Beta Cyclodextrin–Insulin-Encapsulated Chitosan/Alginate Matrix: Oral Delivery System

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ABSTRACT: Cyclodextrins (CD) form inclusion complexes with many drug molecules. The complexed drugs have increased bioabsorption in *in vivo* system. We have attempted to complex insulin with β-Cyclodextrin (BCD) and encapsulate in the chitosan/calcium alginate matrix. For drug release studies insulin complexed with BCD for 20 min and that complexed with BCD for 150 min have been used for encapsulation in the chitosan/calcium alginate matrix. The two matrices seem to have different drug release profiles in simulated intestinal medium (pH 7.4) It appears that drug release from the 20-min BCD complexed system encapsulated in the chitosan/calcium alginate matrix begins only after an hour, where, being released from the 150-min BCD complexed system it begins in the first hour itself. Also, aggregation of the insulin molecules seems to be reduced by the complexation of the drug with BCD. Another noticeable fact is the change in the loading character, which is found to be inversely related to the concentration of BCD when it is above the stoichiometric equivalent of the drug. In an attempt to increase the payload of the drug in the matrix, the pH of the processing medium consisting of calcium chloride and chitosan is varied. It is found that the encapsulation efficiency increases as the pH is decreased from 6.0 to 4.0. Another way of increasing the loading is studied by decreasing the concentration gradient of insulin in the processing alginate solution and the crosslinking medium consisting of chitosan/calcium chloride. Preliminary animal studies on rabbits seem to be promising. J Appl Polym Sci 75: 1089–1096, 2000

Key words: alginate; β-cyclodextrin; chitosan; insulin; oral delivery

INTRODUCTION

Cyclodextrins are cyclic oligo saccharides composed of dextrose units joined through the 1–4 bond. They and their derivatives have been widely used in drug delivery applications due to their capability of forming inclusion complexes with drug molecules. These complexes are capable of altering the release pattern, changing the solubility and increasing the stability of the drugs.[1–5] Cyclodextrins promote dissolution of sparingly soluble drugs.[6] Drug permeation is influenced by the molecular form of the drug. It is also observed that insulin permeation is more with monomeric forms than the hexaemeric form during iontophoresis.[7] In another study, the processing temperature has been reduced to 23°C to avoid the aggregation of insulin molecules.[8] Processing at lower temperature has limitations in large-scale processing.

Cyclodextrins have the capability to enhance the absorption of the complexed drug in the *in*

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vivo system.[9] The mechanism of absorption enhancing effect of CDs and their derivatives have been suggested by Uekama et al.[10] as due to the possibility of cyclodextrins to affect drug absorption through modification of the mucosal membrane. Moreover, the presence of CDs facilitated by the simultaneous release of these molecules and the drugs has been proven to enhance the absorption in the *in vivo* system.[11] The alteration in the enhanced permeability caused by BCD occurred in the transcellular pathway, than the paracellular pathway of the small intestine.[12] BCD and its derivatives have been preferentially used for parenteral applications than Alpha Cyclodextrin or Gamma Cyclodextrin. Also, BCD has more potential than its derivatives in the case of oral application due to the nephrotoxicity caused by its derivatives.

Oral delivery of insulin for the treatment of diabetes mellitus has been tried in diabetic-induced rats by Roques et al.[13] One major problem associated with the oral delivery of insulin has been degradation of the drug due to the proteolytic activity of various enzymes in the GI tract. The advantage of the encapsulated system against degradation of insulin has been studied *in vitro*.[14] Other means of solution to this problem has been tried with the simultaneous release of protease inhibitors along with the drug. But it is reported that for long-term therapy coadministration of peptide and protein drugs along with enzyme inhibitors remain questionable.[15] Hence, alternative methods are sought for the safe delivery, increased bioavailability, and enhanced absorption of the drug.

The release kinetics are important to tailor it to the pharmacological requirement. We have reported earlier on the release of kinetics of insulin in the chitosan/calcium alginate system.[14] At present, our attempt is to incorporate oligo saccharides included with insulin into the chitosan/calcium alginate system, which may be effective in increasing the bioavailability, which in turn, may result in the enhanced absorption *in vivo*.

The time of complexation at room temperature and the release kinetics of the complexed drug are explored. This may help in selecting a system that may release the required amount of the drug for the efficient management of diabetes.

Low loading of the drug in the chitosan/calcium alginate matrix, which comes in the range of 10 to 11 IU per gram of the microspheres, is a weakness of the process . We have attempted to increase the loading by (a) altering the pH conditions of the crosslinking medium consisting chitosan/calcium chloride, and (b) by reducing the concentration gradient of insulin in the crosslinking medium and the alginate gel.

MATERIALS AND METHODS

Sodium alginate (3500 cps for a 2% solution at 25°C) and BCD were procured from Sigma Chemicals (St. Louis , MO; A-2023); chitosan, derived from a prawn shell and having a particle size 1–3 mm, was received from CIFT (Kochi, India). Insulin (bovine) 40 IU per mL injection preparation was from Knoll Pharmaceuticals Ltd. India. All the other chemicals were of AR grade.

Insulin estimation was done as per the Lowry's method of estimation of protein.[16] Absorbance study was done with the help of Shimadzu spectrophotometer at the wave length of 750 nm.

Complexation with BCD and Release Profile

All the experiments are carried out at room temperature and atmospheric pressure, unless otherwise mentioned.

Alginate beads are prepared by the process described by Hari et al.[14] To give here a summary, 2% (w/v) alginate was prepared by the gentle mixing of the alginate in normal saline. Chitosan solution [0.3% (w/v)] was prepared by dissolving it in 0.01 *M* HCl for a prolonged time. Calcium chloride 1.5 g was added to 100 mL of the 0.3% Chitosan solution and is used as the crosslinking medium.

In one experiment 5 mL (200 IU) of insulin solution preparation was mixed with a definite weight of BCD and gently agitated for 20 min for complexation. This is mixed with 45 mL of the 2% alginate solution. This insulin containing alginate is dropped through a 0.15 mm-diameter hypodermic needle into 100 mL of the chitosan/calcium chloride crosslinking medium. The microspheres formed are allowed to harden for 30 min in the same system. They are separated, dried at 10°C, washed with distilled water, and again dried and stored at the same temperature. Three different concentrations of BCD viz 25, 50, and 100 mg are chosen for a comparative study. The microspheres prepared are marked as (A-25), (A-50), and (A-100), respectively.

In another experiment, 5 mL (200 IU) of insulin preparation is gently mixed with a definite quantity of BCD for 150 min. This is mixed with

45 mL 2% alginate solution. Microspheres are prepared as mentioned above. The different amounts of BCD taken for the complexation are 5, 25, and 100 mg. The microspheres thus formed are labeled as (B-5), (B-25), and (B-100) respectively.

The sample (2 g) is taken for each release experiment. They are introduced into 0.1 *M* HCl for 4 h, washed in distilled water and are put into the SIM [simulated intestinal Medium (pH = 7.4), without enzyme, as per the US Pharmacopoeia]. A definite volume of the release medium is withdrawn at particular time intervals. An equal amount of the SIM is added to keep the volume of the release medium constant. Each sample is analysed for its peptide content by Lowry's method. All the experiments are carried out in triplicate or more.

Complexation Time

To 5 mL of the insulin solution, 10 mg of BCD is added. After 5, 30, 60, 120, and 150 min, representative samples are withdrawn , diluted with distilled water, and the drug quantity is estimated as mentioned previously.

Effect of pH of the Crosslinking Medium and Time of Crosslinking on Loading

The pH of the chitosan/calcium chloride crosslinking medium is adjusted to 4.0, 5.0, and 6.0 using 0.1 *N* HCl and NaOH. 5 mL (200 IU) insulin preparation is added to 45 mL of 2% alginate and gently mixed. Ten milliliters of this insulin containing alginate is extruded through a 0.15 mm-diameter hypodermic needle into 100 mL of each of the three different chitosan/calcium crosslinking media, with pH values of 4.0, 5.0, and 6.0. At the end of 10 and 60 min 50 numbers from each are collected separately and put in a 2-mL solution of SIM of pH = 7.4. They are analysed for their drug content as previously mentioned. In a separate study, the average weight of 50 dry beads are estimated to be 0.0323 ± 0.0014 g.

Loading Enhancement by Reducing the Concentration Gradient

The higher concentration gradient of insulin existing between the alginate gel and the crosslinking medium at the time of crosslinking may be one of the reasons for the poor loading. For this study, alginate microspheres are prepared as follows: 200 IU of insulin is gently mixed with 45 mL of 2% alginate. Ten milliliters of this is extruded through a 0.15 mm-diameter hypodermic needle into 100 mL of the crosslinking medium mentioned under "a," to which 200 IU of an insulin preparation is also added. After 30 min hardening time, 50 microspheres from each are collected and introduced into 2.0 mL SIM. For comparison similar microspheres are prepared with another 10 mL of the same alginate extruded into a crosslinking medium consisting of chitosan/calcium chloride alone. The drug content of each is estimated after 24 h.

RESULTS AND DISCUSSION

Drug Release

It is found that all microspheres prepared in this study would swell in the intestinal medium of pH = 7.4. The spherical shape of the particles is maintained for at least 15 days in all the cases, due to lack of apparent dissolution. The release behavior from these particles may be assumed to be diffusion controlled. The first comparison of the release behavior as shown in Figure 1 is of those microspheres containing insulin–BCD complexes, which are allowed to react for 20 min. The relative proportion of insulin and BCD during complexation is 200 IU of insulin to either 25, 50, or 100 mg of BCD. The release of drug from the alginate matrix into the SIM may be summarized as follows. During the first hour the release is negligible from all the three samples. About 6–8 IU of insulin per gram of alginate is released from all the three samples during the second hour. Drug release comes down to 2–4 IU per gram during the third hour. Thereafter, the release is negligible.

Marginally higher loading (about 10 IU) is observed in the samples where a smaller amount of BCD is used.

In the second comparison (Fig. 2), the time of complexation is increased to 150 min, keeping the relative proportion of BCD, as 5, 25, and 100 mg for 200 IU of Insulin [(B-5), (B-25), (B-100)], respectively]. About 7–8 IU of insulin is released in the first hour from (B-5), (B-25), and (B-100) systems . From the (B-100) system only 1–2 IU insulin is released thereafter. From the (B-25) system, about 5 IU is released in the second hour, another 2 IU in the third hour, and thereafter it registered a reduction; the reason for this is unclear. The

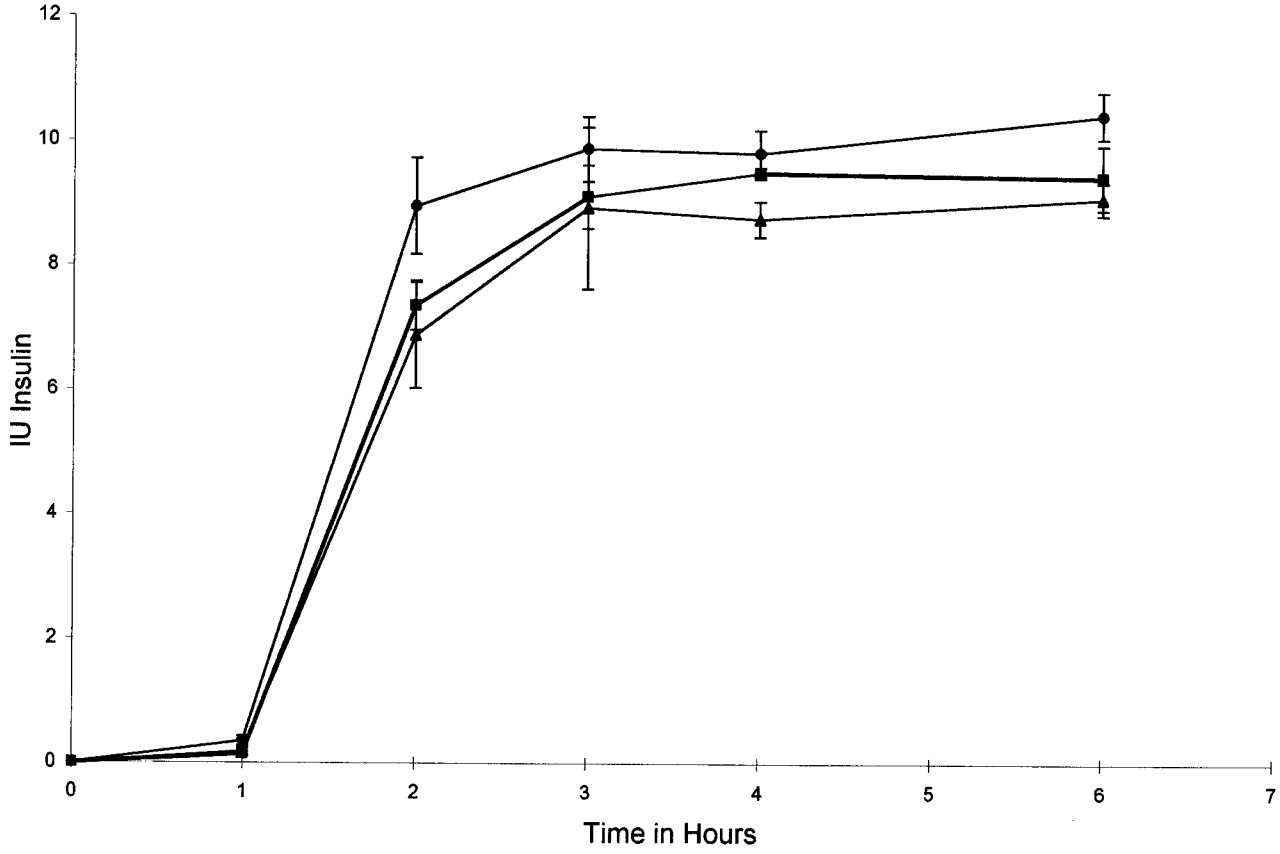

Figure 1 Release profile of Insulin from microspheres formed by mixing 100 mL alginate with 5 mL (200 IU) Insulin complexed with different quantities of BCD for 20 min. ●—200 IU insulin complexed with 25 mg BCD (A-25); ■—200 IU insulin complexed with 50 mg BCD (A-50); △—200 IU insulin complexed with 100 mg BCD (A-100).

(B-5) system registered about 1–2 IU during the second hour up to the sixth hour.

Here, also, the loading of drug seems to be higher (16 IU) in the samples where a smaller amount of BCD [i.e., (B-5)] is used for complexation.

Complexation Time

Figure 3 is a combination of the drug release from the systems complexed for different periods of time. It is interesting to note that the release pattern of the drug complexed with the same amount of BCD and different time of complexation are different. For all the 150-min BCD complexed systems, the release from the matrix begins in the first hour itself, whereas for all 20-min BCD complexed systems, release is observed only after an hour. The latter system behaves similar to the system without BCD.[14] The faster rate of drug release from the 150-min complexed system can be attributed to the smaller size of the non-aggregated molecules of insulin on complexation with BCD.[17] The (A-100) and (B-100) systems have similar amount of drug encapsulated, and they differ only in the initial time of drug release. A second inference is the enhanced loading in the (B-5) system. The lower insulin loading in (A-100) and (B-100) systems may be due to the presence of excess BCD in the matrix, which acts as a filler. This may reduce the crosslinking of alginate. A smaller amount of crosslinking may reduce the retention of the drug, and hence, a smaller amount of loading.

Effect of pH of the Crosslinking Medium and Time of Crosslinking on Loading

The insulin units that could be encapsulated by this process from our previous study seems to be about 11 IU per gram of microspheres,[14] which means that the efficiency of the process has to be improved. Although the initial amount used for

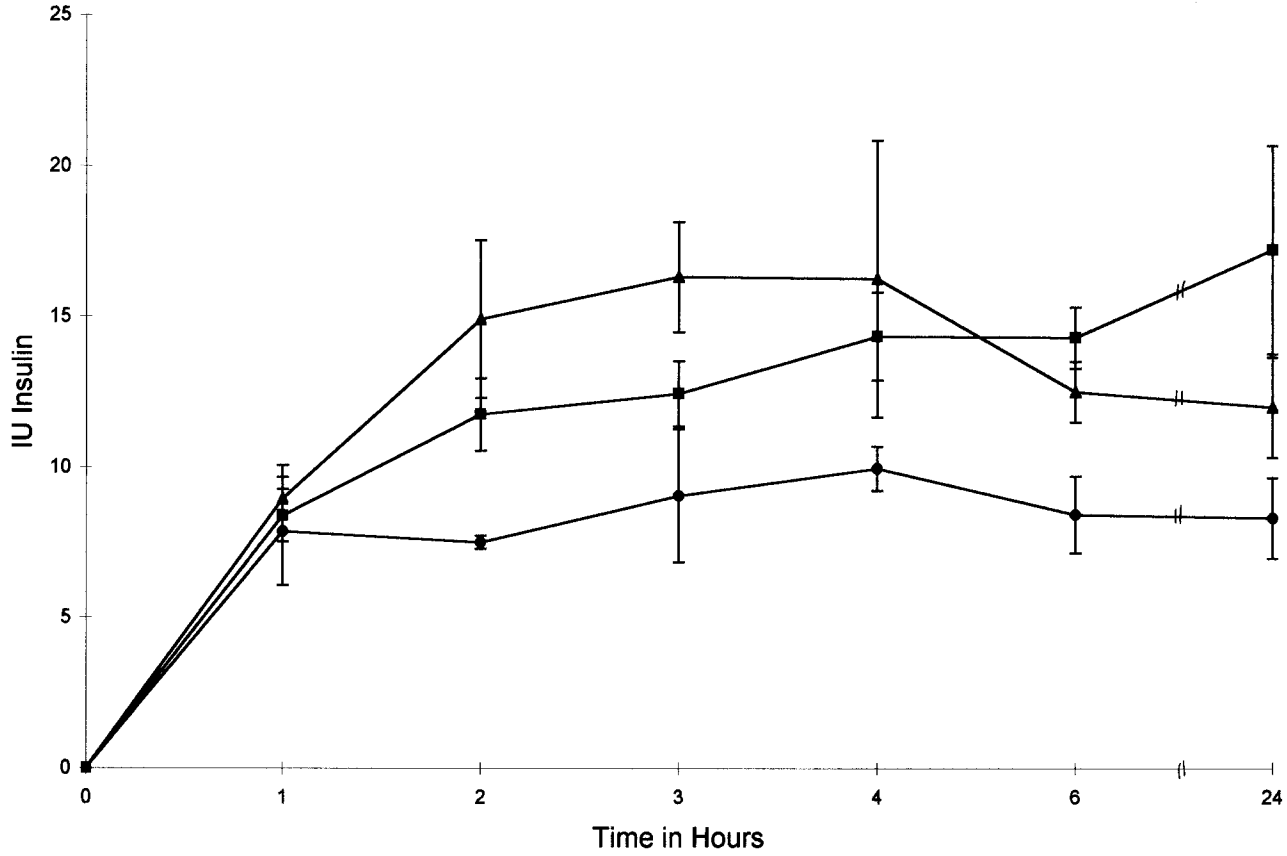

Figure 2 Release profile of Insulin from microspheres formed by mixing 100 mL alginate with 5 mL (200 IU) insulin complexed with different quantities of BCD for 150 min. ■—200 IU complexed with 5 mg BCD (B-5); △—200 IU insulin complexed with 25 mg BCD (B-25); ●—200 IU insulin complexed with 100 mg BCD (B-100).

the process is 200 IU per gram of alginate, the final concentration of the drug goes down to 11 IU. By increasing the efficiency of loading, the quantity of the microspheres for actual application can be reduced.

Alginates are pH sensitive molecules. They shrink in the acid medium and the crosslinked beads swell in the neutral pH. To find out the effect of pH of the crosslinking medium, the pH of the chitosan/calcium chloride solution has been altered. The pH of the crosslinking solution is adjusted to either 4.0, 5.0, or 6.0 for three different experiments. Because there may be loss of insulin from the matrix while drying, the drug content of the microsphere in the wet condition is quantified. Figure 5 shows the concentration of the drug with respect to pH and time. It is observed that both these parameters have profound influence in loading. At a pH of 4, the initial concentration of the drug is low, but increases considerably afterwards. This can be proposed as due to slower crosslinking rate of the alginate by calcium and chitosan at a lower pH. It can be assumed that during completion of homogenization of the matrix by both the cation molecules, they may be trapping the near by molecules of insulin due to their charge. In the second pH of 5, there is only a marginal increase in the drug concentration at higher processing times. In the case of the third pH of 6.0, there is a drop in the concentration after 60 min. This may be due to multiple reasons. At the higher pH, there is partial precipitation of chitosan that provides only a small concentration of the molecule available for crosslinking with alginate. Again, the solubility of alginate is higher at a higher pH. This also weakens the matrix from holding the drug[14] for a longer time.

From these, it is assumed that a lower pH with a longer time of processing can be advantageous. But then other factors like degradation of the drug and *in vivo* absorption also have to be looked into before coming to the final selection for oral application.

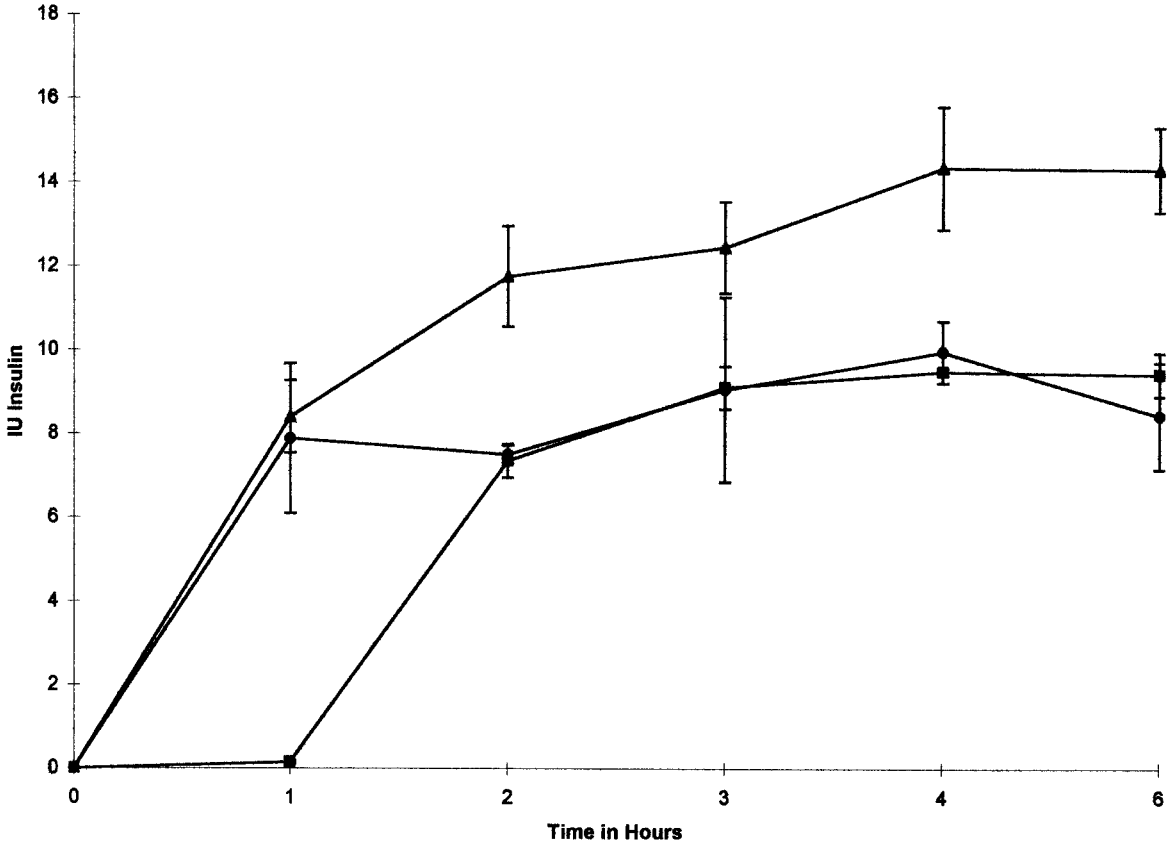

Figure 3 Comparative release pattern of drug from the alginate matrix—200 IU insulin complexed for 20 min with 100 mg BCD and that complexed for 150 min with 5 mg and 100 mg of BCD. ▲—5 mg BCD with Insulin complexed for 150 min (B-5); ●—100 mg BCD with insulin complexed for 150 min (B-100); ■—100 mg BCD complexed with Insulin for 20 min (A-100).

Loading Enhancement by Reducing the Concentration Gradient

Another reason for the poor loading of insulin is the concentration gradient between the drug in the nascent jelly beads, during processing, and that in the surrounding crosslinking medium consisting of chitosan and calcium chloride, before completion of homogenization. It is found that as the drug concentration of the crosslinking medium is increased, the amount of loading is also increased. In our study, after adding 5 mL (200 IU) insulin and dilution, the crosslinking medium has a drug content of 0.7596 IU per mL. Fifty microspheres processed in this have a drug content of 0.2423 ± 0.021 IU per mL after dissolution in SIM and dilution. The microspheres prepared in the crosslinking medium without insulin addition have 0.0867 ± 0.057 IU per mL on dilution with the same amount of SIM and similar dilution. There is about a 275% increase in the amount of insulin loaded in the wet microspheres. This suggests that by decreasing the concentration gradient between the alginate solution and the crosslinking medium, the loading of the drug can be improved. In continuous processing, the drug used up will be that encapsulated inside the microspheres. It also indicates that the system can further accommodate higher amounts of the drug, which can be materialized by using crystalline insulin. It is also observed that aggregation or clumping of the microsphere is less during this process.

CONCLUSION

The release kinetics of the insulin complexed with BCD and encapsulated in the alginate microspheres crosslinked by calcium and chitosan, differ with the complexation time. Insulin complexed with

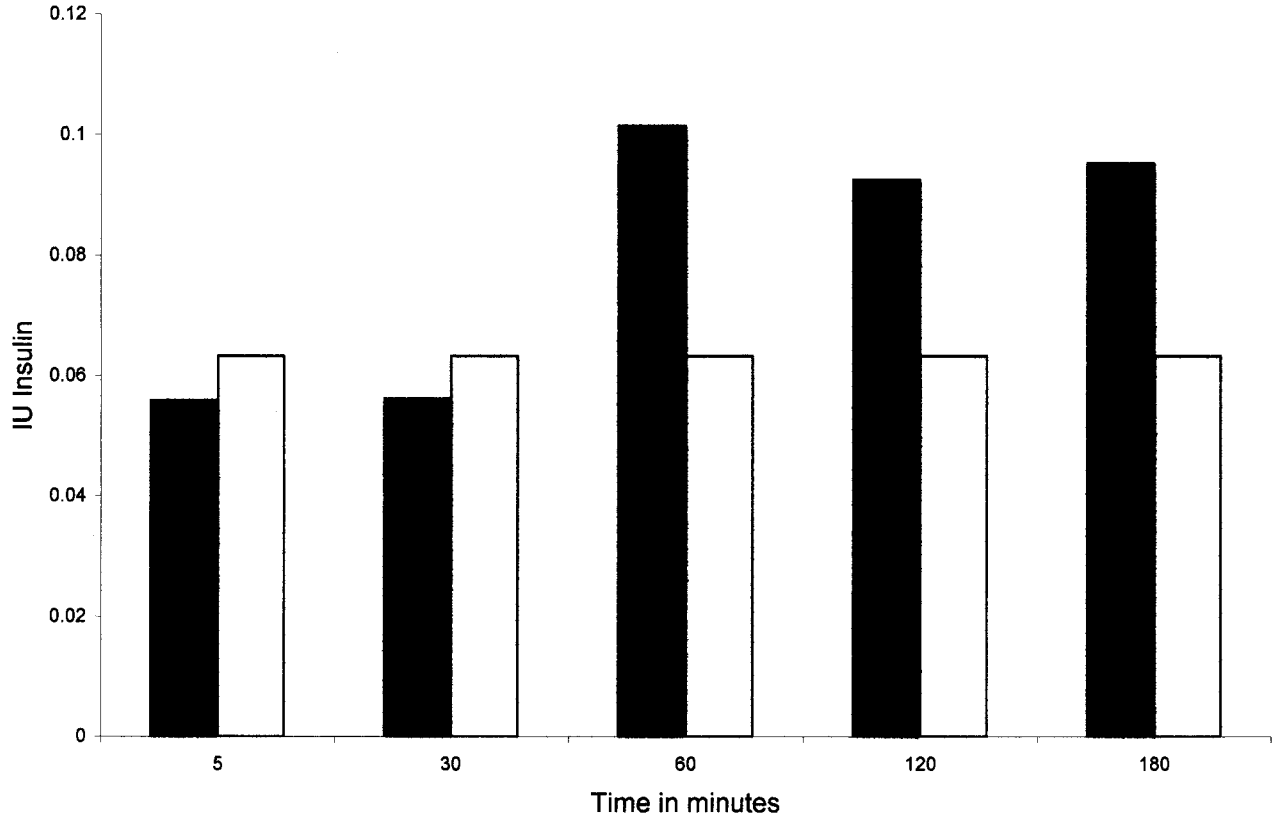

Figure 4 Effect of time of complexation of insulin with BCD. Insulin preparation alone and that complexed with BCD for different time intervals: □—insulin alone; ■—insulin–BCD complexed.

BCD for 20 min behaves like an uncomplexed system in the *in vitro* drug release kinetics. The 150-min complexed system has a faster release. The minimum time of complexation required for faster release of the drug from the matrix is about 1 h. The amount of available insulin can also be increased by complexation with BCD.

The loading of insulin and similar drugs can be increased by lowering the pH of the crosslinking medium. The time of crosslinking process is also a deciding factor in the drug loading. However, for physiological applications, it is advisable to keep the pH closer to the pI of the drug.

There is no necessity for using a higher amount of BCD than the stoichiometric equivalent of insulin. The aggregation of insulin can be reduced at 37°C itself by the use of complexing agents like BCD.

Loading can also be increased by reducing the concentration gradient between the crosslinking medium and the alginate solution containing the drug. This process reduces the microsphere aggregation also.

The different systems available with varied release kinetics may help in tailoring a system suitable for the oral delivery of insulin for the management of diabetes. The controlled release of the drug may prevent extreme conditions of hyperinsulin and hyperglycaemia. The bioavailability may be increased by the complexation with BCD. The absorption can be enhanced by complexation with BCD. It has been suggested that after oral administration, the cyclodextrins undergo degradation in the GI tract, which probably occurs in the colon.[18] Hence, prolonged availability of the drug until reaching the colon may be possible by complexing it with BCD. *In vivo* preliminary studies with 2 g of microspheres containing about 30 IU of insulin fed to diabetic-induced Albino rabbits showed that there is a 30% reduction in the blood glucose level upon oral administration. Such *in vivo* studies are in progress.

More *in vivo* studies are required to ascertain the effectiveness of the system. Size optimization, further improvement of the loading , shelf life of the drug in the system, storage, etc., will have to be studied before an actual system can emerge.

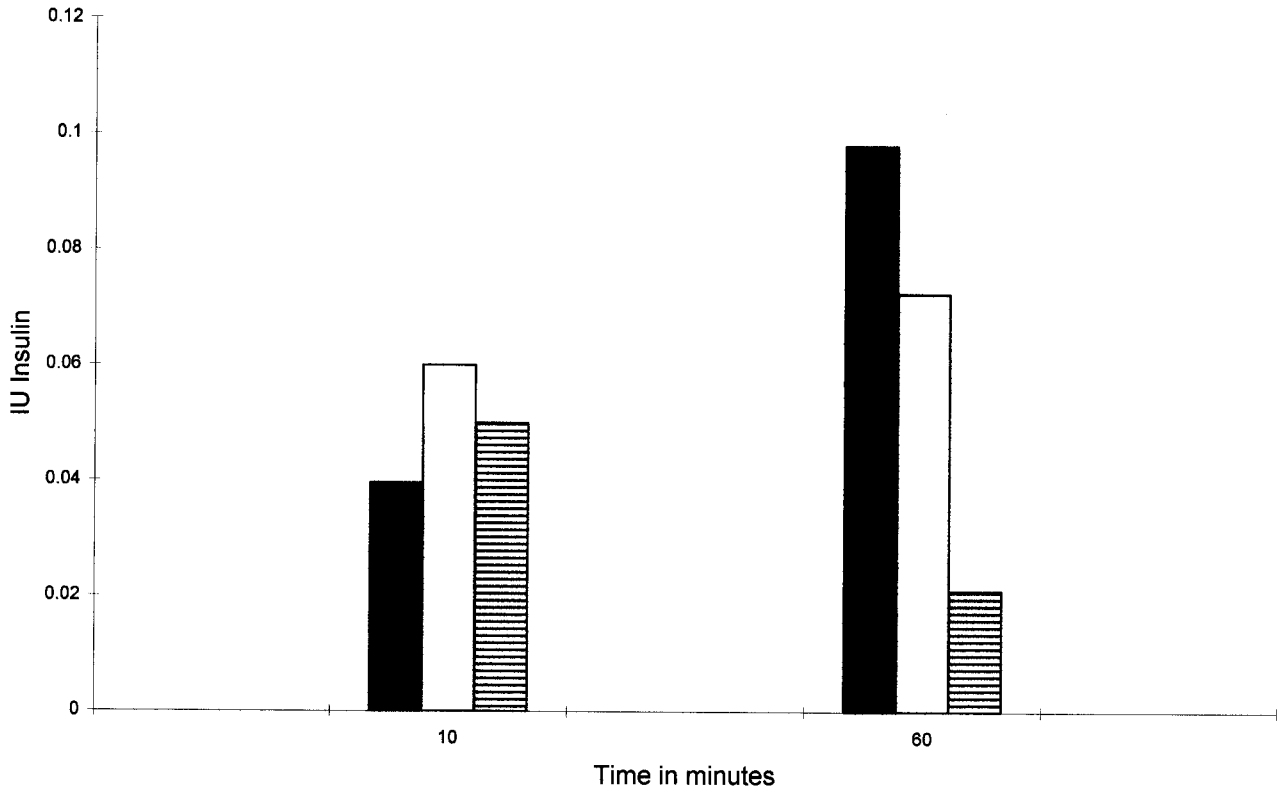

Figure 5 Effect of pH variation of crosslinking medium containing chitosan/calcium chloride and time of crosslinking on loading of Insulin in the alginate matrix: ■—pH 4; □—pH 5; ▤—pH 6.

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