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Preparation and characterization of indomethacin loaded ionically cross-linked microspheres using chitosan

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ABSTRACT

Microspheres loaded with Indomethacin were prepared by employing ionotropic gelation by using sodium tri polyphosphate (Na-TPP) as the crosslinking agent. Chitosan microspheres produced by an emulsification crosslinking process with the chemical crosslinker, glutaraldehyde has problems associated with its use as it may cause toxic reactions and other undesirable effects. The FT-IR spectroscopic analysis indicated the absence of any drug-polymer interaction during the microsphere preparation. Surface morphology of the prepared microspheres was confirmed by Scanning Electron Microscopy (SEM). The prepared microspheres were subjected for evaluation of particle size distribution, drug loading and entrapment efficiency. The results of the in vitro drug release shows that with increase in the crosslinking density the rate of the drug release decreases. Indomethacin was present in the amorphous state and dispersed uniformly in the polymeric matrix of the microspheres as shown from the results of the XRD and DSC studies. From the preliminary studies, it can be concluded that the method developed and adopted for the preparation of drug loaded microspheres is simple and reproducible and also avoids the use of special precautions and complex apparatus.

Keywords: Indomethacin, Chitosan, Ionic gelation, Microspheres, In-vitro release.

INTRODUCTION

A large number of oral chitosan microsphere preparations have been prepared by using a suspension or emulsion crosslinking procedure. For several years, chitosan has been largely evaluated as a potential vehicle for drugs administered orally [1]. Chitosan due to its antacid and anti-ulcer characteristics prevents or weakens drug irritation in the stomach [2]. The gel forming

property of chitosan at low pH along with its antacid and anti-ulcer properties makes it an interesting agent to prevent irritation in the stomach induced by some active compounds [1].

Chitin is one of the most abundant polysaccharides in nature second only to cellulose. The principal derivative of chitin, namely chitosan can be prepared by the N-deacetylation of chitin [1]. The amino group of the polysaccharide responsible for its pH dependent solubility could represent a potential for oral drug delivery. Polysaccharides fabricated into hydrophilic matrices remain popular biomaterials for controlled release dosage forms. The gel and matrix forming abilities of chitosan makes it useful for solid dosage forms such as granules and microparticles. Depending on the nature of the crosslinker, the main interactions forming the network are covalent or ionic bonds. Most of the crosslinkers used to perform covalent croslinking may induce toxicity if found in free traces before administration. An approach to overcome this problem is to prepare hydrogels by reversible ionic crosslinking. Reversible ionic crosslinking instead of chemical crosslinking was used to prepare chitosan microspheres[3]. Networks formed by ionic crosslinking of chitosan are pH dependent drug delivery systems; their properties can be controlled by the experimental conditions during preparation. Chitosan microspheres were prepared by an emulsion phase separation technique without the use of chemical crosslinking agents where ionotropic gelation was employed in a w/o emulsion as an alternative. Three kinds of anions, tripolyphosphate, citrate and sulphate to interact with chitosan were investigated [3]. TPP/Chitosan beads possessed better mechanical strength than sulfate/chitosan or citrate/chitosan beads [4].

In this study, chitosan microspheres were prepared by a modified ionic gelation method which was simple and inexpensive, where Na-TPP was utilized as the crosslinking agent. The use of organic solvents was also minimized when compared to the suspension or emulsion crosslinking procedures. Additionally no special precautions are needed. Indomethacin, a basic drug having poor water solubility with ulcerogenic properties was incorporated as a model drug. The effect of different experimental factors on microsphere size and size distribution, entrapment efficiency and in vitro release pattern was investigated.

MATERIALS AND METHODS

Materials

Indomethacin was obtained as a gift sample from Fourrts (India) Pvt Limited, Chennai, India, Chitosan with a degree of deacetylation of 91% & viscosity of 5 cps at 1% (w/v) in 1% (v/v) aqueous acetic acid at 20°C, was supplied by India Sea Foods, Cochin as a gift sample and were used as received. Na-TPP was obtained from Fluka Chem, Buchs, Switzerland. Lactic acid, formaldehyde, acetone and other chemicals were from E Merck Limited (Mumbai, India).

Preparation of Indomethacin Loaded Chitosan Microspheres

The specified quantity of chitosan was added to an aqueous solution of lactic acid (2.4% v/v) and stirred for one hour. A quantity of indomethacin solution (volume one-fifth that of chitosan solution) in ethanol (Absolute alcohol) was added to the above mentioned aqueous acidic chitosan solution and stirred for 15 minutes to obtain a uniform dispersion of indomethacin in the hydroalcoholic acidic solution of chitosan.

To 20 ml of sodium tripolyphosphate solution in a beaker, 10 ml of the above mentioned dispersion of indomethacin in the hydroalcoholic acidic solution of chitosan was added using a glass syringe with a 18G needle. The resultant particles formed due to the ionic crosslinking of chitosan by sodium tripolyphosphate were stirred for 15 minutes. Then formaldehyde (1.3% w/v) was added and the stirring was continued for a further period of 15 minutes. The particles were then separated from the reaction medium by means of filtration using a Whatmann No.1 Qualitative filter paper. The particles retained in the filter paper were then treated with 10 ml of acetone. They were then transferred to 20 ml of acetone in a beaker, mixed and poured into a petridish and allowed to dry in ambient conditions for 18 hours. The indomethacin loaded chitosan microspheres so obtained were transferred into suitable containers and stored at room temperature. The above procedure was adopted to obtain microspheres prepared with different concentrations of chitosan and sodium tripolyphosphate (Table 1).

Table-1: Formulation Design for the preparation of Indomethacin loaded chitosan microspheres

Formulation code	Chitosan concentration (% w/v) (polymer)	TPP concentration (% w/v) (crosslinking agent)
AI	1	3
BI	2	3
CI	3	3
EI	1	5
FI	2	5
GI	3	5

FT-IR Spectroscopic Analysis

Fourier transform infrared (FTIR) spectral data were taken to find out the chemical stability of the drug in the crosslinked microspheres. FTIR spectra of Indomethacin (pure drug), Chitosan, blank (unloaded) microspheres and indomethacin loaded chitosan microspheres were obtained for the same. FTIR spectral measurements were performed using a JASCO, model 4200 (Japan) FT-IR spectrophotometer. The samples were finely ground with KBr and FTIR spectra were taken in the range 4000-400cm^{-1}.

Particle Size Distribution Analysis and Surface Morphological Studies

Samples of indomethacin loaded chitosan microspheres were analyzed for particle size by an optical microscope fitted with a calibrated ocular micrometer. The prepared microspheres were placed on a clean glass slide and observed under 10X magnification. About 300 particles were counted for each batch and the average particle size was determined. All experiments were carried out in triplicate.

The surface morphology and the size of the indomethacin loaded chitosan microspheres were characterized from the micrographs taken with the scanning electron microscope (Jeol, JSM-840A Scanning Electron Microscope, Japan).

Determination of Drug Loading and Entrapment Efficiency

10 mg of the Indomethacin loaded chitosan microspheres were added to 25 ml of phosphate buffer (pH 7.2) and mixed occasionally. After 24 h the microspheres were separated from the medium by means of filtration using a Whatmann No.1 Qualitative filter paper. The absorbance of the filtrate was measured at 319 nm using a spectrophotometer (Varian Carry 50 Bio, USA).

From the measured absorbance the drug content of the indomethacin loaded chitosan microspheres was calculated. All experiments were performed in triplicate.
Drug Loading was calculated using the formula in Equation 1.

Drug Loading in % = W/W_t x 100 ….. …… Eq 1

where,
W = drug content of the microspheres
W_t = weight of the microspheres

Entrapment Efficiency was calculated using the formula in Equation 2.

Entrapment Efficiency in % = W_c/W_o x 100 …… Eq 2

where,
W_c = total drug present in the microsphere batch
W_o = theoretical drug loading

Theoretical drug loading was determined by calculation assuming that the entire drug present in the polymer solution gets entrapped in microspheres and no loss occurs at any stage of preparation of the microspheres.

***In-Vitro* Drug Release Studies**
Drug release studies on the indomethacin loaded chitosan microspheres were carried out using a USPXXI dissolution rate test apparatus for 3 h at a stirring speed of 100 rpm. An amount of microspheres equivalent to 5 mg of indomethacin was placed in the dissolution medium, phosphate buffer (pH 7.2) maintained at a temperature of 37± 0.5ºC. A 5 ml of sample aliquot of the dissolution medium was withdrawn at different time intervals and fresh dissolution medium was simultaneously used to replace the quantity withdrawn. The samples were then filtered using a Whatmann No. 1 qualitative filter paper and assayed spectrophotometrically (Varian Carry 50 Bio, USA) at 319 nm to estimate the drug concentration. All experiments were performed in triplicate.

Differential Scanning Calorimetry (DSC) Analysis
The physical state of indomethacin in the crosslinked microspheres was analyzed by differential scanning calorimetry (DSC). DSC measurements were done on a Mettler-Toledo star 822^e system, Switzerland. Samples were placed in pierced aluminum pans and heated from 30 to 300°C at the rate of 10°C min^{-1}. Differential scanning calorimetry (DSC) was performed on Indomethacin, chitosan, blank (unloaded) microspheres, physical mixture of indomethacin & blank microspheres and the indomethacin loaded microspheres.

X-Ray Diffraction Analysis
Crystallinity of pure Indomethacin and Indomethacin loaded microspheres were evaluated by X-ray diffraction (XRD) measurements recorded for Indomethacin, chitosan, blank (unloaded) microspheres and Indomethacin loaded chitosan microspheres using a X-Ray Diffractometer (Miniflex goniometer, Japan). The scanning angle ranged from 10 º to 100 º of 2θ.

RESULTS AND DISCUSSION

FT-IR Spectroscopic Analysis

FT-IR spectroscopy was used to characterize possible interaction between the drug and the carrier in the solid state (Figure 1). The spectrum of indomethacin showed two characteristic bands of the –OH group that were found at 3372 cm^{-1} (free –OH) and 2900-3321 cm^{-1} (-OH involved in intermolecular association). The carbonyl stretching peaks appears as a very strong band at 1721 cm^{-1} (acid group) and 1692 cm^{-1} (amide group). In the IR spectra of indomethacin loaded chitosan microspheres, the principal peak for indomethacin in the formulation appeared at 1689 cm^{-1} indicating the presence of amide group and less profoundly at 1721 cm^{-1} corresponding to the carbonyl stretching. This inference rules out the possibility of any drug polymer interaction during the preparation of the microspheres.

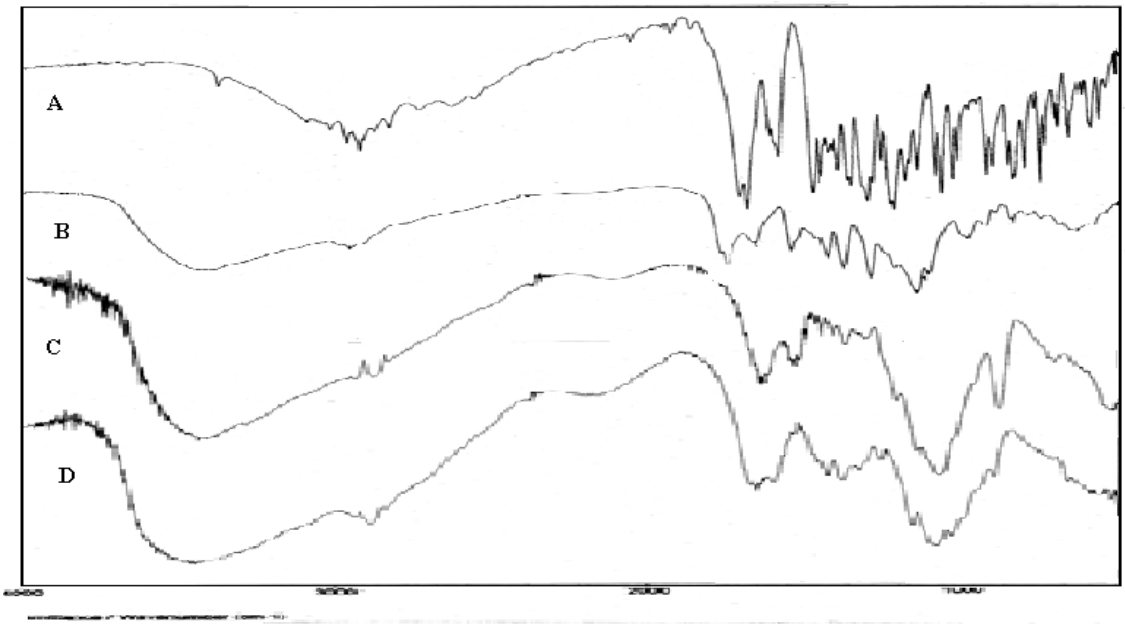

Fig 1. Fourier Transform Infrared (FTIR) spectrum (A) Indomethacin (B) Indomethacin loaded microspheres (C) Blank microspheres (D) Chitosan

Particle size distribution analysis and surface morphological studies

The mean particle size of the indomethacin loaded chitosan microspheres was in the range of 44µm to 51µm and their particle size distribution is presented in Table 2 and Fig 3.

The surface morphology of the prepared indomethacin loaded chitosan microspheres was studied by scanning electron microscopy and the SEM photographs are given in Fig 2. It can be observed that the microspheres were irregular in shape and it has been reported in previous studies that even in the pH region where anions interact with chitosan, irregular particles were obtained in the case of conventional emulsification and ionotropic gelation method [3].

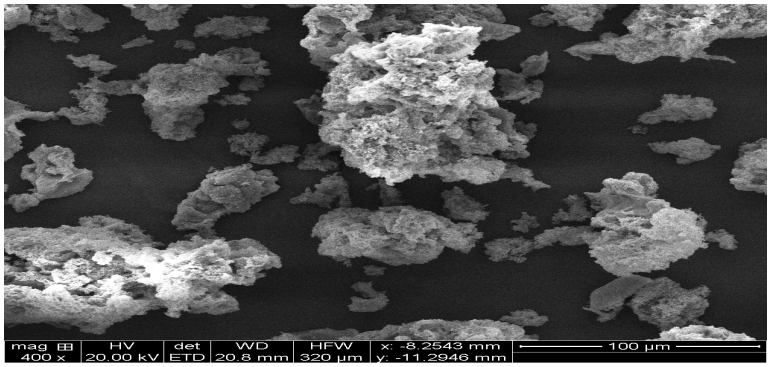

Fig 2. Scanning Electron Micrographs (SEM) of indomethacin loaded microspheres

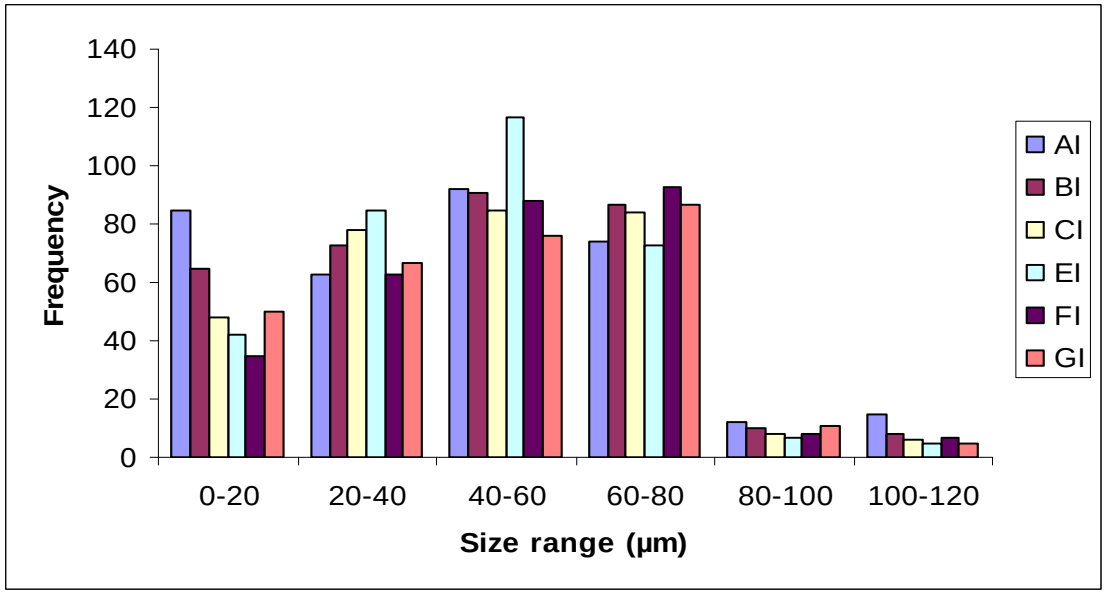

Fig 3. Frequency distribution of Indomethacin microspheres

Table-2: Particle size, percentage yield, drug loading and entrapment efficiency of Indomethacin loaded microspheres

Batch code	Average particle size (µm) ± SD	Percentage yield ± SD	% Drug loading ± SD	% Entrapment efficiency ± SD
AI	44.21± 1.53	19.24 ± 0.87	2.25 ± 0.035	13.38 ± 0.76
BI	46.16 ±1.90	34.75 ± 1.52	4.12 ± 0.065	50.87 ± 2.65
CI	50.16 ± 6.04	45.41 ± 1.69	5 ± 0.035	9.87 ± 2.90
EI	47.09 ± 2.23	12.73 ± 0.545	2.28 ± 0.1	14.17 ± 0.032
FI	50.58 ±1.31	23.5 ± 0.33	4.50 ± 0.047	56.45 ± 1.08
GI	45.88 ± 1.94	33 ± 0.31	5.13 ± 0.05	97.88 ± 0.83

Determination of drug loading and Entrapment efficiency

Increase in the concentration of chitosan and Na-TPP resulted in higher drug loading and entrapment efficiency. This may be due to increased matrix density [5]. Batch GI, prepared with the highest concentration of chitosan and Na-TPP had a drug loading and entrapment efficiency of 5.13% and 97.88%. With an increase in the concentration of chitosan an increase in the

entrapment efficiency was observed (Table 2). This results from an increase in the yield of the microspheres with an increase in the concentration of chitosan.

In-Vitro Drug Release Studies

The release profile of indomethacin from the prepared drug loaded chitosan microspheres is represented in Figure 4. It was observed that there was a burst effect for the different batches studied. The burst effect can be described with the dissolution/diffusion of drug from the surface regions of chitosan microspheres [6] (Akbuga and Bergisadi 1999). The particle size, the properties of the crosslinked matrix and solubility of the drug in the dissolution medium would be expected to influence the rate of release of the drug from the microspheres [7]. With an increase in the cross-linking density, the release of indomethacin from the microspheres decreased. Swellability of the microspheres having higher cross-linking densities was less, which in turn leads to a slower release [8].

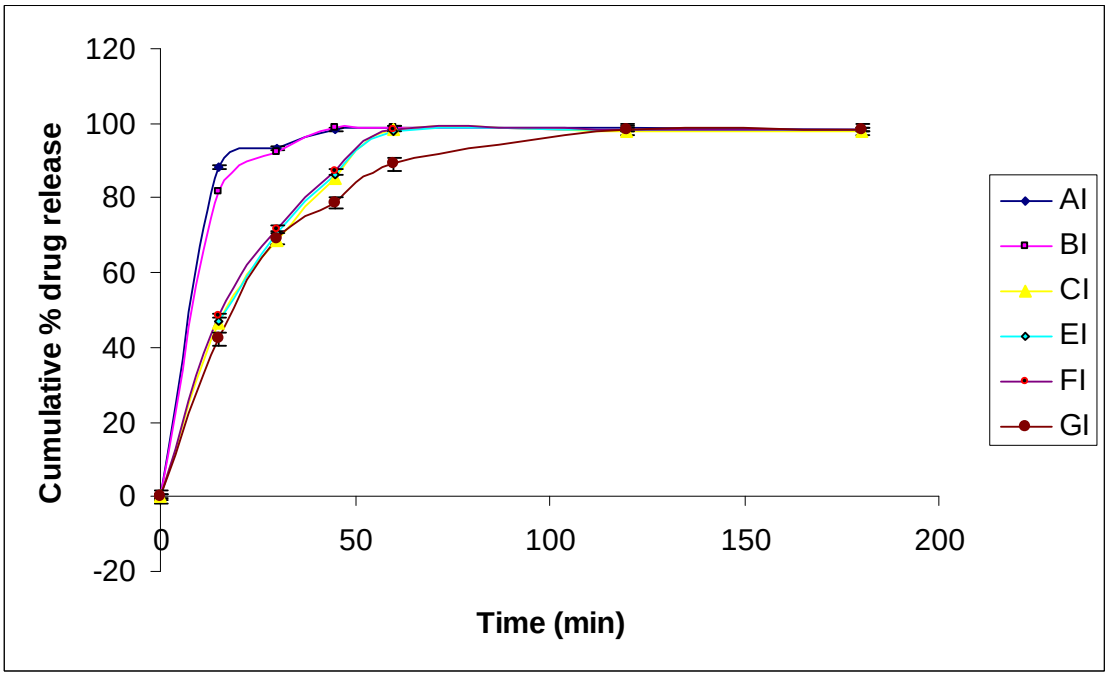

Figure 4: In vitro release of Indomethacin microspheres

Differential Scanning Calorimetry (DSC) Analysis

Fig 5 shows the DSC thermograms of indomethacin (pure drug), chitosan, blank microspheres and the drug loaded microspheres. In the case of indomethacin, a sharp endothermic peak due to the melting of indomethacin was observed at 162.2 ^{0} C corresponding to the melting of crystalline indomethacin. The peak of the drug did not appear in the thermogram of the formulation (indomethacin loaded chitosan microspheres). It indicates that the drug was uniformly dispersed at the molelcular level in the polymeric matrix.

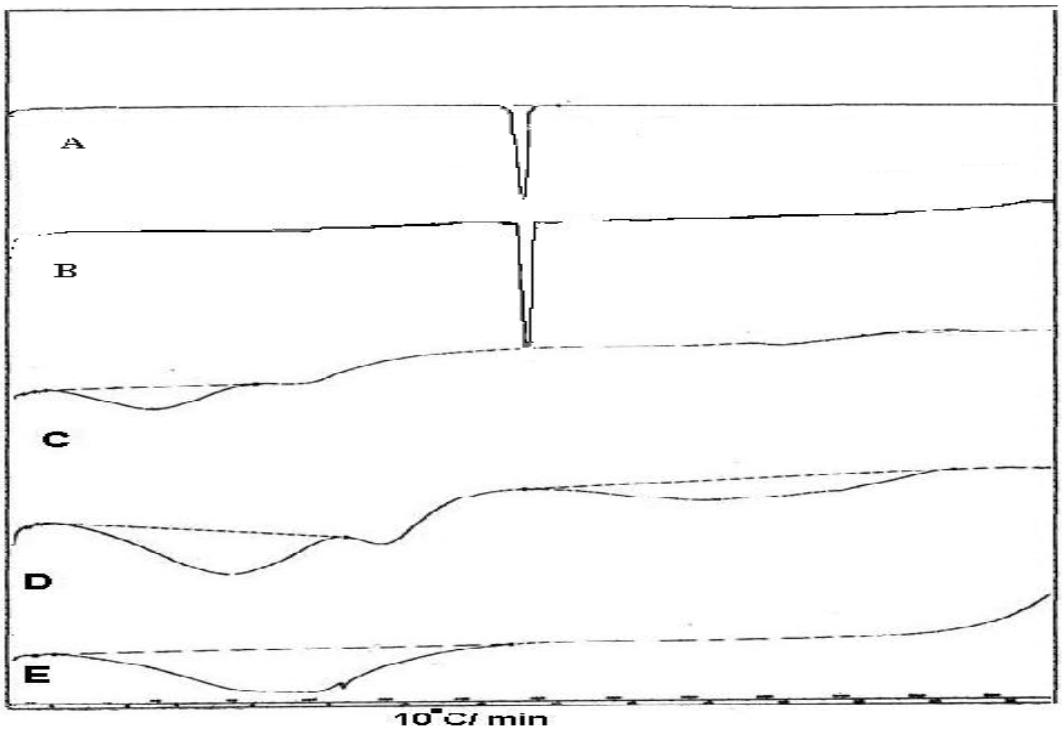

Fig 5. Differential scanning calorimetry (DSC) thermograms (A) Indomethacin (B) Physical mixture of indomethacin and blank microspheres (C) indomethacin loaded microspheres (D) blank microspheres (E) chitosan

X-Ray Diffraction Analysis

The X-ray diffractogram of indomethacin, chitosan, blank (unloaded) microspheres and indomethacin loaded microspheres are displayed in Fig 6. The diffraction spectrum of pure indomethacin showed that the drug was of crystalline nature as demonstrated by numerous distinct peaks. The prominent peak for the pure indomethacin was at 2θ of 21.81^0 . No characteristic X-RD pattern was observed in the case of drug loaded microspheres. Thus from the X-ray diffraction data of the drug loaded microspheres, it can be inferred that the drug was not present in the crystalline state in the microsphere matrix. It was in the amorphous state. This clearly indicated changes in the crystalline state of the drug occurred during the preparation of the microspheres by the ionotropic gelation method.

CONCLUSION

From the present study, it can be concluded that indomethacin loaded chitosan microspheres can be prepared by the ionotropic gelation method with Na-TPP as the cross-linking agent based on the information reported herein. The prepared microspheres were subjected to various studies and results were found to be satisfactory. The method developed and adopted for the preparation of the Indomethacin loaded chitosan microspheres was simple and reproducible and avoids the use of complex apparatus and special precautions.

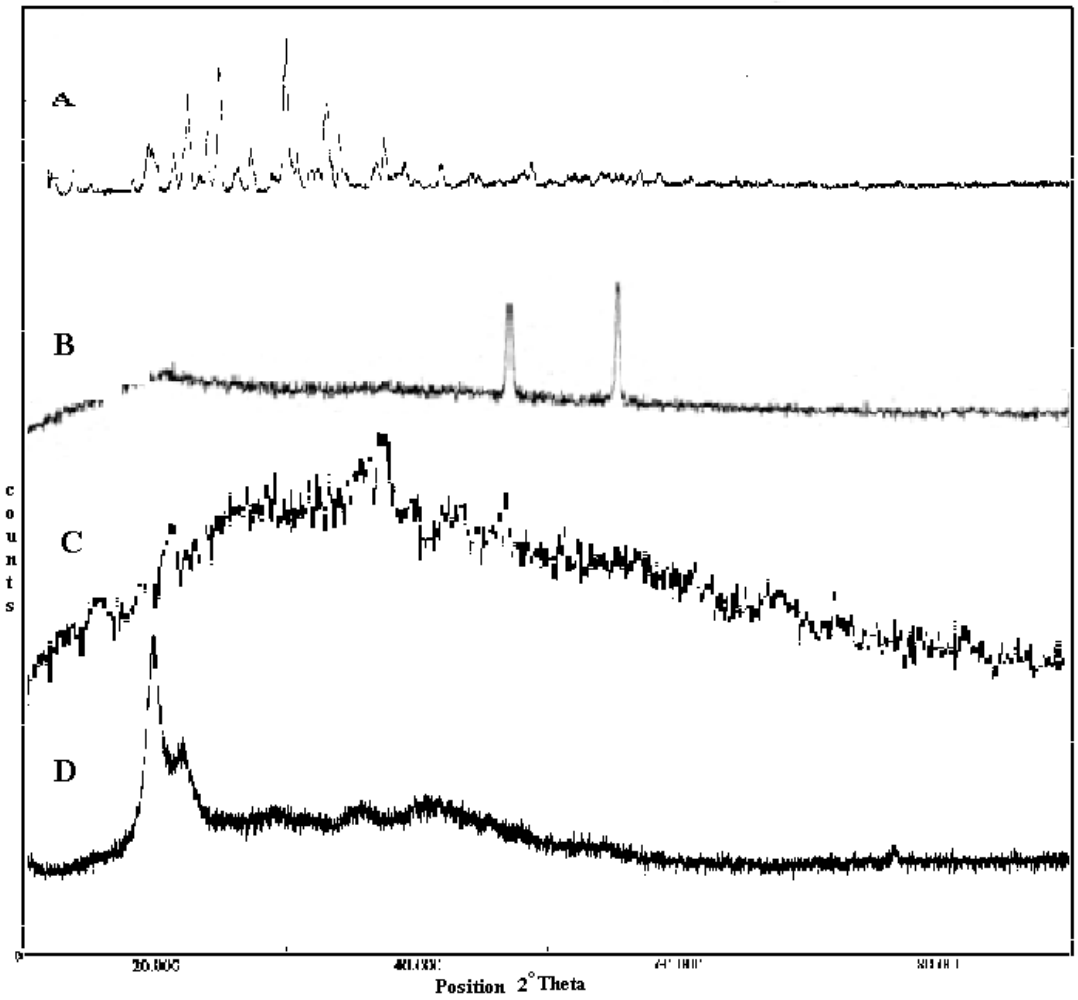

Fig 6. Xray diffractograms of (A) Indomethacin (B) Indomethacin loaded microspheres (C) Blank microspheres (D) Chitosan

REFERENCES

[1] O. Felt, P. Buri, R.Gurny, *Drug Dev Ind Pharm.*, **1998**, 24 ,11, 979-993.
[2] W.M. Hou, S.Miyazaki, M.Takada, T.Komai, *Chem Pharm Bull.*, **1985**, 33, 3986-3992.
[3] X.Z Shu, K.J. Zhu, *J Microencapsul*., **2001**, 18, 237-245.
[4] X.Z Shu, K.J. Zhu, *Int J Pharm*., **2002**, 233, 217-225.
[5] E.E. Hassan, R.C. Parish, J.M.Gallo, *Pharm Res.*, **1992**, 9, 390-397.
[6] J. Akbuga, N. Bergisadi, J.*Microencapsul*, **1999,** 16, 697-703.
[7] P. He, S.S. Davis, L. Illum, *Int J Pharm.*, **1999**,187, 53-65.
[8] E.B. Denkbas, M. Seyyal, E. Piskin, *J Microencapsul*, **1999**, 16,741-749.