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FORMULATION AND EVALUATION OF FLURBIPROFEN LOADED IONICALLY CROSS-LINKED MICROSPHERES USING CHITOSAN

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Abstract:
Aim: *To prepare and evaluate flurbiprofen loaded ionically cross-linked microspheres using chitosan.*
Materials and methods: *Flurbiprofen loaded chitosan microspheres were prepared by the ionic gelation method using tetra sodium pyrophosphate (TSPP) as the crosslinking agent. The use of ionotropic gelation avoids the possibility of the occurrence of the toxic and undesirable 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.*
Results: *With an increase in the crosslinking density the rate of drug release decreased. The results of the DSC and XRD analysis confirmed that flurbiprofen existed as a molecular dispersion in the polymeric microsphere matrix in an amorphous state.*
Conclusion: *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*
Key words: *microspheres, Tetra sodium pyrophosphate, crosslinking agent*

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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]. Tetra sodium pyrophosphate, 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 TSPP /Chitosan showed the most excellent mechanical properties in the undried state and this was due to the stronger interaction of TSPP 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- TSPP matrix was poor in the case of chitosan-TSPP microspheres [11].

A low drug loading efficiency was observed with more water-soluble drugs during microsphere preparation [12]. Flurbiprofen, a water insoluble acidic 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 TSPP as the crosslinking agent.

MATERIALS AND METHODS:

Flurbiprofen drug was gifted by Hygro chemicals pharmatec Pvt limited, Bollaram, Chitosan with a degree of deacetylation of 91% & viscosity of 5 cps at 1% (W/V) in 1% (V/V) glacial acetic acid solution at 20^0 C was supplied by India Sea Foods, Cochin as a gift sample and were used as received. tetra sodium pyrophosphate (TSPP), Lactic acid, formaldehyde, acetone and other chemicals were from E Merck Limited (Mumbai, India)

FORMULATION OF FLURBIPROFEN LOADED CHITOSAN MICROSPHERES

A solution of chitosan was prepared by adding the specified quantity of chitosan to glacial acetic acid solution (3% v/v) followed by stirring for one hour. Separately, flurbiprofen 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 flurbiprofen dispersion in chitosan solution. The ratio of flurbiprofen solution to that of chitosan was 1:5. 20 ml of tetra sodium pyrophosphate (TSPP) solution was placed in a beaker and 10 ml of the flurbiprofen 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 flurbiprofen loaded chitosan microparticles. Different concentrations of chitosan and TSPP were used to prepare various formulations as given in Table 1.

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

S. No.	Formulation code	Chitosan concentration (% w/v)	TSPP concentration (% w/v)
1	F1	1	3
2	F2	2	3
3	F3	3	3
4	F4	1	5
5	F5	2	5
6	F5	3	5

FTIR SPECTROSCOPIC ANALYSIS

FT-IR spectroscopic studies of flurbiprofen (pure drug), chitosan (polymer), blank (unloaded) microspheres and clobazam loaded chitosan microspheres were done by recording the respective FT-IR spectra.

PARTICLE SIZE ANALYSIS AND MORPHOLOGICAL STUDIES

The mean particle size of the flurbiprofen loaded chitosan microspheres were determined by optical microscopy using a calibrated micrometer. About 300 (three hundred) microspheres were analyzed 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.

DETERMINATION OF DRUG LOADING AND ENTRAPMENT EFFICIENCY

An accurately weighed sample (10 mg) of the flurbiprofen loaded chitosan microspheres was placed in 25 ml of a solvent system consisting of methanol and 7.4 pH mixed phosphate buffer 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 247 nm.

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

Drug Loading in % = W/Wt x 100

where,

W = drug content of the microspheres

Wt = weight of the microspheres

Entrapment Efficiency was calculated using the formula

Entrapment Efficiency in % = Wc /Wo x 100

where,

Wc = total drug present in the microsphere batch

Wo = 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

Flurbiprofen loaded chitosan microspheres equivalent to 5 mg of flurbiprofen were subjected to in-vitro drug release studies to ascertain the drug release pattern of flurbiprofen from the prepared microspheres formulations. Drug release studies were carried out in USP Dissolution test apparatus II (paddle type) a dissolution test apparatus for 12 hrs in phosphate buffer (pH 7.4) at 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 λ_{max} at 247 nm 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 flurbiprofen (pure drug), chitosan (polymer), blank (unloaded) microspheres and flurbiprofen loaded chitosan microspheres were carried out over a temperature range of 30 to 300º C at a scanning rate of 10º C/min.

RESULTS AND DISCUSSION:

PARTICLE SIZE ANALYSIS AND MORPHOLOGICAL STUDIES

The mean particle size of the clobazam loaded chitosan microspheres ranged from 30.33µm to 38.56µm (Table 2). The changes in the concentration of chitosan and TSPP 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 TSPP affected the particle size distribution of the prepared flurbiprofen loaded chitosan microspheres. Microspheres prepared with 3% W/V TSPP had a higher number of particles in the size range 36.41 to 38.56 µm when compared to microspheres prepared with 5% W/V which had a greater number of particles in the size range of 36.41 to 37.23 µm. Figure 1 shows the scanning electron photomicrograph of flurbiprofen loaded chitosan microspheres. Microspheres which were irregular in shape were obtained using the method developed and adopted for preparing the flurbiprofen loaded chitosan microspheres. In the pH region where anions can interact with chitosan, irregular particles were obtained in the case of conventional emulsification and ionotropic gelation method [7].

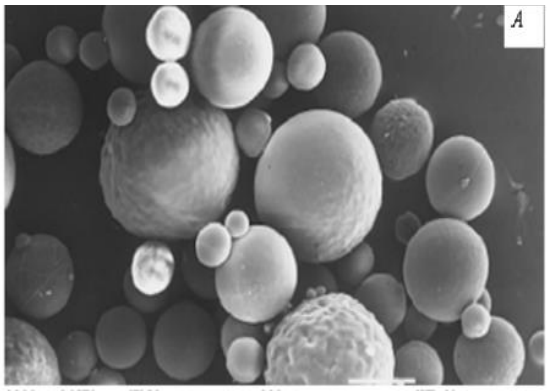

Figure 1: Morphology of microspheres by scanning electron microscopy

DETERMINATION OF DRUG LOADING AND ENTRAPMENT EFFICIENCY

The concentrations of chitosan and TSPP had an effect on the drug loading and entrapment efficiency of flurbiprofen loaded chitosan microspheres. Maximum drug loading and entrapment efficiency were 5.77% & 81.65% and 6.22% & 92.29% for the formulations F1 and F6 respectively (Table 2). Lower drug loading and entrapment efficiency observed with the prepared flurbiprofen 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 TSPP and chitosan. Increase in the concentration of chitosan increases the yield of the prepared microspheres and thereby resulting in higher drug entrapment levels of flurbiprofen. When the concentration of TSPP 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)

S. No.	Formulation code	Average particle size(µg)±SD	% drug loading	% Entrapment efficiency
1	F1	30.33±0.33	5.77±0.11	15.86±0.27
2	F2	32.25±0.62	7.12±0.55	62.53±0.93
3	F3	35.89±0.47	8.22±0.31	81.65±1.22
4	F4	36.41±0.22	6.22±0.25	18.35±0.52
5	F5	37.23±0.55	6.99±0.47	66.22±0.43
6	F6	38.56±0.59	9.85±0.54	92.29±0.53

***IN-VITRO* DRUG RELEASE STUDIES**

Release of flurbiprofen 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 2). And most probably, the drug was located at the outer layer of the microspheres during particle formation, which resulted in a burst effect [15].

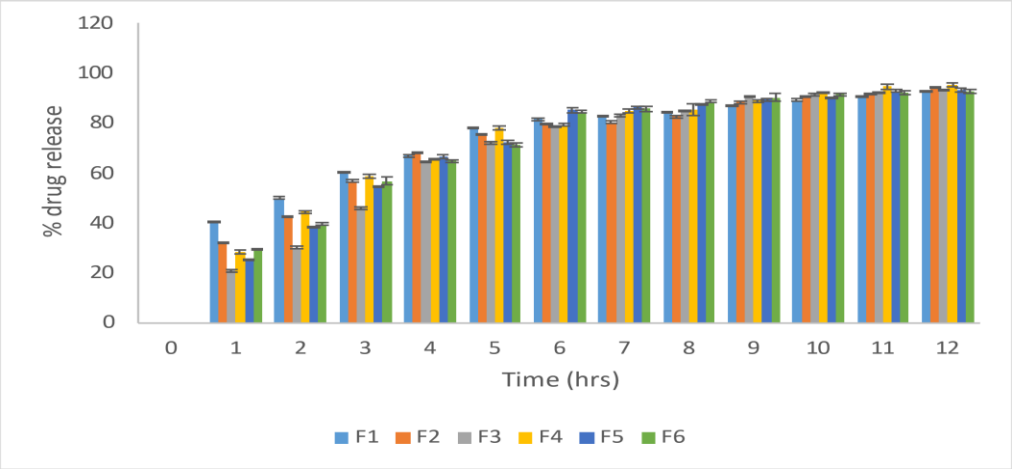

Figure 2: Cumulative percent release of flurbiprofen loaded microspheres

FTIR SPECTROSCOPIC ANALYSIS

The FTIR spectra of flurbiprofen, chitosan, blank microspheres and flurbiprofen loaded chitosan microspheres are illustrated in Figure 3. The FT-IR spectrum of flurbiprofen (pure drug) matches with that of its reference spectrum of the British Pharmacopoeia (1999). flurbiprofen shows a very strong IR absorption near 1694.7 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 flurbiprofen at almost the same wave number. Thus, it can be confirmed that no interaction exists between the drug and polymer.

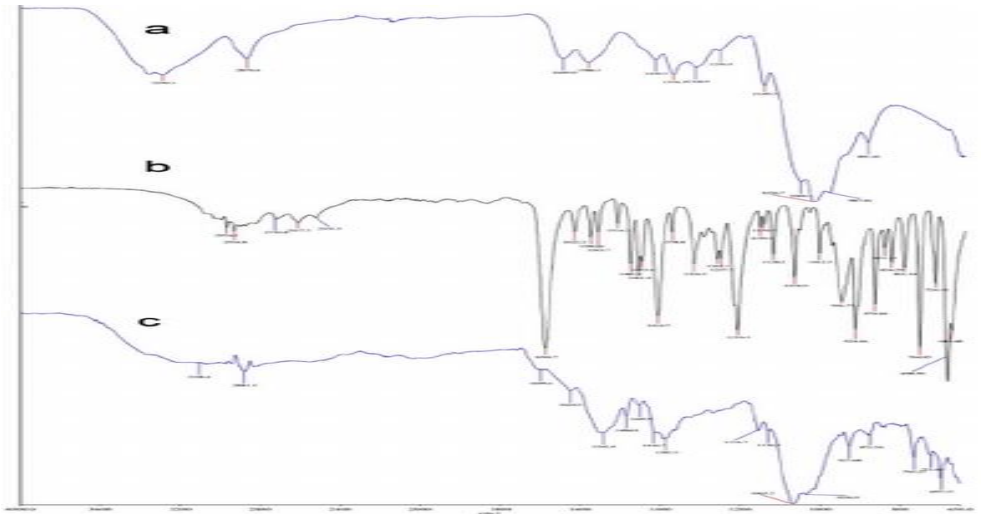

Figure 3: Fourier transform infrared spectroscopy spectrums of (a) chitosan (b) flurbiprofen and (c) chitosan-flurbiprofen microspheres

DIFFERENTIAL SCANNING CALORIMETRIC ANALYSIS

Figure 4 illustrates the DSC thermograms of flurbiprofen (pure drug), chitosan (polymer), blank (unloaded) microspheres and flurbiprofen loaded chitosan microspheres. A sharp endothermic peak corresponding to the melting of crystalline flurbiprofen was found at 254^0C. The melting endotherm of flurbiprofen was not observed in the drug loaded chitosan microspheres. This indicates that flurbiprofen was uniformly dispersed and present in an amorphous state in the polymeric matrix

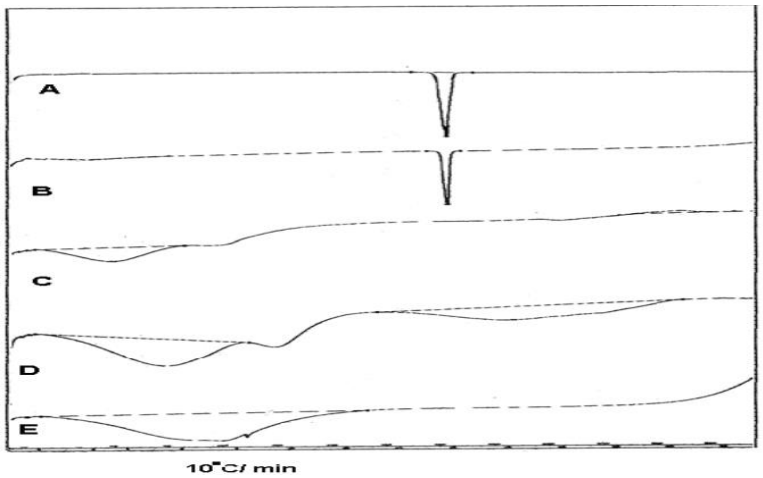

Figure 4: Differential scanning calorimetry (DSC) thermograms (A) flurbiprofen (B) Physical mixture of flurbiprofen and blank microspheres (C) flurbiprofen loaded microspheres (D) blank microspheres (E) chitosan

X RAY DIFFRACTION ANALYSIS

Figure 5 illustrates the comparative x-ray powder diffraction pattern of clobazam (pure drug), chitosan (polymer), blank (unloaded) microspheres and flurbiprofen loaded chitosan microspheres. Pure flurbiprofen 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.

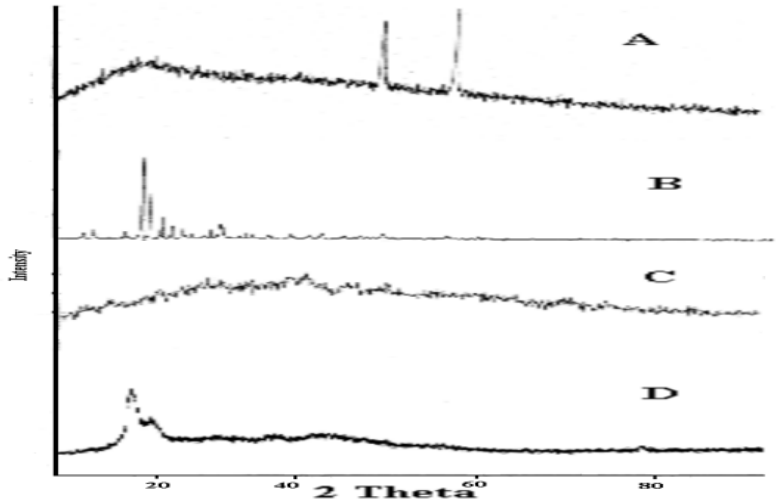

Figure 5: X-Ray Diffraction pattern of A) Clobazam loaded microspheres B) Clobazam C) Blank microspheres D) Chitosan

CONCLUSION:

The flurbiprofen loaded chitosan microspheres were prepared by ionic gelation using TSPP 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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