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Preparation and *in vitro* evaluation of Chitosan-Carrageenan, Chitosan-Alginate beads for controlled release of Nateglinide

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ABSTRACT

The aim of the study was to prepare and evaluate beads of chitosan-carregeenan, chitosan-alginate for the controlled release of nateglinide, an oral antidiabetic agent used in the management of Type 2 diabetes mellitus. Preparation of alginate-chitosan beads and hydrogel beads of chitosan-carrageenan was carried out using ionic gelation technique. The beads were evaluated for their properties like particle size distribution, drug loading, swelling behavior etc., Surface characteristics were determined using SEM (Scanning Electron Microscopy). FT-IR and XRD analysis was carried out to determine the chemical compatibility of the polymers with the drug. The polymers alginate and carrageenan showed considerable controlled release in the presence of the polymer chitosan. The ability of these polymers to retard the release of drug can be attributed to gelation medium concentration, the pH of dissolution medium and curing times. The beads were incubated in simulated gastric fluid (SGF) for the first 4 h then the SGF was replaced using simulated intestinal fluid (SIF) and the drug release was observed for the next 20 h. The chitosan coated alginate and carrageenan beads showed a release of 70% and 76% of the drug in the first 6h itself. The beads continued to release the drug for the reminder of the time.

Key words: Carrageenan, Chitosan, Alginate, Controlled release, Ionic gelation, FT-IR and XRD,

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INTRODUCTION

Increasingly natural polysaccharides are being utilized in the research because they exhibit biodegradability, biocompatibility, versatility, and are found abundant in nature. The diversity of natural polysaccharides provides the chemist with a broad spectrum of raw materials that can be used in many biological applications. Chitosan is a natural polysaccharide that possesses

excellent biological properties. Biodegradable, bioadhesive and biocompatible chitosan has emerged as a multifunctional excipient that has been extensively explored for use in several modified release dosage forms including hydrogels, microspheres, wafers, beads/microgranules and transdermal drug delivery systems **[1].**Carrageenan is an anionic polymer extracted from marine red algae. Its structure is a linear heteropolysaccharide with ester sulfate groups. The main chain consists of alternative copolymer of 1, 4-α and 1, 3-β-D-galactopyranose and 3,6-anhydro-D-galactopyranose.Because of its gelling, viscosity enhancing, and proven safety properties, carrageenan can be used as a sustained-release composition **[2].** Alginate is an anionic copolymer of 1,4-linked-β–D mannuronic acid and α-L-guluronic acid residues. In the presence of divalent cations such as calcium, alginate forms a gel due to the stacking of guluronic acid blocks with the formation of "egg-box" calcium-linked junctions **[3,11].** The interaction between alginate and chitosan had been systematically investigated[4]. Their polyelectrolyte complex has been widely used to obtain devices for the controlled release of drugs **[5,6,7,8,9].** Employing these polymers using ionic gelation technique we have tried to produce beads encapsulating the drug, nateglinide.

Nateglinide is an oral anti diabetic agent used in the management of type 2 diabetes mellitus. Nateglinide, (-)-N-[(trans-4-isopropylcyclohexane) carbonyl]-D- phenylalanine , is structurally unrelated to the oral sulfonylurea insulin secretagogues. Nateglinide is the drug of interest because it has poor biopharmaceutical properties like the half life ($t_{1/2}$), 1.5 h.Following oral administration immediately prior to a meal, nateglinide is rapidly absorbed with mean peak plasma drug concentrations (Cmax) generally occurring within 1 h (Tmax).There by extending the release of the drug might help reduce the maintenance dose of 120 mg thrice daily before meals and also might help improve the bioavailability of the drug.

The present study aims at formulating and evaluating novel polymeric mixtures that can be used efficiently in preparing beads encapsulated with nateglinide. Furthermore the study tries to explain the effect of other parameters like curing times, gelation medium concentration and pH of the surrounding environment in the formation of the beads and release of the drug.

MATERIALS AND METHODS

Materials

The following materials were used in the present study. Chitosan (Deacylation degree 77%, High molecular weight, Sigma Aldrich) ; Sodium Alginate (Grade LF 240 D, is a gift sample from M/s.Aurobindo Pharma, Hyderabad); Carrageenan (Grade 812, is a gift sample from M/s . Aurobindo Pharma, Hyderabad); Nateglinide (Molecular weight 317.43 is a gift sample from M/s. Aurobindo Pharma , Hyderabad) ; Acetic acid (SD Fine Chem., Mumbai, India) ; Sodium tripolyphosphate (Sigma Aldrich.; Ethanol (Shymlakhs International, New Bond Street, London); Calcium Chloride (Rankem, Ranbaxy Fine Chem, New Delhi, India); Sodium Dihydrogen phosphate (Qualigens Fine Chemicals Ltd., Mumbai, India); Sodium Hydroxide (SD Fine Chem., Mumbai, India) ; Dichloromethane (SD Fine Chem, Mumbai, India).

Preparation of alginate-chitosan beads

The preparation of beads was carried out by ionotropic gelation technique following the method adopted by Yongmei Xu et al., and Catarina M. Silva et al., The blend solution containing

sodium alginate and chitosan was prepared with various mass proportions. Firstly a certain amount of sodium alginate was dissolved in 30 mL distilled water at 40 °C under mechanical stirring for 5 min; chitosan powder was added into the solution and mixed homogeneously. Then chitosan was dissolved by adding 0.3 ml acetic acid into the mixture; pH was adjusted to 5.0 by NaOH (0.1 mol/L) solution; homogeneous blend solution of two polymers was formed under stirring at 40°C. Then the blend was added with various ratios of nateglinide solution which was previously dissolved in dichloromethane. Lastly the blend solution was dripped through a 18 gauge injection needle into the 100 mL solution of calcium chloride; beads were formed under mechanical stirring , beads were then washed with distilled water three times and dried under vacuum at room temperature for 24-48hrs **[2, 10]**.

Preparation of hydrogel beads with various ratios of chitosan: carrageenen

The preparation of these beads was carried out by adopting the method followed by Nongnuj Muangsin *et al*., A solution of 2.5% vol/vol carrageenen was prepared by dissolving carrageenan in deionized water at 70±5°C, a constant 1% (wt/vol) of nateglinide was added after the drug was thoroughly dissolved in a mixture of alcohol: water (1:1) which was added to the carrageenan solution. A solution of chitosan 2.0% (wt/vol) in 2% (vol/vol) acetic acid was added to the mixture of carrageenan and nateglinide solution. The mixture was further stirred until becoming homogenous.

The mixture was extruded in the form of droplets, using a 18 gauge needle, into 100 mL of 0.3% KCl/5.0 %(wt/vol) NaOH as coagulant solution. The formulation of chitosan solution and carrageenan solution were extruded into 5% NaOH solution and 0.3% KCl solution, respectively. The solutions were maintained at 10^{o}C for 5 h to let the beads harden. Then, the beads were filtered and washed with cold deionized water to remove excess of NaOH and potassium ion. Finally, the hydrogel beads were freeze dried at -42°C for 24 h [11].

RESULTS

Evaluation of beads:

Observation under scanning electron microscopy

The surface morphology and appeareance of randomly selected beads of chitosan-carrageenan, chitosan-alginate and carrageenan-alginate were examined by scanning electron microscopy. HITACHI S300 N microscope (Made in Japan) was employed for this purpose. The micrographs revealed detailed surface morphology. Scanning electron micrographs of nateglinde loaded beads revealed the following details. The shape of the drug loaded alginate-chitosan beads was spherical whereas the carrageenan beads were tear shaped.

Swelling study

The swelling behavior study of the bead was performed in 3 dissolution systems.

1. 0.1N HCl (pH 1.2);
2. Phosphate buffer saline pH 7.4; and
3. The pH-alternating system. **[12, 13].**

Samples of beads of known weight (10 mg) were placed in a petridish containing 20 mL of swelling medium and allowed to swell at room temperature. The swollen beads were periodically (every 1 h) removed and weighed. The wet weight of the swollen beads was determined by

blotting them with filter paper to remove moisture adhering on the surface, immediately followed by weighing on an electronic balance. The swelling ratio of the beads was calculated from the formula given below.**[14]**

$$\text{Swelling Ratio} = W_t / W_o$$

Where W_t is the weight of the beads at the defined time and W_o is the initial weight of the beads. All the experiments were performed in triplicate.

Loading efficiency

Accurately weighed amount (10 mg) of drug loaded beads were pulverized using mortar and pestle and incubated in 10 mL 0.02 mol phosphate buffer (pH 6.8) at room temperature for 24 h for complete digestion and the drug was extracted with ethanol (99% v/v). The solution was filtered through a filter disc and the filtrate was assayed spectrophotometrically for drug content at 210 nm. The same method was employed to prepare the blank. All the experiments were performed in triplicate and then the percentage of drug loading and incorporation efficiency was calculated using the following formula:

$$\text{Drug Loading (EDL) in \%} = L/L_o \times 100$$

Where L is the actual drug content in the weighed quantity of the beads and L_o is the weighed quantity of beads.

FT-IR Spectroscopic analysis

FT-IR spectroscopic analysis of nateglinide, sodium alginate, chitosan, carrageenan and physical mixtures of nateglinide with the same polymers individually were performed. Individual beads were analyzed by adapting the procedure given below.

Individual beads were crushed in a mortar and pestle. The crushed material was mixed with potassium bromide in 1:100 proportions and dried at 40°C. The mixture was compressed to a 12 mm semitransparent disk by applying a pressure of 10 tons for 2 min. The FT-IR spectra over the wavelength range 4000 to 400 cm^{-1} was recorded using a FT-IR spectrometer. For this purpose Bench type Thermo Nicolet Nexus 670 spectrometer was employed with a resolution of 4 cm^{-1}, detector type – DTGSKBr, beam splitter – KBr.

X-ray powder diffraction analysis

X-ray powder diffraction (XRD) analysis of the samples of nateglinide, sodium alginate, chitosan and physical mixtures of nateglinide with various polymers was carried out. For this purpose diffractometer Made, Brooker, Model AXS D_8 Advance, Made in Germany was employed. The analyses was carried out at 40Kv/30mA over a range of 2-60 2θ, using Cu Kα radiation wavelength 1.5406 A^o. Step time was 0.011^o-10.3S.

Invitro drug release studies

Invitro release of nateglinide from beads was carried out using ELECTROLAB TDT 08L 8 basket dissolution apparatus.The nateglinide release study of the beads from each formulation was performed in the simulated gastrointestinal condition by the pH-change method at 37

°C.[12,13]he media of pH 1.2 (0.1N HCl) was chosen to represent the gastric condition; pH 6.6 was a compromise condition between the pH of the gastric and the small intestine, and the condition in the small intestine was represented by pH 7.4. 100 milligrams of beads were enclosed in a teabag and placed into a beaker that contained 250mL of the dissolution medium. The beaker was placed on a horizontal shaking water bath at a speed of 50 rpm and incubated at 37±2°C. In the dissolution model with pH-change, the pH of the dissolution medium was kept at 0.1N HCl (pH 1.2) (SGF) for the first 4 h (at 30 min time intervals). Then, the dissolution medium was changed to phosphate buffer saline pH 6.6 for 1 h (at 15 min time intervals). At different time intervals, 5 mL of the dissolution medium was withdrawn. Finally, the release dissolution medium was changed to pH 7.4 and maintained up to 24 hours. In this solution, at various time intervals, 2 mL of the dissolution medium was withdrawn. Each sample solution was centrifuged and diluted to a suitable concentration if necessary. The release rate of nateglinide was assayed by UV-Vis spectrophotometry at 210 nm. All experiments were performed in triplicate. The amount of nateglinide released was calculated by interpolation from a calibration curve containing increasing concentrations of nateglinide. A cumulative percentage correction was made for the previously removed sample to determine the total amount of drug release.The sample was filtered through filter disc during each with drawl and analyzed for drug content at 210 nm on a spectrophotometer (UV-Vis Perkin-Elmer Spectrophotometer). The results are shown as graphical plots.

DISCUSSION

Study of the effect of coagulant conditions on surface morphology

The effect of various coagulant conditions on the surface morphology of beads was studied. Increase in concentration of gelation medium increases the porosity of the formed chitosan-alginate beads. But the increase in porosity was observed till 5% conc of the gelation medium only. Any further increase from this concentration, the bead surface became smooth and non porous. This may be of disadvantage as porosity of the beads is responsible for the effective effective entry of the medium into the inner crevices of the bead.

Similarly the chitosan carrageenan beads showed increase in porosity upto a concentration of 3% NaOH/0.2% KCl.After this concentration the surface of the beads showed no porosity and became very non porous.

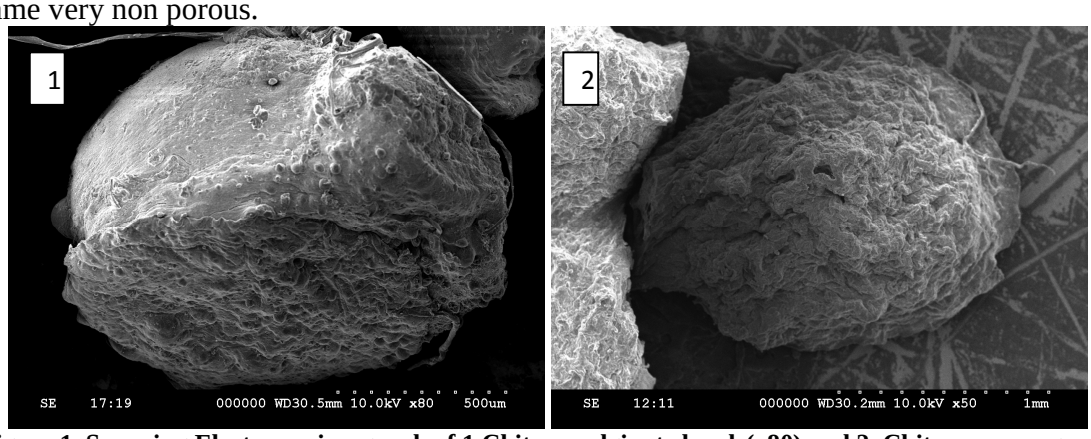

Figure 1. Scanning Electron micrograph of 1.Chitosan alginate beads(x80) and 2. Chitosan carrageenan beads(x50). The chitosan alginate beads showed ovoid shape whereas the chitosan-carrageenan beads showed a tear shape

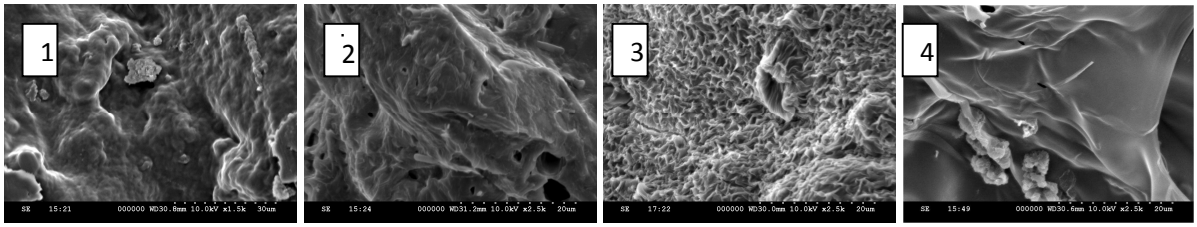

Figure 2.Surface scanning electron micrographs (x2.5k) of chitosan-alginate beads showing an increase in porosity as the concentration of gelation medium increases.1. Surface of the chitosan alginate at 1% gelation medium, 2. Surface at 3% gelation medium, 3. Surface at 5% gelation medium, 4. Surface at 7% gelation medium

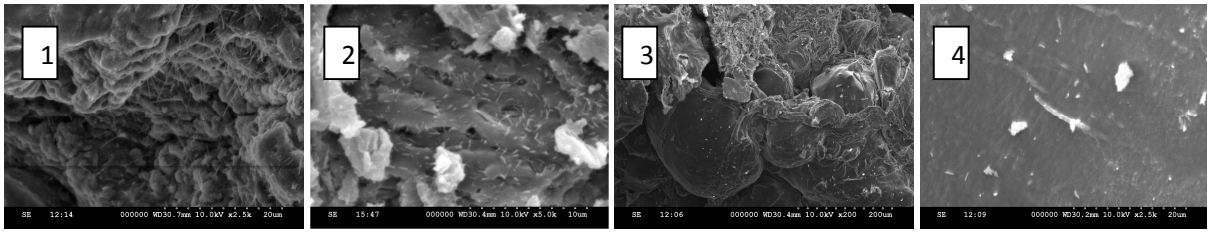

Figure 3. Surface scanning electron micrographs (x2.5k) of chitosan-carrageenan beads showing an increase in porosity as the concentration of gelation medium increases. 1. Surface of a chitosan- carrageenan bead at 1% gelation medium, 2. Surface at 3% gelation medium, 3. Surface at 5% gelation medium, 4. Surface at 7% gelation medium

So, optimum concentrations of gelation medium are necessary for the porous characteristics of the beads and subsequent release of the drug depends on these concentrations.

Swelling behavior

The swelling ratio of the beads in 0.1N HCl (pH 1.2), phosphate buffer saline pH 7.4, and the pH-alternating system was not significantly changed. The beads were not significantly swollen and eroded in the 3 dissolution systems. Thus, from these results, it could be assumed that the drug release was not under the control of the swelling behavior but rather was controlled by the dissolution of nateglinide in the dissolution medium and diffusion of nateglinide through polymer matrix.

Entrapment efficiency

The percentages of entrapment efficiency (%EE) of the nateglinide-loaded beads prepared from various compositions are shown in Table 1. The %EE was obtained in the range of 65% to 97%. Chitosan-alginate beads gave the highest %EE of 96.9%. The chitosan/carrageenan beads showed the %EE to be around 84.7% to 89.7%, which was higher than the pure chitosan bead (Formulation A, 79.6%). The results might indicate that the $–SO_4^{2-}$ group of carrageenan exhibited electrostatic interactions with the $–NH^{3+}$ group of chitosan in the PEC formation, resulting in the drug entrapped in PEC, forming a better encapsulation than the pure chitosan bead. For few of the formulations, the %EE of the beads could not be determined because the obtained beads were fragile, and the shape was not well formed, hence they were broken into pieces after being dried. The %EE was increased from 84.7% to 96.9% when the nateglinide content was increased from 1% to 2% (wt/vol). It was because the drug was better entrapped in the viscous hydrogel. But when the drug content was increased from 3% to 5% (wt/vol), the %EE was decreased from 76.4% to 89.3%. This is probably because the excess drug could not be entrapped in the beads.

The loading efficiency can be determined by comparing these values with the standard curve. The standard curve was prepared by taking the absorbances of increasing concentrations of nateglinide at 210nm.

Table 1: Gelation medium compositions, loading effiencies of various formulations

FORMULATION	GELATION MEDIUM	TEMPERATURE	LOADING EFFICIENCY (%LE±S.D)
Chitosan beads	Tripolyphosphate sodium (5%)	Room temperature (RT)	79.6±0.5
Alginate beads	Calcium chloride (2%)	(RT)	67.3±0.4
Chitosan-alginate beads(AB_1)	Tripolyphosphate sodium (5%)	(RT)	96.9±0.3
Chitosan-carrageenan beads(AC_1)	NaOH (5%)/ Kcl (0.3%)	(RT)	89.7±0.3

LE:- Loading Efficiency; **S.D.:- Std Deviation**

FT-IR Spectroscopy

FTIR of Nateglinide shows the principle peaks at the wave numbers of 1213-1386 cm^{-1} justifying the presence of carboxyl, carboxylate groups, and carbonyl at 1646 cm^{-1}, C-H stretching between at 2857-3030 cm^{-1}, C=O vibration at 1,723 and NH stretching appeared at 3296 cm^{-1}.

In the FTIR spectrum of sodium alginate powder shows various distinct peaks of alginate are evident from hydroxyl groups appeared at 3,428 cm^{-1}, carbonyl at 1,623 cm^{-1}, and carboxyl and carboxylate groups appeared between 1,000 to1,400 cm^{-1}. The absorption band around 2933, 1623, 1419 and 1031 cm^{-1} corresponds to the stretching of –CH, COO-, -CH and C-O-C, respectively. In the FTIR spectrum of drug loaded alginate-chitosan bead formulation the band 2876 cm^{-1} corresponding to chitosan pure was shifted to 2932 cm^{-1} indicates the confirmation of complex formation between chitosan and alginate.

The IR spectrum of chitosan/carrageenan beads showed an absorption band at 1447 cm^{-1} which is corresponds to $–NH^{3+}$ group. A decreased in intensity of $–SO_4^{2–}$ group absorption band at 1018 cm^{-1} is the evidence of the forming of strong polyelectrolyte complex (PEC).

The FTIR Spectrum of various physical mixtures was also subjected to analyses and from the spectrum the effect of various polymers on nateglinide can be studied. The encapsulation can also be determined from the FTIR data in figure 4. Loss of crucial peaks from the spectrograph is indicative of the encapsulation of the nateglinide.

X-ray diffraction analysis

The XRD pattern of nateglinide showed the diffractogram of a crystalline product, and the XRD profile of sodium alginate and carrageenan indicated the presence of a completely amorphous material. Chitosan on the other hand showed few crystalline peaks with very low intensity. Difference in XRD patterns was observed in various physical mixtures as shown in figure 5. This was possibly due to the fact that a decrease in the degree of crystallainity of the drug that might have occurred when the drug is well dispersed in the polymer matrix.

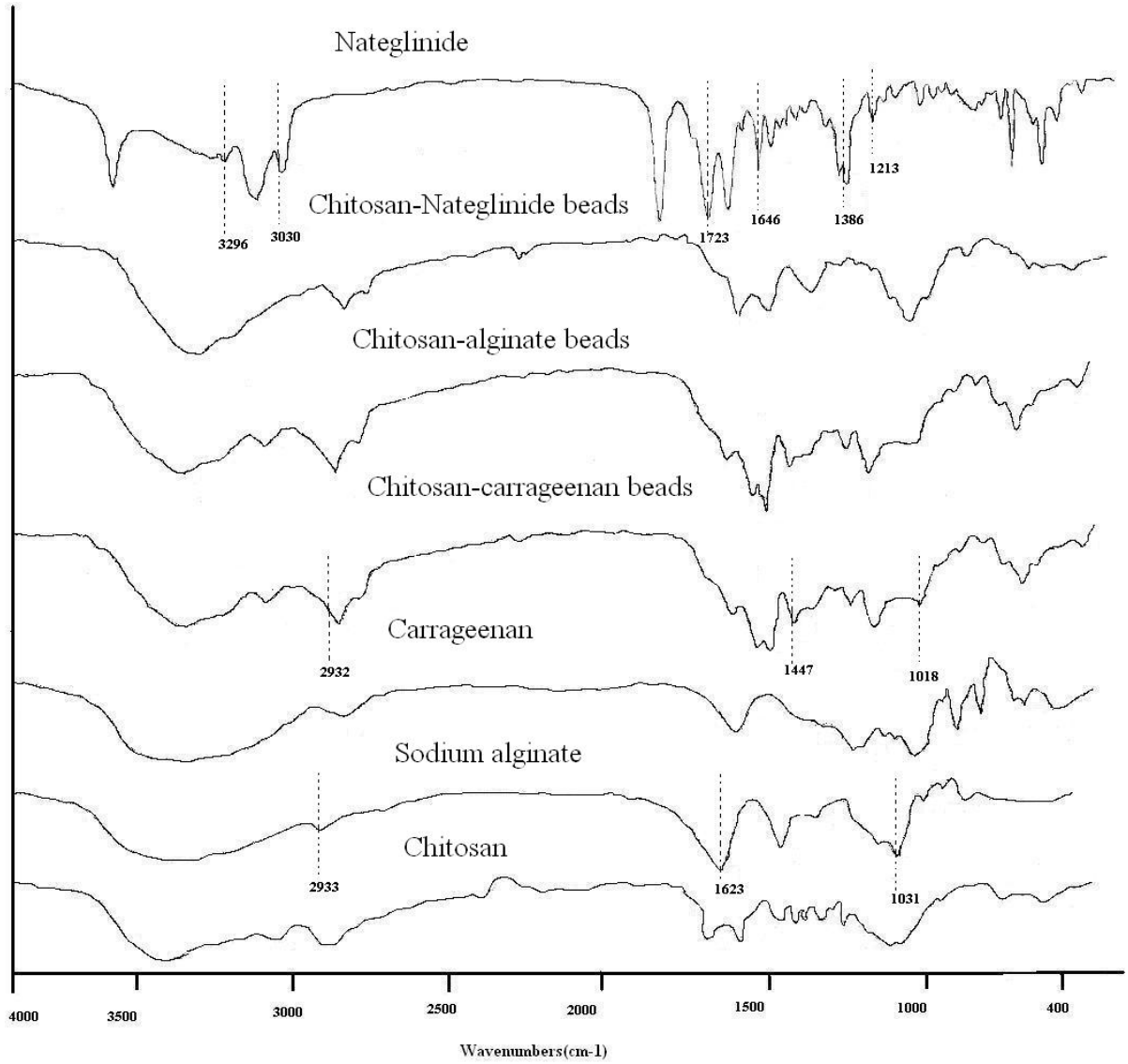

Figure 4. Fourier transform infrared spectroscopy of beads and individual components of beads: (A) Nateglinide; (B) Chitosan- nateglinide beads; (C) Chitosan-alginate beads; (D) Carrageenan; (E) Sodium alginate; (F) Chitosan.

Invitro drug release studies

Comparing the nateglinide release from drug loaded alginate-chitosan and chitosan-carrageenan; one should expect that the chitosan treatment would lessen the release profile of the drug. Apart from the effect of chitosan concentration, the factors such as gelation medium concentration, curing times and pH of the surrounding medium affect the release rate of the drug. Beads prepared with 5 % (w/v) calcium chloride showed increase in the release rate than the beads prepared with 2 % (w/v) calcium chloride; this may be due to increase in the porosity and swelling ratio of the beads with increase in concentration of the calcium chloride.

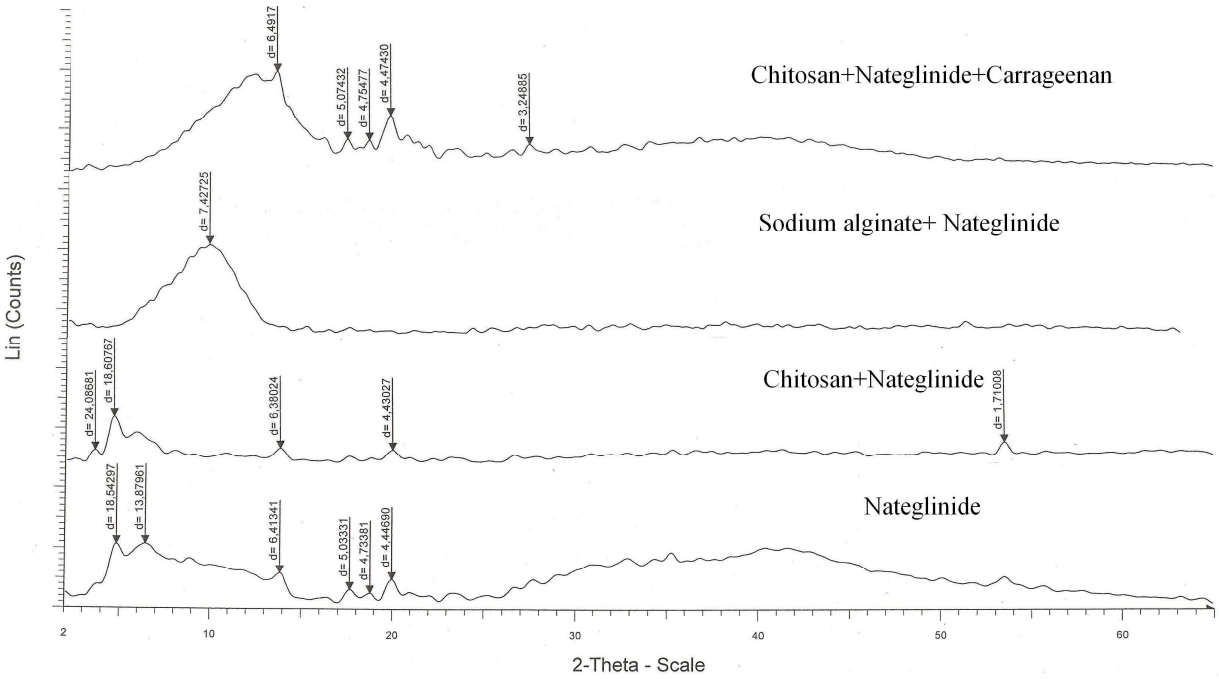

Figure 5. The X-ray diffraction patterns of polymers with nateglinide. The nateglinide crystalline peaks at the bottom of the graph disappear as the nateglinide is mixed with the polymers, chitosan, carrageenan and sodium alginate.

The degradation rate of the beads depended on the pH of the test medium. In acidic medium i.e., 0.1N HCl, pH 1.2 the degradation was found to be negligible at pH 7.4. The faster degradation may be attributed to the solubility of chitosan matrix which is soluble in acidic environment.

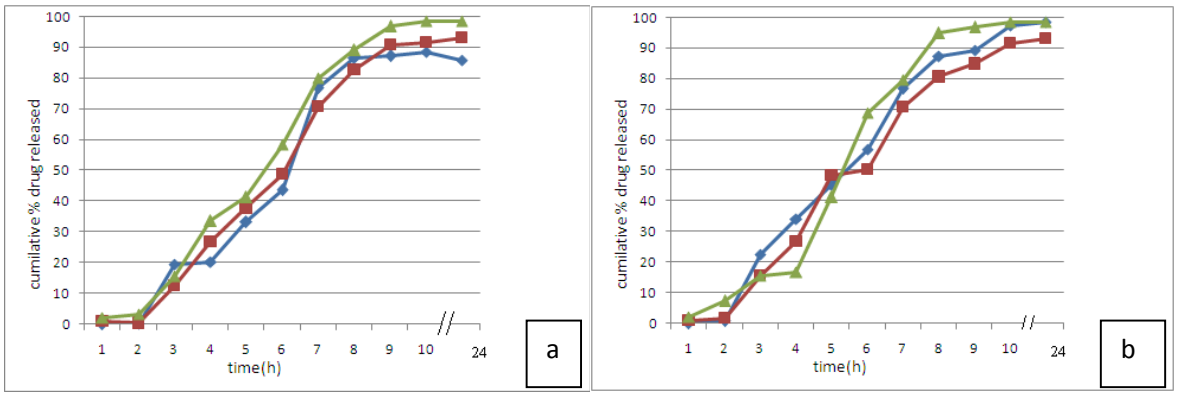

Figure 6. Effect of pH of dissolution medium on invitro nateglinide release. The above graphs show drug release profiles of (chitosan beads), (chitosan-alginate beads), (chitosan-carrageenan beads). In (a) SGF (0.1N HCl, pH 1.2), (b) SIF (pH 7.4)

The release of nateglinide is shown in figure 6. The amount of drug released at pH 1.2 was slightly higher in comparison to pH 7.4. This pattern of release is indicative of the pH sensitivity of the beads. In 4 h around 40-50% of the drug was released and by the end of 6 h there is a 70% release of drug. In SIF the beads showed slow drug release this may be due to electrostatic

attractive interactions between the polymer and anions. These attractive forces harden the beads and may contribute to the controlled release of nateglinide from the bead matrix.

CONCLUSION

In conclusion, this study shows that the drug loading, swelling ability and release characteristics of nateglinide loaded calcium alginate-chitosan beads and beads of carrageenan-chitosan is dependent on the presence of the polyelectrolyte complex between alginate and chitosan; carrageenan and chitosan. Gelation medium concentration, the pH of dissolution medium and curing times has a profound effect on the overall behavior of the beads. The FTIR studies revealed that no drug polymer interaction occurred during the preparation of the formulations. XRD studies revealed that the crystalline peaks of the drug nateglinide significantly disappeared in the bead formulations and this is indicative that the drug is well dispersed in the polymer matrix at molecular level. In vitro release study revealed the drug release of alginate beads could be reduced considerably by treating mixing alganite, carrageenan with chitosan; carrageenan and coating of the beads prolonged the release of nateglinide to a little extent.

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