COMPARATIVE STUDIES ON THE DISSOLUTION PROFILE OF FLURBIPROFEN FROM COATED AND UNCOATED ALGINATE MICROSPHERES

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Summary

The aim of this study is to develop Alginate microspheres and coat them with Chitosan to form a biocompatible matrix, where the drug is retained either in a liquid or solid core and the shell allows permeability control over substrates. The effect of polymer concentration on the release profile of the drug was investigated. The microspheres core was formed by cross-linking sodium alginate with either calcium or barium ions. The cross-linked alginate core was uniformly coated with a chitosan layer, cross-linked with Gluteraldehyde. Flurbiprofen was chosen as a model drugs, and was encapsulated in the alginate core. Scanning electron microscopy was used for morphological observation. The coated microcapsule had poorer shape and rougher surface morphology when compared to uncoated one. Using carbopol along with alginate improved entrapment efficiency of the drug in the alginate core. The ability to release the active substance from the beads by chitosan coating was examined as a function of some parameters related to the chitosan density on bead surface, polymer concentration and pH of dissolution medium. The release of drug is prevented at acidic pH, while it showed controlled release when pH is raised up to 7.4. The alginate/chitosan ratio and the nature of the gelifying cation allow a control on the release rate of the drug. The release of the drug from the microcapsules was prolonged for 7-8 hours and the drug release followed Fickian diffusion mechanism with n value in the range of 0.26-0.31. This study illustrates a new method of drug encapsulation for biotechnology applications using liquid or solid core and shell microcapsule technology and to design a sustained release formulation for poorly water soluble drug like Flurbiprofen in the form of microcapsules using hydrophilic polymer which not only ensures a large effective surface area and increase the dissolution rate, but also achieves sustained release of the drug and maintain the plasma concentration with in the therapeutic range.

Keywords: Chitosan, Sodium alginate, Microcapsules, Flurbiprofen, alginate/chitosan ratio, biocompatible matrix, Gluteraldehyde.

Introduction

The efficacy of a drug in a specific application requires the maintenance of appropriate drug blood level concentration during a prolonged period of time. In the recent years, considerable attention has been focused on the development of Novel Drug Delivery Systems (NDDS). A 'microcapsule' is defined as a spherical particle with size varying from 50 nm to 2 mm, containing a core substance. Recently, the use of natural polymers in the design of drug delivery formulation has received much attention due to their excellent biocompatibility & biodegradability [1, 2]. Among them, alginate & chitosan are very promising & have been widely exploited in pharmaceutical industry for controlled drug release [3, 4]. Microcapsules of calcium alginate coated with a polycation have been widely investigated for applications like immunoprotective containers in cell transplantation [5, 6], enzyme immobilization [7, 8] & drug release systems [9, 10]. Alginate is a seaweed extract composed of chains of alternating a-l-guluronic acid & b-d-mannuronic acid residues [11]. Alginate supports are usually made by crosslinking the carboxyl group of the a-l-guluronic acid with a solution of a cationic crosslinker such as calcium chloride, barium chloride, or poly(l-lysine) [12], which takes place mainly at junctions in the G–G sequence rich chain region known as the 'egg box junctions' [13,14].. However uncoated Alginate matrices crosslinked with Ca^{2+} ions, however, are unstable in the physiological environment or in common buffer solutions with high concentration of phosphate & citrate ions that can extract Ca^{2+} from the alginate & liquefy the system. [13], these ions exchange with the no cooperatively bound calcium ions, leading to swelling of the gel. Chitosan is another natural polymer that has gained tremendous interest for use in immobilization technologies [15, 16]. Chitosan is a naturally occurring polysaccharide comprising glucosamine & N-acetylglucoamine with unique polycation characteristics [17, 18]. Chitosan is obtained by N-deacetylation of chitin, which is the second most abundant naturally occurring polymer found in the exoskeleton of marine crustaceans [19]. A major disadvantage of chitosan is its complete lack of solubility near physiological pH [20]. Since the pK_a of the d-glucosamine residue is around 6.5 [21], chitosan is soluble only in dilute acetic acid or hydrochloric acid solutions. To overcome these limitation, several investigators have suggested ionic complexation of chitosan (having a positive charge) with alginate (having a negative charge) to form a gel or microcapsule system for drug encapsulation [22, 23]. The polycationic nature of chitosan leads to a strong electrostatic interaction with negatively charged alginate where, carboxylic groups of alginate interact with amine groups of chitosan resulting in the formation of a membrane [24]. This membrane must be strong enough to resist an osmotic swelling pressure built up inside the capsules. There are many advantages of the chitosan coating, such as the improvement of drug payload & bioadhesive property, prolong drug release properties and also to reduce & control the permeability of the capsules which often is desirable when the capsules are used for encapsulation [25]. Flurbiprofen is a propionic acid derivative with extensive hepatic metabolism. It is effective in the treatment of rheumatoid arthritis, ankylosing spondylitis, osteoarthritis and gout [26]. The objective of the present study was to describe the development of a novel core-shell microcapsule technology for sustained drug delivery systems for water insoluble drugs.

Materials and Methods

Materials:
Flurbiprofen (KAPL, Bangalore, Karnataka, INDIA), Chitosan (Sigma-Aldrich, USA), Alginic acid-sodium salt (Sigma-Aldrich, USA), Carbopol (Loba-Chemie Pvt ltd, Mumbai, INDIA), Gluteraldehyde (SD fine-chemicals limited, Mumbai, INDIA) and all other chemicals and reagents were of analytical grade.

Preparation of alginate microspheres:
Complex coacervation method: Two step procedure, initially it involves the preparation of alginate core micro spheres, where Calcium/barium alginate beads were formulated by using the needle extrusion/external gelation method. All the formulations were prepared using 20 ml of sodium alginate in varying concentrations (2%, 3% and 4% w/v) containing 200mg of drug. This solution was loaded in to a syringe fitted with 24 G needle and was added drop wise in to calcium/barium chloride aqueous solution of varying concentrations (3%, 5% and 7% w/v) and then the beads were allowed to crosslink for 10mins.

Preparation of alginate-chitosan core shell microcapsules:
Second step involves the Preparation of alginate-chitosan core shell microcapsules, where the prepared alginate micro spheres will be dispersed in to chitosan solution in 0.2M acetic acid, of varying concentrations (0.75%, 1% and 1.5% w/v) to form chitosan shell of varying thickness for about 10mins. Thus formed beads were transferred in to aqueous solution of Gluteraldehyde and allowed to crosslink for 10mins. Thus formed microcapsules are washed with deionised water and dried at room temperature.
The prepared microcapsules were characterized by Fourier transformed infra red spectroscopic analysis, Differential scanning calorimetric analysis and Scanning electron microscopic analysis and evaluated for Content uniformity / drug loading, Swelling studies, Particle size analysis, Entrapment efficiency and *In vitro* drug release studies.

Characterization

Fourier Transform- Infrared Spectroscopy:
The FT-IR spectral measurements were taken at ambient temperature using a Shimadzu, Model 8033 (USA). About 2 mg of the pure drug and optimized formulations were selected separately. Pure drug and formulations were dispersed in KBr powder and the pellets were made by applying 6000 kg/cm^2 pressure. FT-IR spectra were obtained by powder diffuse reflectance on FT-IR spectrophotometer.

Differential scanning calorimetry:
DSC analysis was done to ascertain the compatibility of drug with the excipients. It was performed on a DSC Dupont 9900, differential scanning calorimeter with a thermal analyzer. About 100 mg of the powered sample was placed in a sealed aluminum pan, before heating under nitrogen flow (20ml/min) at a scanning rate of $10^{o}C\ min^{-1}$, from $25^{o}C$ to $250^{o}C$. An empty aluminum pan was used as reference.

Scanning electron microscopy:
SEM was done using Joel SEM analysis instrument Japan. It was done to study external morphology of microcapsules. Prior to observation, samples were mounted on aluminum mount, using double-sided adhesive tape and sputtered by gold under vacuum and were scanned at an accelerating voltage of 15kV before observation.

Evaluation

Content uniformity / drug loading:
The prepared microcapsules were powdered and passed through sieve no (85/120). The powder retained on the sieve 120 was taken for content uniformity studies. A weight of powder containing 100 mg of the drug was taken in a 100ml standard volumetric flask. To this of 0.1 N w/v NaOH solution was added and made up to the mark with 0.1 N w/v NaOH solution and kept overnight. The final solution was filtered using what man filter paper. From this 10 ml was pipetted out into a 100 ml standard volumetric flask and made up to the volume with 0.1 N w/v NaOH solution and estimated spectrophotometrically for drug content.

Swelling studies:
The swelling properties of the microcapsules were carried out using 30mM sodium phosphate buffer (pH 7.4). The microcapsules of known weight were placed in 50 ml of the buffer solution for 24hr. At time intervals of 15mins for first one hour, 30mins for next two hours and one hour for next four hours, the microcapsules were removed, excess surface liquid was removed by blotting paper and their weight was recorded.

The percentage swelling (S) was determined by the following equation.

$$S = \frac{\text{Weight of swollen microcapsules} - \text{weight of dry microcapsules}}{\text{Weight of dry microcapsules}} \times 100$$

Entrapment efficiency:

To evaluate the amount of the drug inside the microcapsules, an indirect method was used. Aliquots from the filtered solutions remaining after removal of the beads were assayed spectrophotometerically. The amount of drug entrapped was calculated from the difference between the total amount of drug added and the amount of drug found in the filtered solution.

About 100 mg of beads were completely dissolved in 500 ml of carbonate buffer solutions (pH 7.4), and stirred for 1h. Then, 2 ml of solution was filtered and the concentration of drug was determined spectrophotometrically by UV. Efficiency of drug entrapment was calculated in terms of percentage drug entrapment (PDE) as per the following formula:

PDE = (Practical drug loading/Theoretical drug loading) ×100

***In-vitro* drug release studies:**

The *In vitro* release of drug from the microcapsules was carried out in Paddle type dissolution tester-USP XXII, TDT-08L, with auto sampler containing 900 ml of pH 7.4 carbonate buffer, maintained at 37 $\pm$ 0.5^0 C and at a stirring speed of 50 rpm. About 10ml of the Sample was withdrawn for every one hour and analyzed for drug content spectrophotometerically. The released data obtained were fitted into various mathematical models to know which mathematical model is best fitting the obtained release profile.

Stability studies of optimized formulations:

Stability is defined as the ability of a particular drug or a dosage form in a specific container to remain with its physical, chemical, therapeutic and toxicological specifications. Two optimized formulations of the microcapsules of drug were selected for stability studies. Formulations were packed in an aluminum foil and studies were carried out by keeping at

- 30 $\pm$ 2^oC and 65 $\pm$ 5% relative humidity
- 40 $\pm$ 2^oC and 75 $\pm$ 5% relative humidity

The 65% relative humidity was obtained by saturated solution of sodium nitrite while 75% relative humidity was obtained by saturated solution of sodium chloride. The duration of studies was a period of 60 days. Samples were withdrawn on 0^{th}, 15^{th}, 45^{th}, 60^{th} day and were analyzed for drug content and drug dissolution studies.

Calculation of Maintenance Dose:

Drug loaded beads were designed for 8 hours maintenance therapy with a loading conventional tablet of 100 mg (both to be taken together on 1^{st} day followed by beads only on subsequent days), hence, it is assumed that with a loading dose tablet of 100 mg steady state is reached and beads with appropriate dose can maintain that steady state for rest 8 hours. To calculate the dose of the drug, the principle as iv infusion was utilized.

In case of I.V. infusion, Rate of drug input = Rate of drug output

Rate in = D K_E
$K_E = 0.693 / t_{1/2}$
Maintenance dose M.D = (D X 0.693 X T) / $t_{1/2}$
Where, D is the initial dose of the drug present in the microcapsules.
T is the time interval for which release is required (i.e. 8 hours).
$t_{1/2}$ is the half life of the drug.

Results and Discussion

Preparation of alginate-chitosan core-shell microcapsules:

The procedure followed for the preparation of microcapsules produced good yield of microcapsules. As shown in Table 1, the percentage of yield was 86, which indicated low loss of microcapsules during the preparation and recovery. The formulated microcapsules were with free flowing nature. In these microcapsules the concentration of alginate was varied from 1-2% w/v while the concentration of barium chloride and calcium chloride was varied from 1.5-3.5% w/v respectively to study their effects on the drug release and swelling properties. Here calcium chloride or barium chloride was used as a crosslinking agent for alginate. Carbopol another polymer was used to increase the drug loading in to the microcapsules. The concentration of carbopol was kept constant. i.e. 1% w/v. Chitosan is coated around the alginate microcapsules by dispersing the microcapsules in the chitosan solution of varying concentrations .i.e. 0.75-1.5% w/v and its effect on the release behavior of the drug from the microcapsules was studied.

Gluteraldehyde was used as crosslinking agent to crosslink chitosan, to form a film around the alginate microcapsule. The Gluteraldehyde concentration was optimized to 4% v/v. The amount of drug was kept constant i.e. 100 mg in all the formulations. The objective of varying the concentration of crosslinking agents and the polymers was to achieve optimized formulations, which would give a sustained release of the drug over a period of 8 hours.

Characterization:

FT-IR:

Flurbiprofen and one of the formulations of each were subjected to FT-IR spectroscopic analysis, to ascertain whether there is any interaction between the drugs and the polymers used, it was observed that similar characteristic peaks appear with minor differences, at frequencies 2977.89 cm^{-1} (C-H stretch of CH_3), 1697.24 cm^{-1} (C=O stretch of acid), 1654.81 cm^{-1} (C=O stretch of Ketone), 1595.02 cm^{-1}(C=C stretch of aromatic ring), 1448.44 cm^{-1} and 1417.58 cm^{-1} (C-H deformation of CH_3).

DSC:

The DSC thermograms obtained are reported that there was no polymer drug interaction as the pure drug Flurbiprofen displayed a single sharp endothermic peak at 119.93 ^{o}C corresponding to the melting point of the drug, and a similar peak was also observed in the formulation.

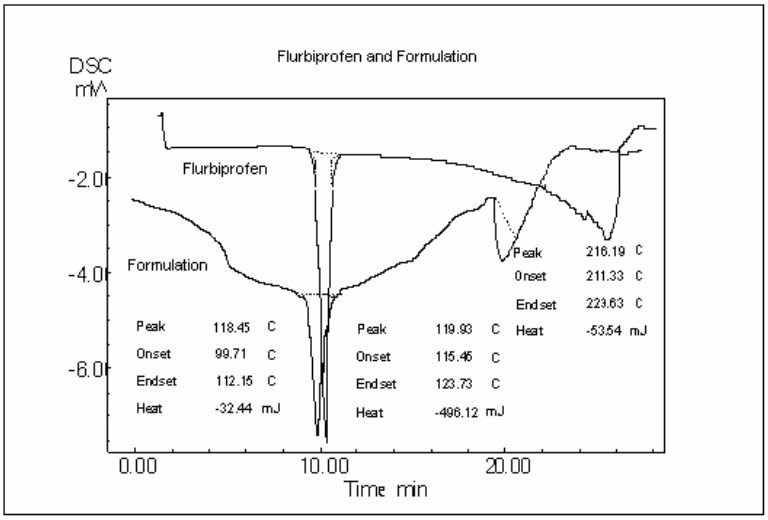

Fig. 1. DSC thermograms of Flurbiprofen and formulation

SEM:

Scanning electron microscopy was carried out in order to characterize surface morphology, texture and porosity of the microcapsules. In this study the morphological observations were carried out to study the surface morphology of coated and uncoated microcapsules. It was observed that microcapsules were spherical in wet conditions, irregular after drying (Fig. 2).

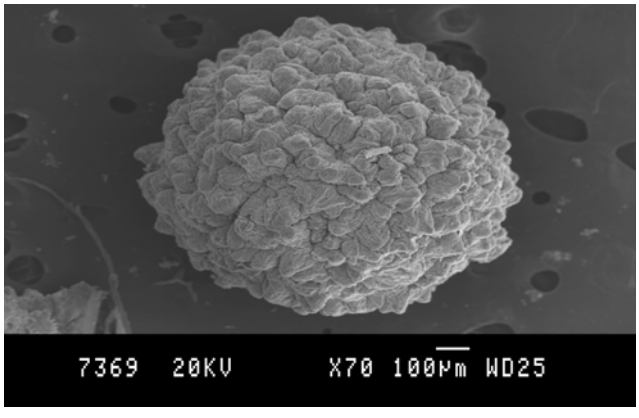

Fig 2: Chitosan coated calcium-alginate microcapsule at ×70

The chitosan membrane was continuous in nature and it was observed that by increasing the concentration of chitosan, the thickness of coat was also found to increase (Fig.3).

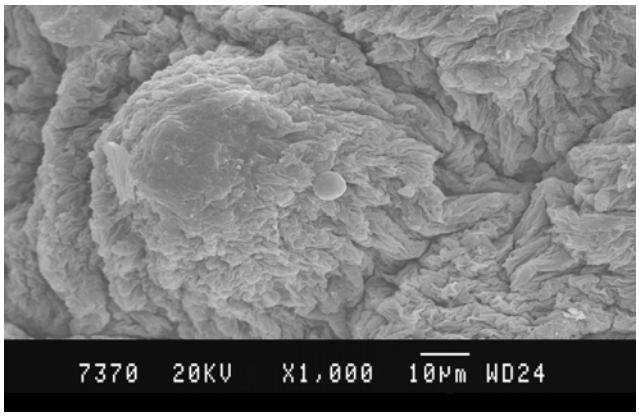

Fig 3: Surface morphology of the coated microcapsule at X1000

Cross-sectional view indicated the presence of a continuous chitosan coat around the alginate-drug core matrix. The surface morphology indicated that the chitosan coat was continuous while that of uncoated one showed presence of pores.

Evaluation:

Swelling Studies:

The swelling studies were carried out in pH 7.4 buffer to study the dynamic weight change of the alginate beads cross linked with calcium/barium chloride. It is clear from the figure, that the beads exhibited maximum water uptake in pH 7.4 buffer within 4 hours, after attaining maximum water uptake; the beads begin to loose weight and finally dissolve.

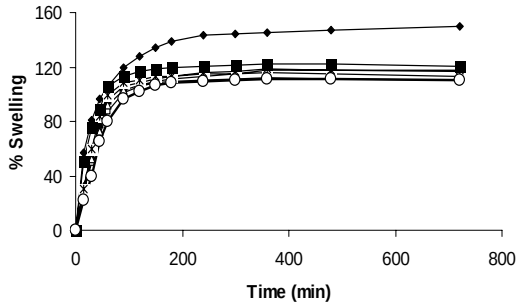

Fig 4: The Swelling ratio of Alginate-Chitosan Microcapsules of formulations F1 (◆), F2 (□), F3 (▲), F4 (△), F5 (×), F6 (■) and F7 (○) in pH 7.4 carbonate buffer

When microcapsules are placed in the pH 7.4 buffer, the Na^+ ions present in the external solution undergo ion-exchange process with Ca^{2+} ions, which are binding with COO^- groups mainly in the polymannuronate sequences. As a result the electrostatic repulsion among negatively charged COO^- groups increases, which ultimately causes the chain relaxation and enhances the gel swelling. Thus it can be said that in the initial phase of the swelling process the Ca^{2+} ions present in polymannuronate units are exchanged with Na^+ ions, thus causing the beads to swell along with uptake of water. In the later stage of swelling process, the Ca^{2+} ions, which are binding with COO^- group of the polyguluronate units and thus form the tight -egg-box- structure, which also start to exchange with Na^+ ions of the buffer medium. This consideration is plausible because polyguluronate sequences have a strong auto-cooperative binding of Ca^{2+} ions and serve as a stable crosslinking structure within the gel. Finally, the alginate beads begin to disintegrate when Ca^{2+} ions in the egg box buckled structure diffuse out into the medium. Therefore, the beads start to loose their weight and finally dissolve. It is also clear that as the extent of crosslinking increases, the maximum water uptake decreases.

Encapsulation Efficiency:

The encapsulation efficiency was performed to find out the amount of drug that gets encapsulated in the microcapsule so that sufficient amount of drug is present in the microcapsules to ensure the drug remains in the therapeutic range once it enters the systemic circulation.

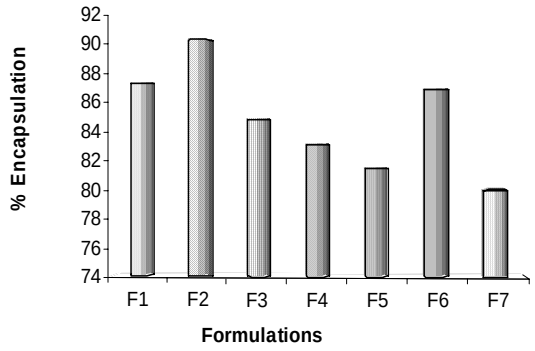

Fig 5: Encapsulation efficiency of different formulations of Alginate-Chitosan Microcapsules

The encapsulation efficiency was found to be higher in case of Barium alginate core (90.4%), when compared to the calcium alginate core (60%) because calcium was leached out of calcium alginate core during the process of preparation of beads. Drug loading was further found to decrease with the increase in the concentration of encapsulating polymer i.e. chitosan. Apart from alginate, Carbopol tend to increase the encapsulation efficiency as it tends to bind with alginate and forms a reservoir for the drug (fig 5). The concentration of Carbopol was optimized to 1%. By increasing the concentration of Carbopol the viscosity of alginate solution was found to increase and it was found to be difficult to be dropped from the hypodermic needle.

***In Vitro* Release Studies:**

The *in vitro* release studies were carried out by buffer change method to mimic the GIT environment. It was carried out for all the formulations in both acidic and basic pH. The release studies were carried out in acidic pH i.e., at 1.2 pH for the first two hours, to mimic the acidic environment prevailing in the stomach. For the next 6 hours, the release studies were carried out in basic pH i.e., pH 7.4 buffer to mimic the basic conditions prevailing in the intestine. The *in vitro* release profiles are given in Figures 6-9. Formulations with varying concentrations of alginate and chitosan were used to study the effect of chitosan and alginate on the drug release from the microcapsules.

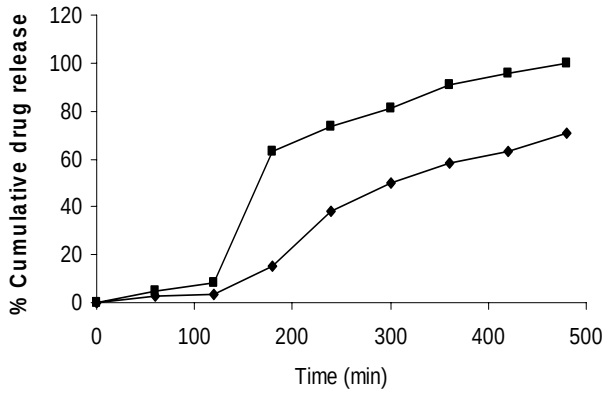

Fig 6: The Effect of coating on the In vitro drug release from microcapsules for F3 (◆) Uncoated microcapsules, F1 (■) Microcapsules coated with Chitosan

For the initial 2 Hours i.e. in 1.2pH buffer the drug release was found to be low (less than 10%) in all the cases, indicating that the outer coat of chitosan was resistant to the acidic media, however when transferred into intestinal media there was slow dissolution of the coat resulting in the slow release of the drug and it can also be attributed to the fact that the swelling of microcapsules in the acidic media is low. However when transferred into intestinal media there was slow dissolution of the coat resulting in the release of the drug. The basic pH of the media was able to penetrate the bead and dissolve the matrix to release the drug by diffusion.

In the Flurbiprofen microcapsule formulations, the amount of drug release was found to decrease with the increase in the concentration of the alginate, which is due to the increase in the density of the beads and also increase in the diffusional path length through which the drug molecule has to travel.

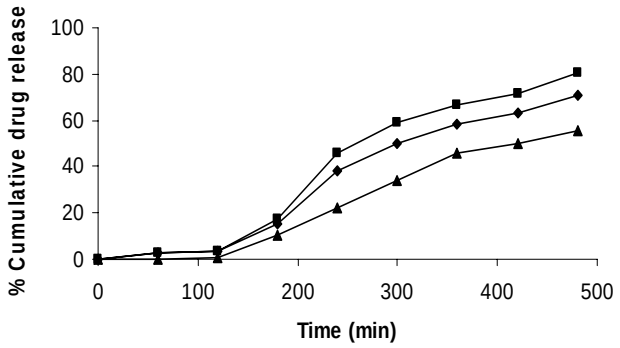

Fig 7: In vitro drug release from microcapsules prepared with different concentrations of Alginate F5 (■), F3(♦), F8(▲)

With the increase in the concentration of Calcium/Barium chloride there was a remarkable decrease in the release of the drug from the microcapsules. This can be attributed to increased crosslinking of alginate with $Ca/BaCl_2$.

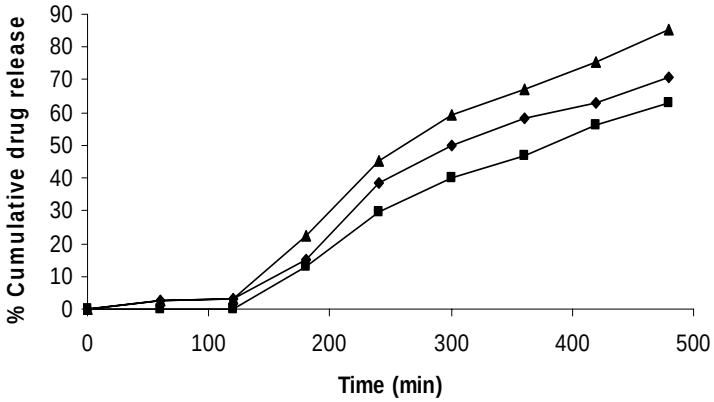

Fig 9: In vitro drug release from microcapsules prepared with different concentrations of Calcium/ Barium chloride F2 (♦), F6 (▲), F7 (■)

Furthermore the amount of drug release was found to decrease marginally with the increase in the concentration of chitosan, which can be attributed to the increased thickness of the coating membrane.

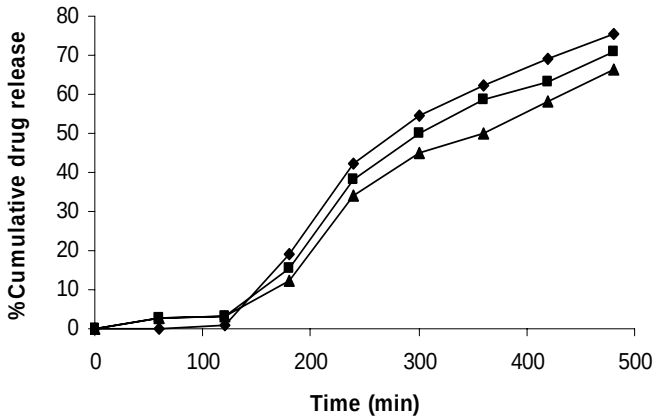

Fig 8: In vitro drug release from microcapsules prepared with different concentrations of Chitosan F2 (♦), F3 (■), F4 (▲)

The results obtained were fitted into various mathematical models to know which mathematical model is best fitting the release profile. The parameters, n and k, were also determined for Koresmeyer-Peppas equation to know the release mechanism.

Stability Studies of the Optimized Formulations:
Stability studies of the optimized formulations of Flurbiprofen were carried out to determine the effect of formulation additives on the stability of the drug and also to determine the physical stability of the formulation under accelerated temperature. Stability studies shown that after 90 days, at the two conditions i.e. 40 $\pm$2^{o}C and 75 $\pm$ 5% relative humidity and 30 $\pm$ 2^{o}C and 65 $\pm$ 5% relative humidity, there was no significant change in the drug content and the drug release.

Pharmacokinetic Data:
The invitro release studies data was fitted in to various mathematical models to determine which is the best-fit model. The various parameters n, the time exponent, k, the release constant and R, the regression coefficient, were also calculated. The results indicate that, the best-fit model in all the cases was found to be Peppas model (fig. 10)
Koresmeyer-Peppas equation:

$$Mt/M\infty = 1- A (\exp -Kt)$$

$$\log (1 - Mt/M\infty) = \log A – k\ t / 2.303$$

Among the Flurbiprofen formulations
F2 to F7 shows Non-Fickian transport (by polymer chain relaxation)
F1 and F8 shows super case-II transport (by more than one mechanism).

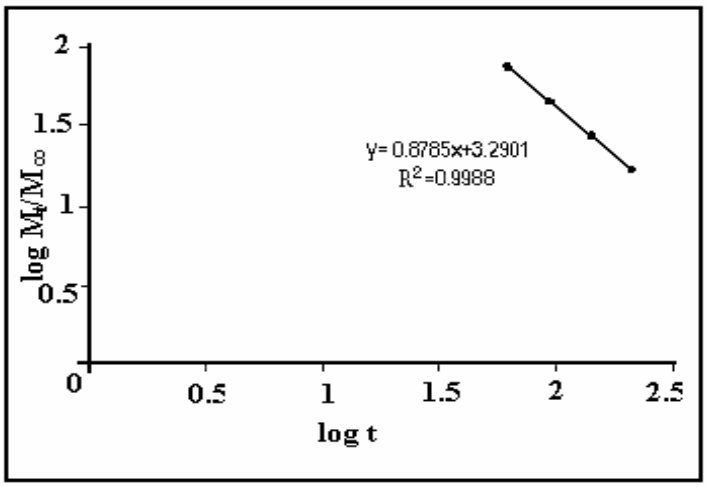

Fig 10: Graph showing the various parameters of model fitting for chitosan coated alginate microcapsules

Conclusion

Alginate–chitosan core-shell microcapsules were prepared as a novel biocompatible matrix system for drug encapsulation where the drug is confined to either a liquid or solid core and the diffusion of the drug was dictated by the permeability of the shell. Using Ca^{2+} and Ba^{2+} ions for crosslinking alginate, we were able to produce the microcapsules with liquid and solid core, respectively. Chitosan was coated over the alginate core using Gluteraldehyde as a crosslinking agent. Flurbiprofen was used as a model drug, 60% loading efficiency was obtained with the Ca^{2+} alginate, while 89% loading efficiency was obtained with the Ba^{2+} alginate. However, the solid core microcapsules, prepared with Ba^{2+} alginate, did improve the stability of the bead at 37^0 C as compared to the liquid core Ca^{2+} alginate microcapsules. The beads formed were irregular and nonporous wall structure as evidenced by SEM photographs. The particle size was not varied significantly either by increasing the exposure time to the crosslinking agent or by increasing the percentage loading of the active ingredient. The use of a coating membrane i.e. chitosan successfully delays flurbiprofen release, which can be tailored to produce a suitable single dose administration per day by adjusting the thickness of the coating membrane and obtain the desired time of complete drug release. Physicochemical characterization shows that flurbiprofen does not interfere with the matrix formation process. Matrix swelling induced by buffer does not result in calcium alginate erosion and destruction for cross-linked beads, which may be the main cause of flurbiprofen retention within the matrix. This study illustrates a novel method for preparing microcapsules to be used in the encapsulation of the drug, such that the drug is protected in the inner biocompatible alginate core and the outer chitosan shell dictates the transport properties. These relatively porous and highly stable microcapsules could also be applied in cell immobilization for biotechnology applications.

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