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Evaluation of clobazam loaded ionically cross-linked microspheres using chitosan

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ABSTRACT

Clobazam loaded chitosan microspheres were prepared by the ionic gelation method using sodium tripolyphosphate (Na-TPP) as the crosslinking agent. The use of ionotropic gelation avoids the possibility of the occurrence of the toxic and undersirable effects associated with the use of glutaraldehyde, a chemical crosslinking agent. The prepared microspheres were evaluated for mean particle size and particle size distribution, drug loading, encapsulation efficiency and in-vitro drug release. FT-IR spectroscopic analysis was performed to ascertain drug-polymer interaction, if any during the microsphere preparation. The surface morphology of the prepared microspheres was studied by SEM. With an increase in the crosslinking density the rate of drug release decreased. The results of the DSC and XRD analysis confirmed that clobazam existed as a molecular dispersion in the polymeric microsphere matrix in an amorphous state. From the results of the present investigation it may be concluded that drug loaded chitosan microspheres can be prepared by a simple technique which avoids the use of complex apparatus and special precautions.

Keywords: Clobazam, Chitosan, Ionic gelation, Microspheres, In-Vitro drug release.

INTRODUCTION

Chitosan has attracted attention in biomedical and pharmaceutical fields because of its reactive groups and favorable properties of biodegradability, low toxicity and biocompatibility [1]. The formulation of chitosan microparticulate delivery systems seem to be particularly advantageous for oral, mucosal and parenteral administration [2]. Chitosan microspheres provide a potentially useful means of delivering drugs, as they are stable physically and chemically, amenable to preparation in large batches, non-antigenic, metabolize within the body and capable of accommodating a wide variety of drug molecules. Studies have shown that glutaraldehyde crosslinked chitosan microspheres are long acting biodegradable carriers suitable for controlled delivery of many drugs. The emulsion-solvent evaporation method has been utilized to obtain microspheres of theophylline, 5– fluro uracil [3] or magnetic microspheres of oxantrazole [4] and cisplatin [5]. Chitosan microspheres produced by an emulsification crosslinking process with chemical crosslinker, glutaraldehyde may cause toxic reaction and other undesirable effects [6]. Passive absorption of drugs on chitosan microspheres crosslinked by glutaraldehyde was performed, but this process was quite time consuming and tedious [7].

Unless safe covalent crosslinkers with well documented biocompatibility and metabolism are available, alternatively ionically crosslinked hydrogels should be preferred [8]. Chitosan forms gels with multivalent counter ions through the formation of intermolecular or intramolecular linkages by ionic interaction [9]. Tripolyphosphate, citrate and sulphate are multivalent anions which may interact with positively charged chitosan to form complexes [7]. Ionic cross-linking is a simple and mild procedure. Moreover, ionically crosslinked chitosan hydrogels are generally thought to be well tolerated and their pharmaceutical applications are numerous since ionic crosslinkers are often biocompatible. Ionically crosslinked chitosan hydrogels offer more possibilities as drug delivery systems compared to covalently crosslinked hydrogels. They can be used for controlled release not only in acidic but also in basic media for rapid release by dissolution. However, their main disadvantages are the possible lack of mechanical stability and the risk of dissolution of the system, due to a pH sensitive swelling [8]. The effect of anion nature on the mechanical strength of chitosan bead was found to be significant [10]. The crosslinking process of TPP/Chitosan showed the most excellent mechanical properties in the undried state and this was due to the stronger interaction of TPP with the chitosan due to its more charge numbers and higher charge density. Sodium Tripolyphosphate/ Chitosan beads exhibited poor mechanical strength which limited its usage in drug delivery [10]. The rigidity of the chitosan- Na-TPP matrix was poor in the case of chitosan-Na-TPP microspheres [11].

A low drug loading efficiency was observed with more water soluble drugs during microsphere preparation [12]. Clobazam, a water insoluble basic drug was incorporated as a model drug for the preparation of the drug loaded chitosan microspheres.

The objective of the present study was to prepare drug loaded chitosan microspheres by a simple technique which avoids the use of complex apparatus and special precautions based on a slight modification of the ionic gelation method using Na- TPP as the crosslinking agent.

MATERIALS AND METHODS

MATERIALS

Clobazam BP was obtained from Fourrts (India) Laboratories Pvt. Limited, Chennai, India as a gift sample, 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^{o}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 CLOBAZAM LOADED CHITOSAN MICROSPHERES

A solution of chitosan was prepared by adding the specified quantity of chitosan to lactic acid solution (2.4% v/v) followed by stirring for one hour. Separately, clobazam was dissolved in absolute ethanol and this solution was added to the previously prepared chitosan solution and stirred for 15min. This resulted in the formation of a clobazam dispersion in chitosan solution. The ratio of clobazam solution to that of chitosan was 1:5.

Table 1: Formulation Design for the preparation of Clobazam loaded chitosan microspheres.

Formulation code	Chitosan concentration (% w/v) (polymer)	TPP concentration (% w/v) (crosslinking agent)
AC	1	3
BC	2	3
CC	3	3
EC	1	5
FC	2	5
GC	3	5

20 ml of sodium tripolyphosphate (TPP) solution was placed in a beaker and 10 ml of the clobazam dispersion in chitosan solution was added by means of a glass syringe attached with an 18 G needle. Stirring was done for 15 min and formaldehyde (1.3% v/v) was added. The resultant system was further stirred for 15 min to obtain the microparticles in the wet state. Filtration, using Whatmann No.1 Qualitative filter paper was done to separate the particles. To the particles in the filter paper 10 ml of acetone was added. Finally, the particles were transferred into 20 ml of acetone in a beaker and mixed for a few minutes. Drying was carried out by placing the particles in a pertridish at room temperature for 18 h to obtain the clobazam loaded chitosan microparticles.

Different concentrations of chitosan and TPP were used to prepare various formulations as given in Table 1.

FTIR SPECTROSCOPIC ANALYSIS

FT-IR spectroscopic studies of clobazam (pure drug), chitosan (polymer), blank (unloaded) microspheres and clobazam loaded chitosan microspheres were done by recording the respective FT-IR spectra in a JASCO, Model 4200 Spectrophotometer (Japan) over a wave number range of 400 – 4000 cm^{-1}.

PARTICLE SIZE ANALYSIS AND MORPHOLOGICAL STUDIES

The mean particle size of the Clobazam loaded chitosan microspheres were determined by optical microscopy using a calibrated micrometer. About 300 (three hundred) microspheres were analysed for each preparation and the mean diameter was calculated. Triplicates were performed for each of the experiments.

The surface morphology and appearance of the microspheres were examined by means of a Scanning Electron Microscope (JSM 840A, Japan).

DETERMINATION OF DRUG LOADING AND ENTRAPMENT EFFICIENCY

An accurately weighed sample (10 mg) of the Clobazam loaded chitosan microspheres was placed in 25 ml of a solvent system consisting of methanol and 0.1N Hydrochloric acid in 2:1 ratio at room temperature for 24 h. The solution was then filtered using a Whatmann No.1 Qualitative filter paper. The filtrate was assayed spectrophotometrically for drug content at 230 nm (Genesys 10 UV Spectrophotometer, Thermo, USA).

The same method was utilized confirm the non-interference of unloaded microspheres in the spectrophotometric determination of drug content prior to the Drug Loading studies. All experiments were performed in triplicate.

Drug Loading was calculated using the formula in Equation 1.

$$\text{Drug Loading in \%} = W/W_t \times 100 \qquad \text{Eq 1}$$

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

Entrapment Efficiency was calculated using the formula in Equation 2.

$$\text{Entrapment Efficiency in \%} = W_c/W_o \times 100 \qquad \text{Eq 2}$$

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

Clobazam loaded chitosan microspheres equivalent to 5 mg of clobazam were subjected to in-vitro drug release studies to ascertain the drug release pattern of clobazam from the prepared microspheres formulations.

Drug release studies were carried out in a USP XXI dissolution rate test apparatus for 3 h in 0.1N Hydrochloric acid (350ml) at 100 rpm and 37± 0.5°C. At different time intervals 5ml of the sample was withdrawn and replaced with the same amount of fresh medium. The withdrawn samples were filtered using Whatmann No.1 Qualitative filter paper and analyzed for clobazam content spectrophotometrically at 228 nm using a Genesys 10 UV Spectrophotometer, Thermo, USA against a reagent blank. All experiments were carried out in triplicate.

DIFFERENTIAL SCANNING CALORIMETRIC ANALYSIS

Differential scanning calorimetry (DSC) analysis was undertaken to characterize the changes, if any, observed during the preparation of the microspheres. DSC of clobazam (pure drug), chitosan (polymer), blank (unloaded)

microspheres and clobazam loaded chitosan microspheres were carried out using a Mettler- Toledo star 822 system over a temperature range of 30 to 300 ºC at a scanning rate of 10º C / min.

X RAY DIFFRACTION ANALYSIS

X-Ray powder diffraction (XRD) analysis of the samples of clobazam (pure drug), chitosan (polymer), blank (unloaded) microspheres and clobazam loaded chitosan microspheres was carried out using a Miniflex goniometer, Japan over a range of 10 – 100 2θ.

RESULTS AND DISCUSSION

FTIR SPECTROSCOPIC ANALYSIS

The FTIR spectra of clobazam, chitosan, blank microspheres and clobazam loaded chitosan microspheres are illustrated in Figure 1. The FT-IR spectrum of clobazam (pure drug) matches with that of its reference spectrum of the British Pharmacopoeia (1999). Clobazam shows a very strong IR absorption near 1690 cm^{-1} attributed to the C=O stretching mode. The principal peaks of clobazam were observed in the spectra of the drug loaded microspheres. The spectra obtained from the drug loaded microspheres indicate the presence of the characteristic bands of clobazam at almost the same wave number. Thus, it can be confirmed that no interaction exists between the drug and polymer.

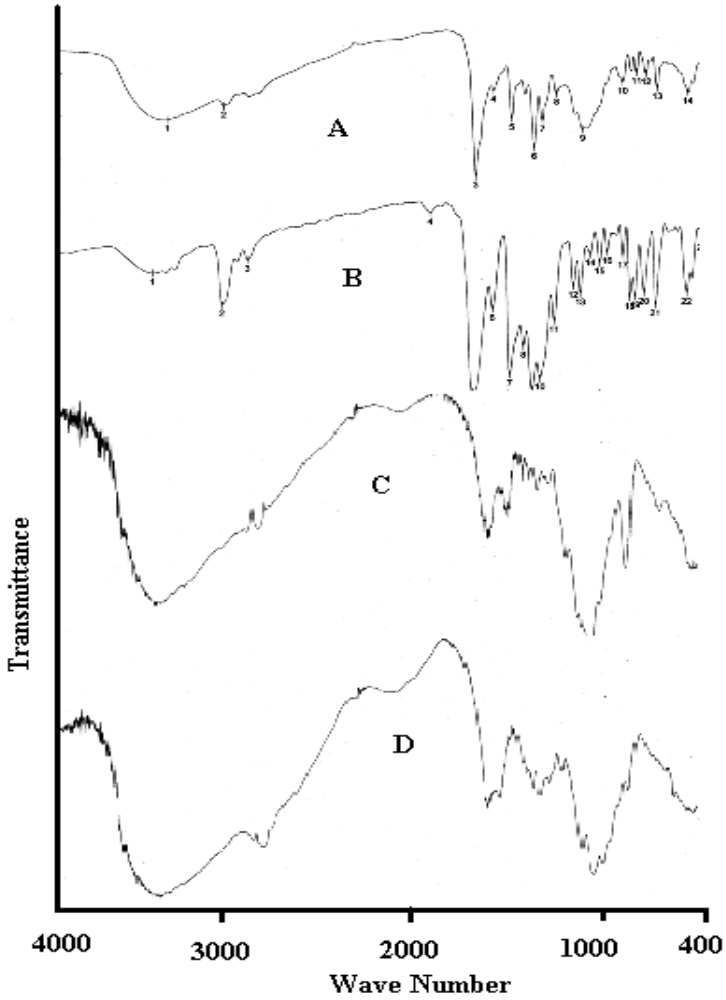

Figure 1: FT-IR spectra of A) Clobazam loaded chitosan microspheres B) Clobazam C) Blank microspheres D) Chitosan

PARTICLE SIZE ANALYSIS AND MORPHOLOGICAL STUDIES

The mean particle size of the clobazam loaded chitosan microspheres ranged from 44µm to 51µm (Table 2). The changes in the concentration of chitosan and Na-TPP did not result in any major differences in the mean particle size of the various batches of the clobazam loaded chitosan microspheres. The concentration of Na-TPP affected the particle size distribution of the prepared clobazam loaded chitosan microspheres. Microspheres prepared with 3% W/V Na-TPP had a higher number of particles in the size range 15 to 20µm & 20 to 40 µm when compared to microspheres prepared with 5% W/V which had more number of particles in the size range of 40 to 60 µm & 60to 80 µm. Figure 2 shows the scanning electron photomicrograph of clobazam loaded chitosan microspheres. Microspheres which were irregular in shape were obtained using the method developed and adopted for preparing the clobazam loaded chitosan microspheres. In the pH region where anions can intereact with chitosan, irregular particles were obtained in the case of conventional emulsification and ionotropic gelation method [7].

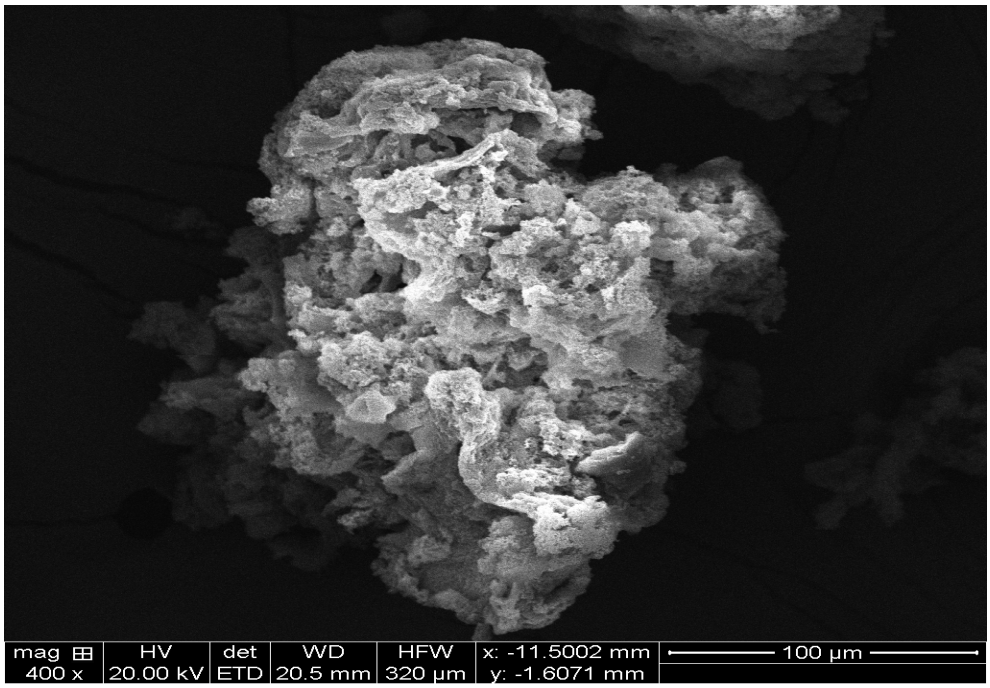

Figure 2: Scanning electron micrographs of clobazam loaded chitosan microspheres at 400X (Batch GC)

DETERMINATION OF DRUG LOADING AND ENTRAPMENT EFFICIENCY

The concentrations of chitosan and Na-TPP had an effect on the drug loading and entrapment efficiency of clobazam loaded chitosan microspheres. Maximum drug loading and entrapment efficiency were 4.59% & 80.12% and 5.02% & 95.50% for the formulations CC and GC respectively (Table 2). Lower drug loading and entrapment efficiency observed with the prepared clobazam loaded chitosan microspheres is due to the lower microsphere matrix density [4, 5 and 13]. Drug entrapment increases with increase in the concentration of both Na-TPP and chitosan. Increase in the concentration of chitosan increases the yield of the prepared microspheres and thereby resulting in higher drug entrapment levels of clobazam. When the concentration of Na-TPP increased from 3%W/V to 5%W/V, the increased entrapment efficiency was possibly due to increased matrix density.

Table 2: Particle Size, Drug loading and Entrapment efficiency of clobazam loaded chitosan microspheres (Mean ± SD, n = 3)

Batch code	Average particle size (µm)± SD	% Drug loading	% Entrapment efficiency
AC	44.16 ± 3.03	2.09 ± 0.035	12.8 ± 0.658
BC	45.02 ± 1.62	3.72 ± 0.065	46.47 ± 2.48
CC	47.07 ± 2.32	4.59 ± 0.026	80.12 ± 2.19
EC	46.85 ± 4.49	2.20 ± 0.02	14.56 ± 0.248
FC	50.47 ± 1.15	4.19 ± 0.04	53.53 ± 1.18
GC	49.23 ± 1.87	5.02 ± 0.096	95.50 ± 1.53

IN-VITRO DRUG RELEASE STUDIES

Release of clobazam from microspheres depends upon the type of matrix and its rigidity. The release of the active agent from the polymeric matrix involves initial swelling followed by diffusion of the drug [11, 14]. The drug release rate decreased with increase in the cross-linking density. A denser matrix of the microspheres might exhibit slower release rates of the drug (Figure 4). And most probably, the drug was located at the outer layer of the microspheres during particle formation, which resulted in a burst effect [15].

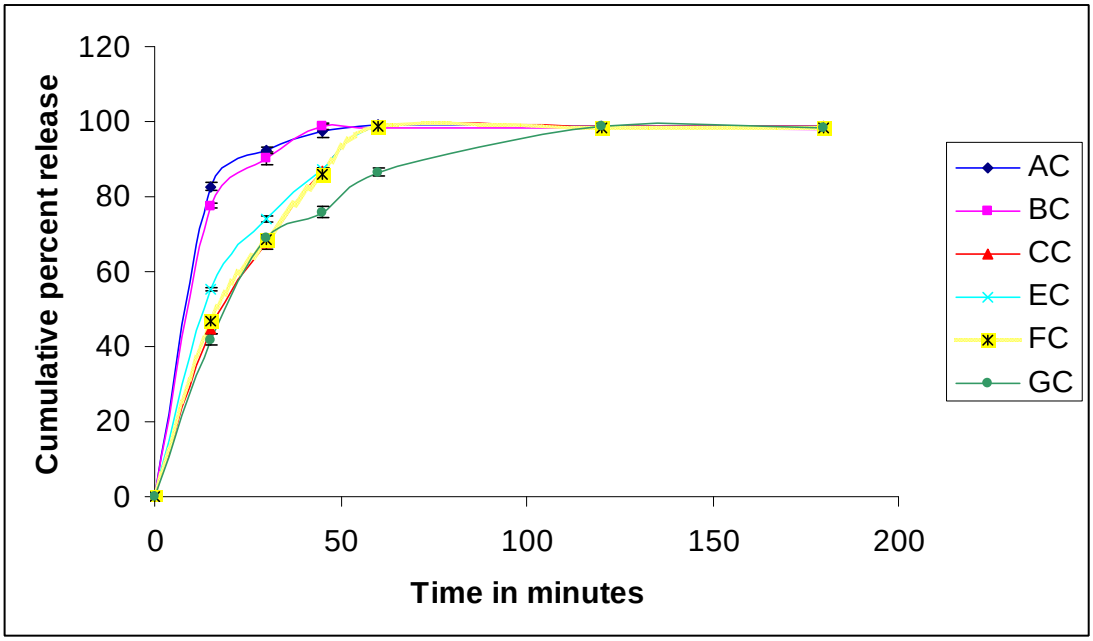

Figure 3: Cumulative percent release of clobazam in 0.1 N Hydrochloric acid (pH 1.2) from drug loaded microspheres

DIFFERENTIAL SCANNING CALORIMETRIC ANALYSIS

Figure 4 illustrates the DSC thermograms of clobazam (pure drug), chitosan (polymer), blank (unloaded) microspheres and clobazam loaded chitosan microspheres. A sharp endothermic peak corresponding to the melting of crystalline clobazam was found at 184°C. The melting endotherm of clobazam was not observed in the drug loaded chitosan microspheres. This indicates that clobazam was uniformly dispersed and present in an amorphous state in the polymeric matrix.

X RAY DIFFRACTION ANALYSIS

Figure 5 illustrates the comparative x-ray powder diffraction pattern of clobazam (pure drug), chitosan (polymer), blank (unloaded) microspheres and clobazam loaded chitosan microspheres. Pure clobazam showed the classical diffractogram of the crystalline substance. The X-RD pattern of the drug loaded chitosan microspheres indicates the presence of drug in the amorphous state.

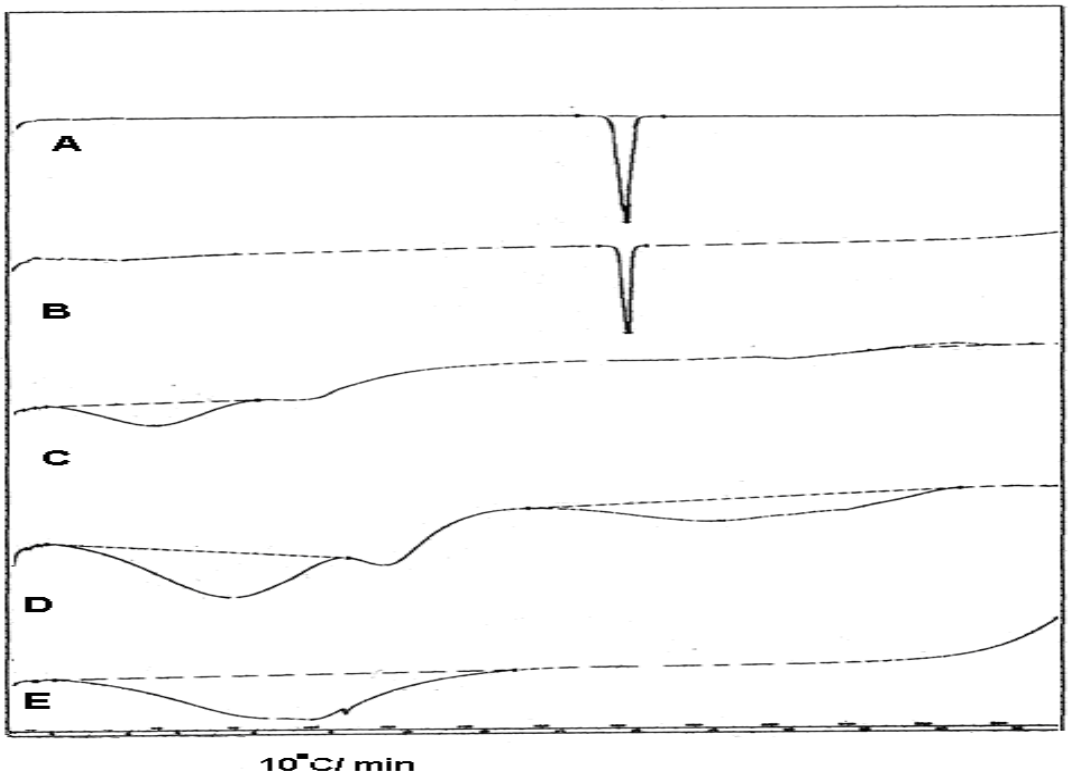

Figure 4: Differential scanning calorimetry (DSC) thermograms (A) clobazam (B) Physical mixture of clobazam and blank microspheres (C) clobazam loaded microspheres (D) blank microspheres (E) chitosan

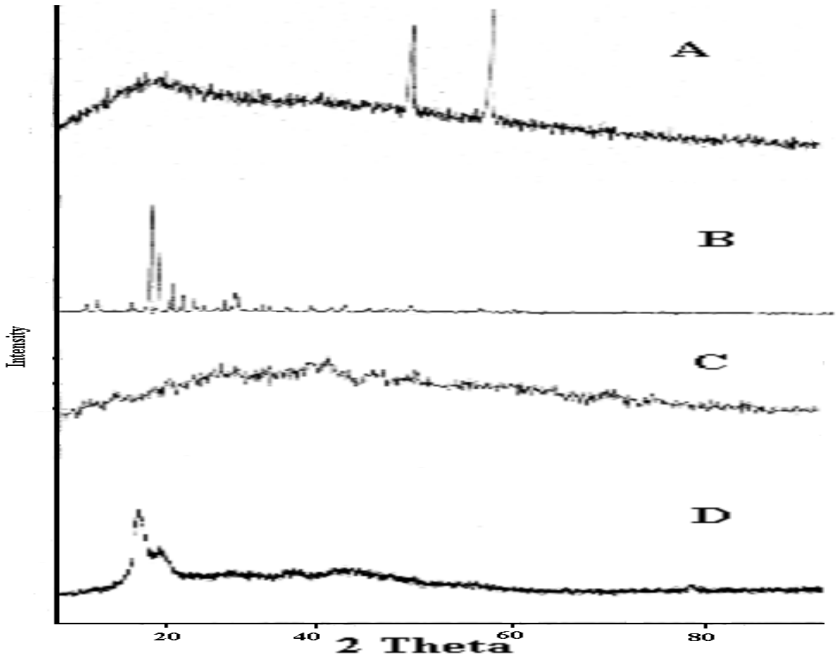

Figure 5: X-Ray Diffraction pattern of A) Clobazam loaded microspheres B) Clobazam C) Blank microspheres D) Chitosan

CONCLUSION

The clobazam loaded chitosan microspheres were prepared by ionic gelation using Na-TPP as the crosslinking agent. The prepared drug loaded microspheres were evaluated for studies such a mean particle size & particle size distribution, SEM, drug loading, entrapment efficiency, in-vitro drug release, FT-IR, DSC and X-ray diffractometry. The results of these studies have been shown to be satisfactory. The microparticulate drug delivery system based on chitosan seems promising for the oral administration of clobazam. Chitosan, a natural biodegradable polymeric carrier is biocompatible and easily available. From the results of the present investigation it can be concluded that drug loaded chitosan microspheres can be produced by a simple and reproducible method in which the use of complex apparatus and special precautions are avoided.

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