% to 3% (w/w).Generally, the particle sizes were in the acceptable size range of microcapsules. It was observed that surface morphology and size of microspheres were dependent on the amount of both the polymers. Also, the microspheres which contained higher proportion of sod. alginate has some rough surface as the proportion of sod. alginate decreases. The presence Na CMC, appears to impart smoothness to the surface.

The percentage practical yield of all the formulations (CA1 –CA6) was found to be within the range of 80.12 to 91.12 % which indicates the suitability of the method in preparing the micro spheres. The percentage practical yield is depicted in Table.2.

Drug entrapment efficacy

The drug entrapment efficacy of all the formulation was in the range of 61.76 – 72.50. The drug entrapment efficacy of microspheres was noted to increases with increase in concentration of hydrophilic polymers. Amongst formulations (CA1-CA6) CA5 and CA6 have shown good entrapment efficiency. Over all, it is observed that there was not much difference in their capacity to entrap drug (Table 2). The highest drug entrapment efficiency was noted in alginate-Na CMC beads at 4.5 % w/v Calcium chloride and Aluminium chloride. The lowest drug entrapment efficiency of KTM was observed at low cross-linking agent concentration (2.5 % w/v CaCl2 and aluminium chloride). At lower concentrations of cross linking agents, the beads showed larger pores due to insufficient cross-linking which results in lower drug entrapment.

Swelling behavior

Swelling behavior is one of the critical factors which influence the drug release profile from the drug loaded beads. Therefore, the swelling behavior of KTM-loaded alginate-CMC beads were evaluated and shown in table 3 and depicted in fig 3 Maximum swelling of beads was noticed at 3–5 hrs in phosphate buffer after which erosion and breakdown started to take place. This type of behavior may result from gradual erosion of crosslinking of alginate backbone into smaller fragments. Also, the exchange of Ca2+ ions present in the microspheres with Na+ ions of the phosphate buffer would cause a prolonged erosion of the microspheres which in turn significantly increases the drug release rate in the phosphate buffer. These results clearly indicate that the beads will swell to a lesser extent in the stomach before they move to the upper intestine where the drug is absorbed and the alginate-CMC beads starts to swell to their maximum extent and behave as hydrophilic matrices for the controlled release of the drug in the intestine.

In vitro dissolution studies

The data from in vitro drug release profile of all the formulations was depicted in the figure 4. From the study, it is revealed that CA5 and CA6 formulations exhibited better drug release retarding ability at the end of 8 hrs releasing of the drug 78.50% and 73.05% respectively, In comparison with other formulations. On the other hand, it is found that the formulation CA1 could release almost all of its drug content at the end of 8 hrs. The release profile of KTM from micro spheres exhibited more sustained nature of release when the sodium alginate and NaCMC were incorporated at 2 % each.

DSC Analysis

The DSC analysis of plain KTM, drug-free beads and a drug-loaded micro spheres was carried out and the results are shown in Figure 2. The drug-free beads have shown an endothermic peak at

100.76 ^{o}C due to associated free and bound water, another peak is seen at 195.9 ^{o}C, may be due to melting temperature of the polymer. Whereas drug-loaded beads have shown an endothermic peak at 91.31 ^{o}C, due to free and bound water present in the matrix. The plain keterolac has shown a sharp endothermic peak at 168.9 ^{o}C due to melting of the drug. However, this peak is observed in the drug-loaded beads at 167 ^{o}C, indicating the stability of drug in the polymer matrix.

X-ray diffraction studies

The x-ray diffraction studies are useful to investigate the crystallinity of the drugs after entrapment into the dosage forms. The X-ray diffractograms of plain KTM, drug free beads and drug-loaded beads are presented in Fig. 3. Keterolac has shown characteristic intense peaks between the 2θ of 10^{o} and 40^{o} due to its crystalline nature. Whereas, in case of drug loaded beads, no intense peaks related to drug were noticed between the 2θ of 10^{o} and 40^{o}. However, a peak at 10^{o} observed in both drug free and drug loaded beads may be attributed to the polymer crystallinity/noise. This indicates the amorphous dispersion of the drug after entrapment into beads.

Results of FTIR

The IR spectrum of pure KTM shows a peak at 3446.79 cm-1 which is attributed to the N-H and NH2 stretching and peaks at 1469.76 cm-1, 1490.97 cm-1 are due to C=C aromatic and aliphatic stretching, peak at 1381.03cm-1 is due to –C-N vibrations, peak at 1049.28 cm-1 is due to –OH bending confirms presence of alcoholic group, peaks at 702.09, 725.23, 763.81 and 798.53 cm-1 confirms the C-H bending (aromatic). Hence, it is thus, conforms the structure of drug KTM (Figure 3). Figure: 7

From the examination of the recorded IR spectral data, it can be seen that all the characteristic peaks of the drug are also seen in the IR spectra of the physical mixture of drug and excipients and some more peaks were observed with physical mixtures, which could be attributed to the presence of polymers(Figure: 11) These results indicate that there is no interaction between the drug and polymers taken up for the investigation.

Table 1: Properties of KTM loaded alginate microspheres formulations

Formulation code	Mean particle size(µm)	% Encapsulation efficiancy	Drug content mg/100 of beads	% Practical yield
CA1	1265±0.53	61.76±1.4	12.35	81.15
CA2	1052 ± 0.45	63.37±1.3	12.67	80.12
CA3	954± 0.59	65.52±1.1	13.10	82.14
CA4	992±0.28	68.75±0.9	13.75	85.10
CA5	901± 0.32	70.89±1.5	14.17	89.15
CA6	842± 0.44	72.50±2.2	29.00	91.13

Table 2. Percentage drug release

Sl.No	Code	1 Hr	2 Hr	3 Hr	4 Hr	5 Hr	6 Hr	7 Hr	8 Hr
1	CA1	37.391	62.915	84.217	90.455	92.950	94.354	95.914	98.409
2	CA2	33.48742812	54.7364789	76.00907809	83.76200406	86.80236718	89.538694	91.21089371	94.25125684
3	CA3	27.79322866	49.09271231	67.0894823	76.04453355	79.5678324	82.50391478	85.58680127	88.81649189
4	CA4	30.73321859	49.5697074	69.05836576	77.60311284	82.50583658	86.14785992	87.82879377	90.35019455
5	CA5	22.47110546	40.13555734	56.50529874	64.79093149	68.86583284	71.98992388	75.52150505	78.50976604
6	CA6	20.85880468	37.01703365	53.79712867	62.0991547	67.08037032	69.73701865	72.06158594	73.05782906

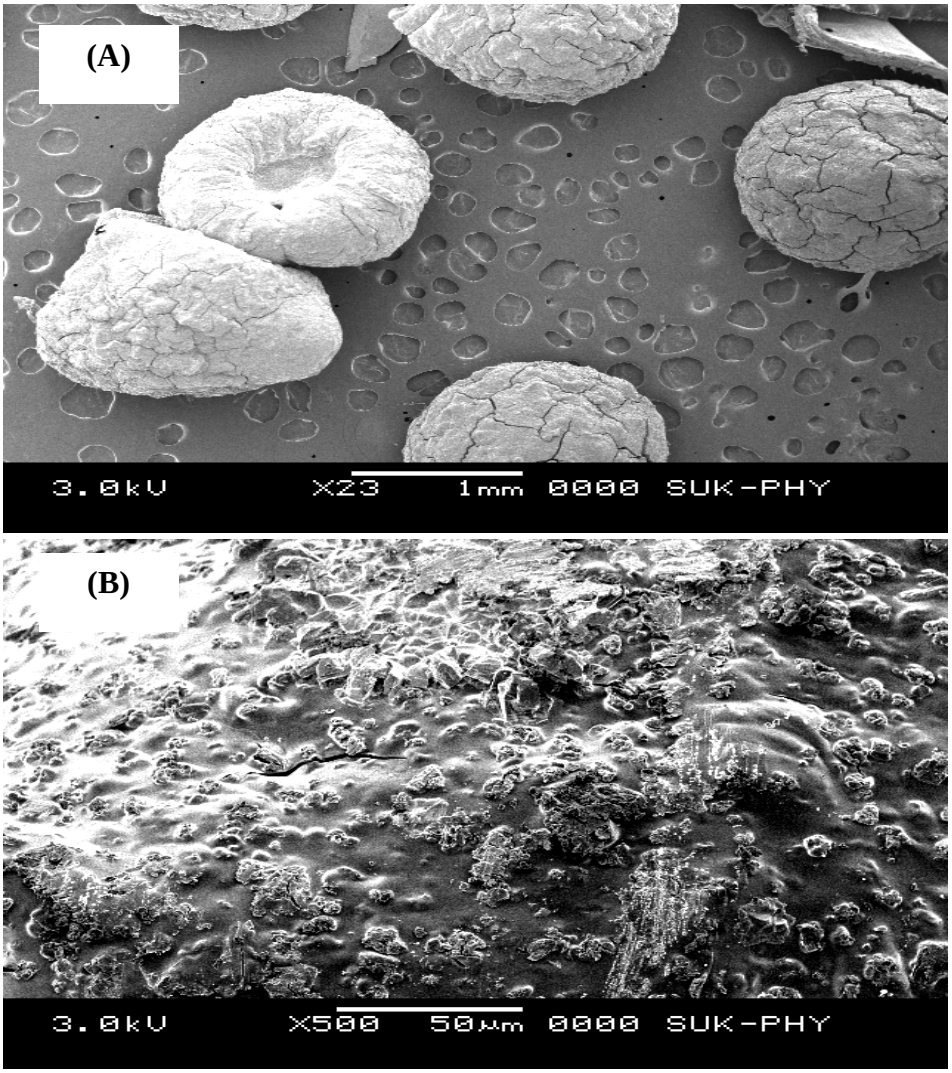

Figure 1. Scanning electron microscopic photographs of microbeads (A) and its surface morphology (B).

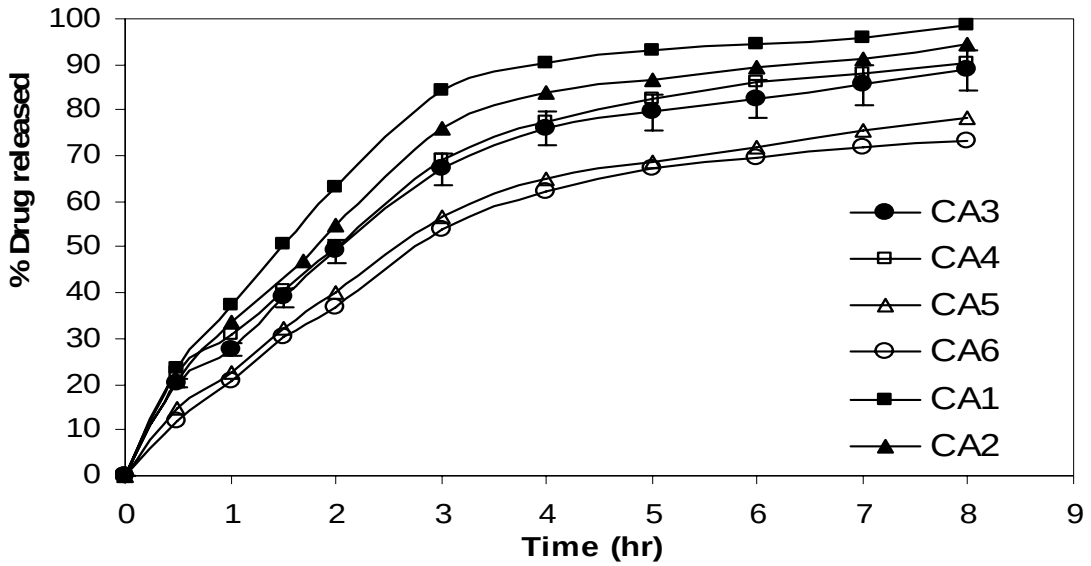

Figure 2 Drug release behavior of beads in pH 1.2 and pH 7.4 solutions

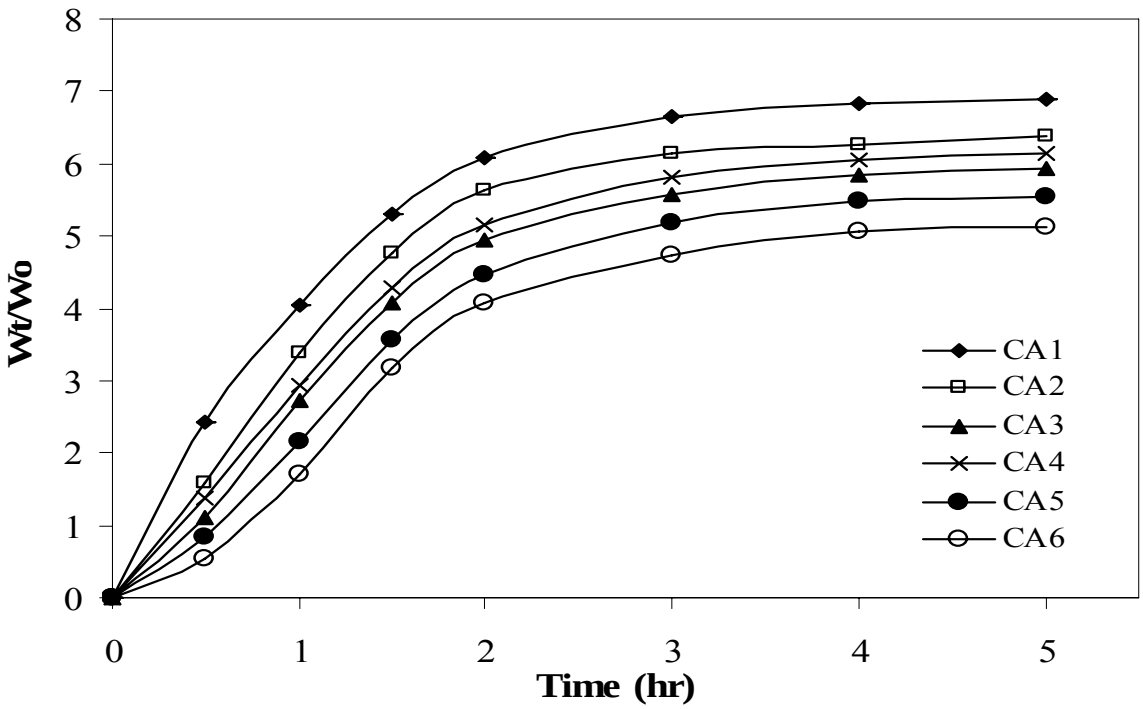

Figure 3 Swelling behaviour of beads in phosphate buffer pH 7.4

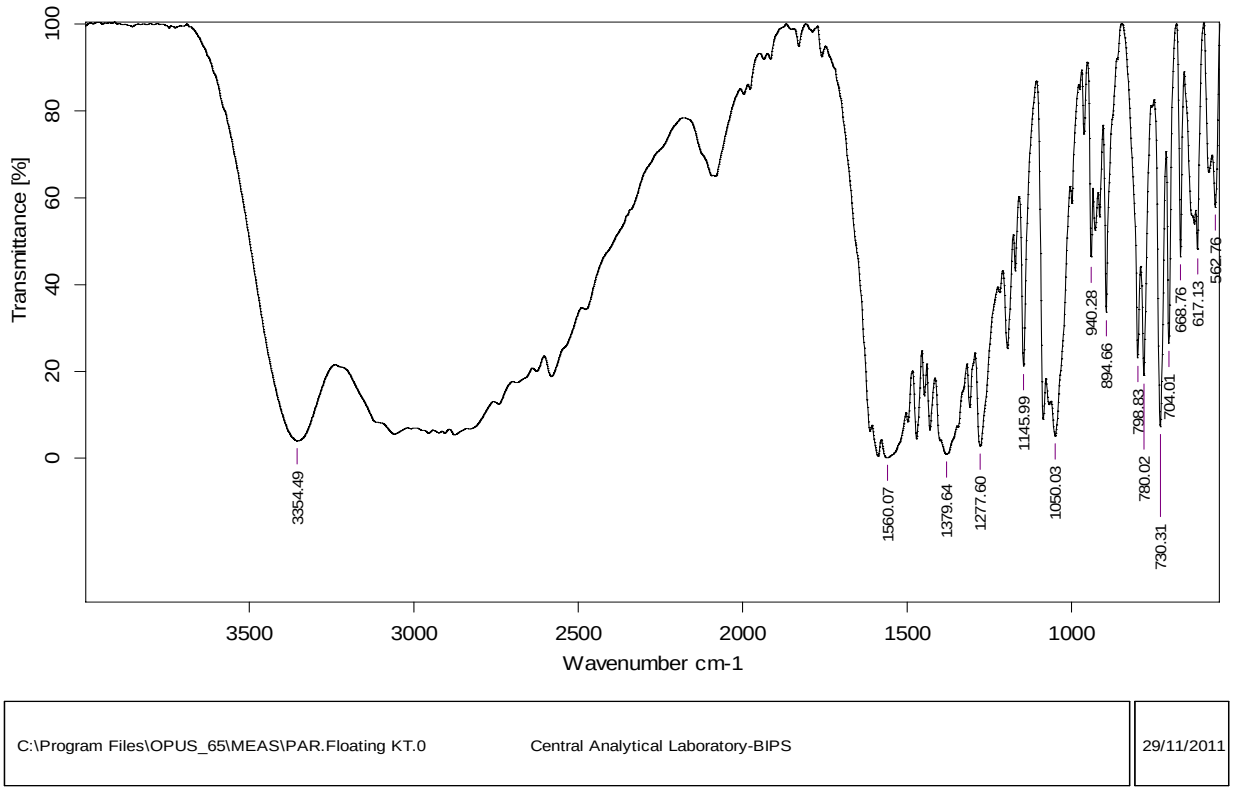

Figure 4. , FT-IR Spectra of KTM.

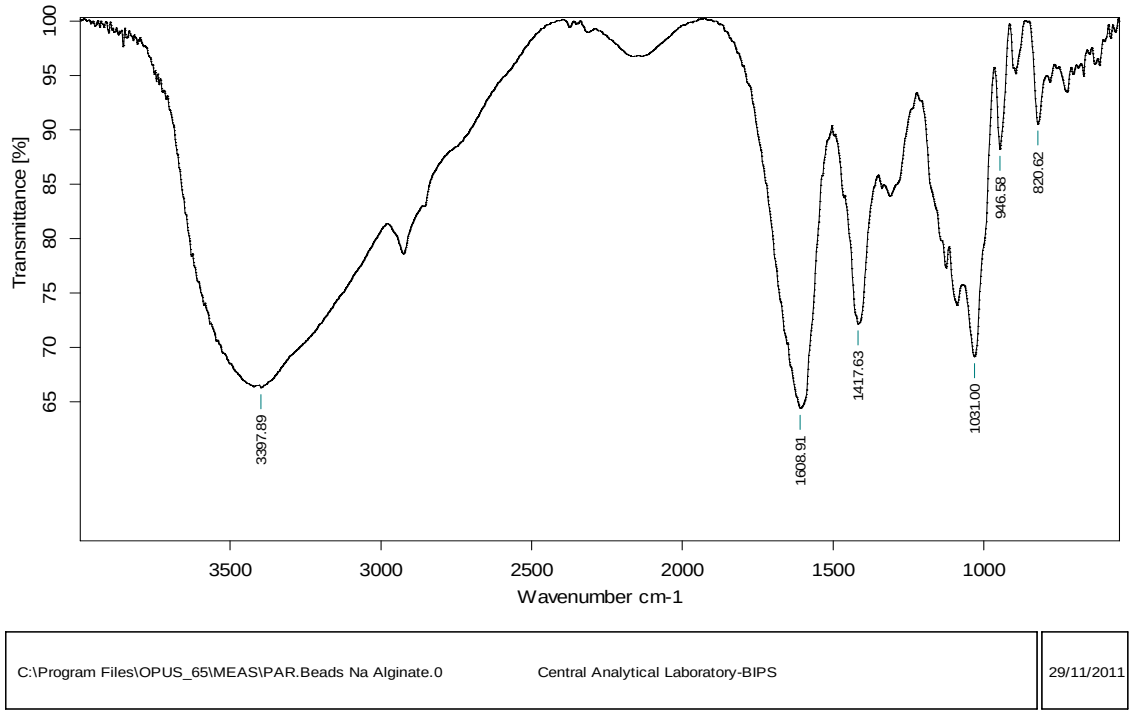

Figure 5. FT-IR spectra of Sodium alginate.

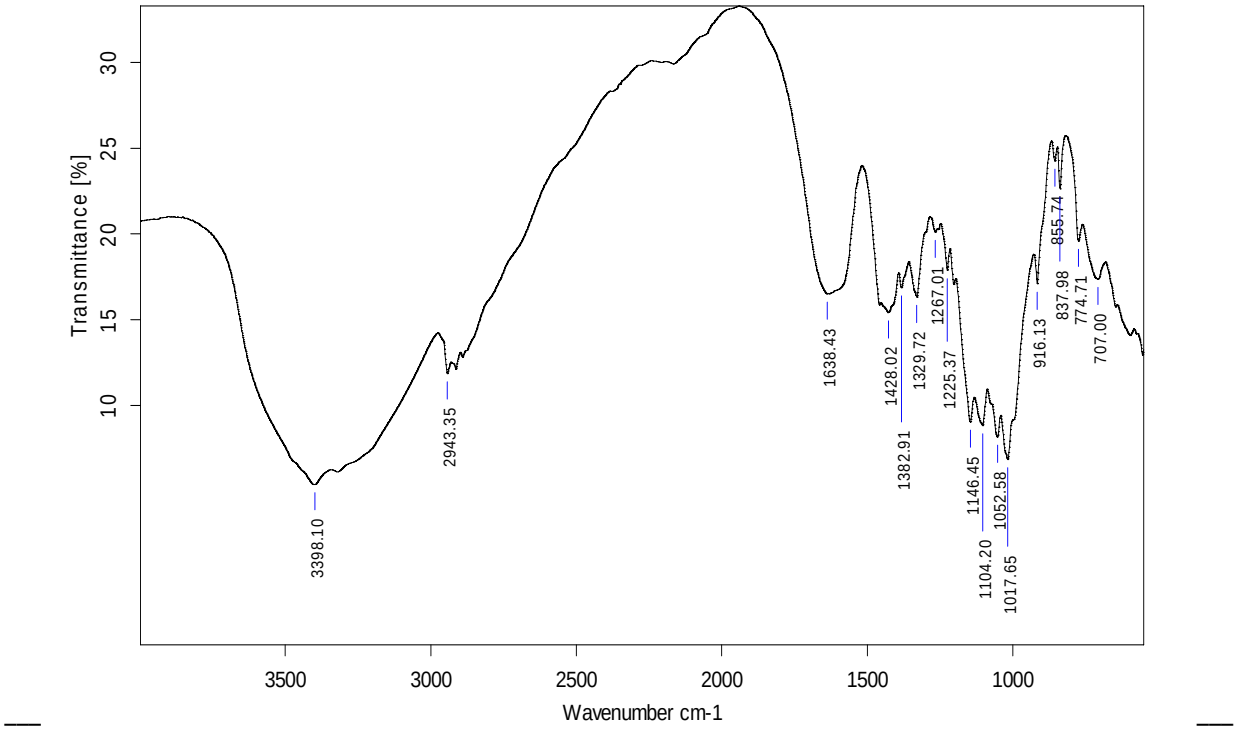

Figure 6 FT-IR spectra of Sodium CMC.

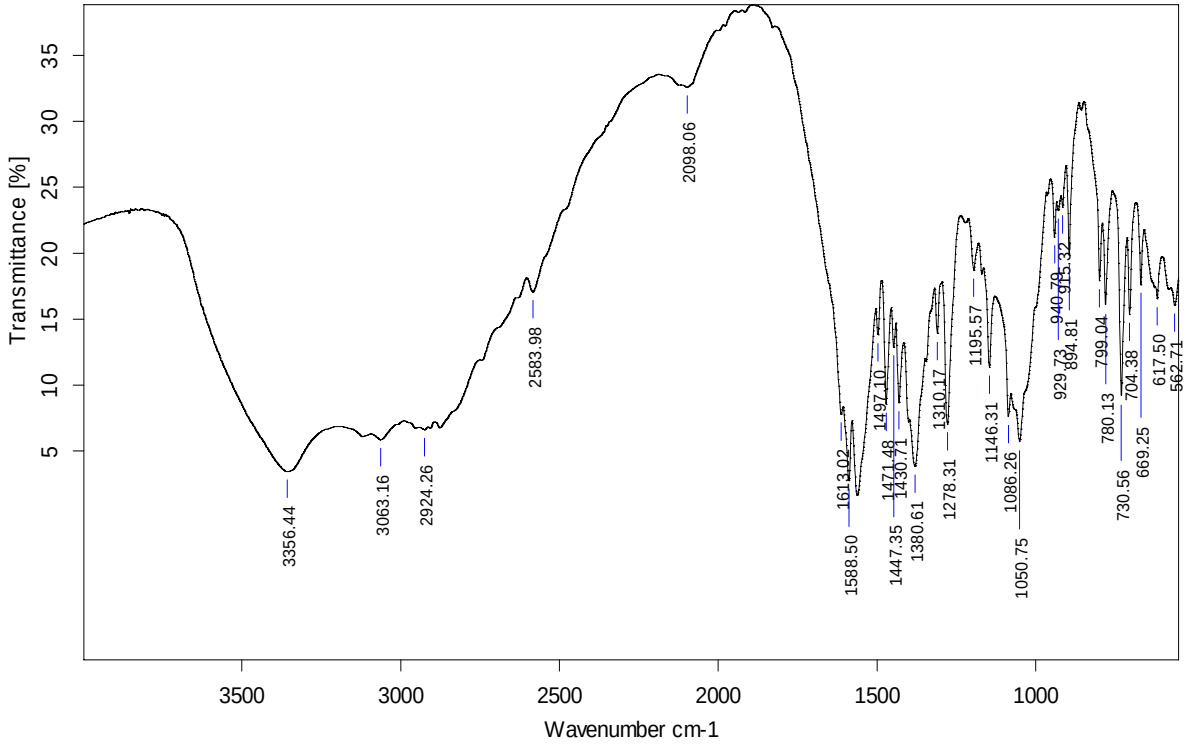

Figure7. FT-IR spectra of drug and polymers

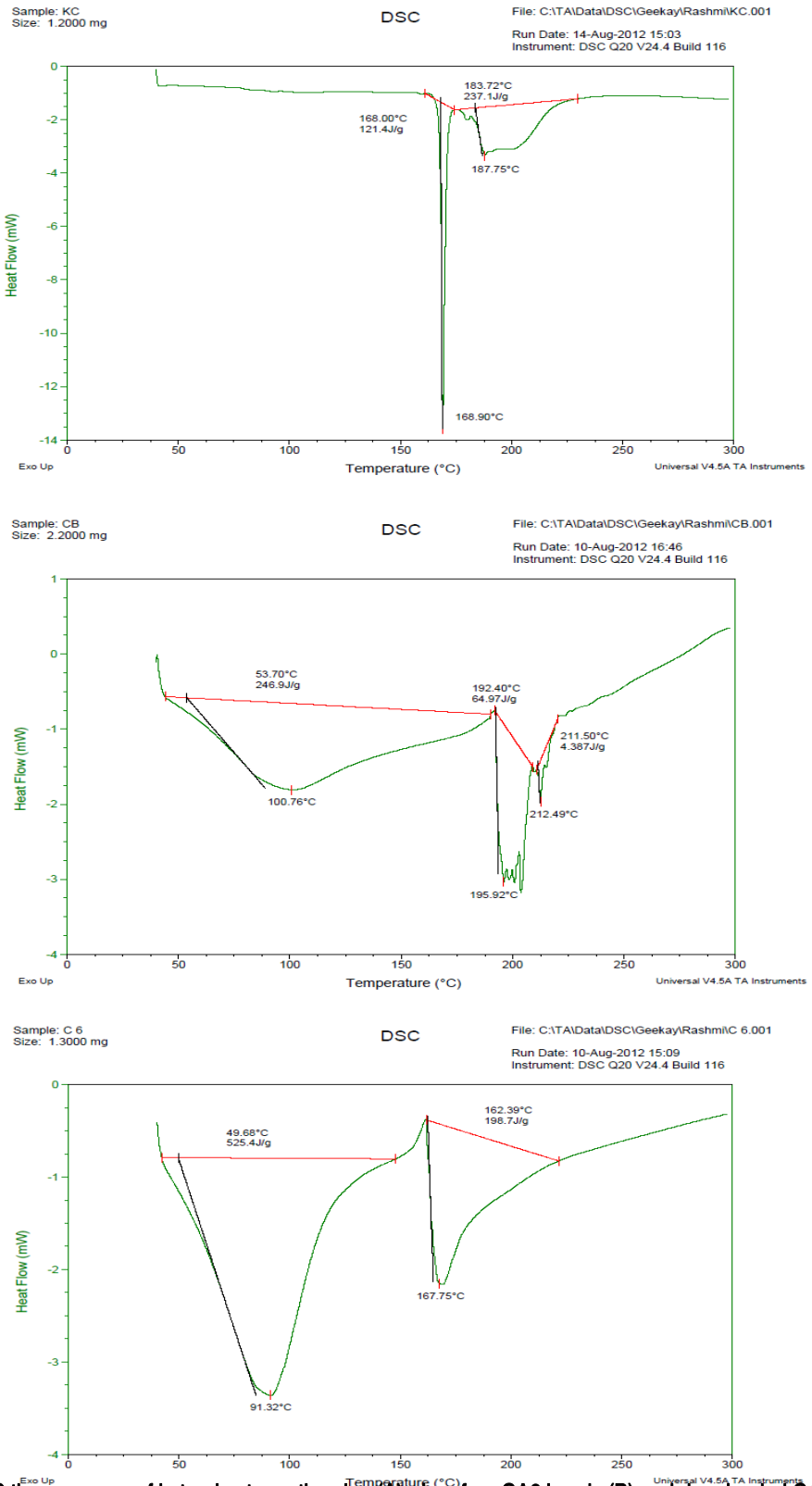

Figure. 2. DSC thermograms of keterolac tromethamine (A), drug free CA6 beads (B) and drug loaded CA6 beads (C).

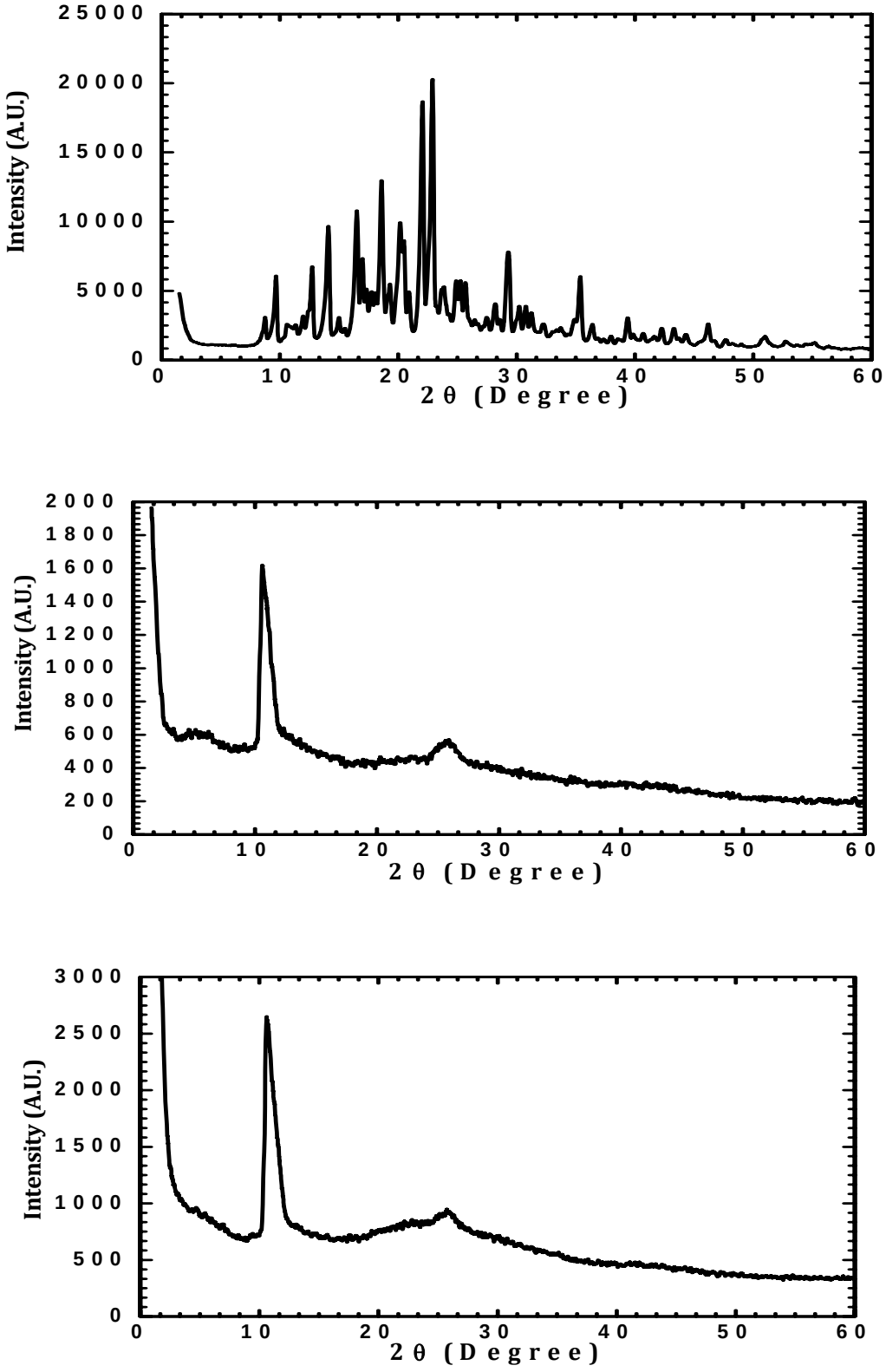

Figure 3. X-ray diffractograms of keterolac tromethamine (A), drug free CA6 beads (B) and drug loaded CA 6beads (C).

Conclusion

The ionotropic gelation method was successfully applied in preparing KTM loaded beads which demonstrated a satisfactory sustained-release characteristics, suggesting that Sod .alginate and Na CMC were effective natural polymer in for MUPs. The drug loaded formulations prepared from sodium alginate and sodium CMC at the concentrations of 2% weight ratios each with 3.5% weight ratios of calcium chloride and aluminium chloride as cross linking agents showed the lowest drug release of 73.07 % at the end of 8 hrs. FTIR Spectroscopy demonstrated that there is no chemical interaction between the drug and polymers. DSC and X-Rd data indicated the no change in the physical state of the drug in the formulations.

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