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Dual crosslinked pectin–alginate network as sustained release hydrophilic matrix for repaglinide

Rajendra Awasthi*¹, Giriraj T Kulkarni², Malipeddi Venkata Ramana³, Terezinha de Jesus Andreoli Pinto⁴, Irene Satiko Kikuchi⁴, Daniela Dal Molim Ghisleni⁴, Marina de Souza Braga⁴, Paul De Bank⁵, Kamal Dua^{6,7}

¹NKBR College of Pharmacy & Research Centre, Meerut – Hapur Road, Meerut – 245 206, Uttar Pradesh, India

²Amity Institute of Pharmacy, Amity University, Sec 125, Noida – 201303, Uttar Pradesh, India

³Amity Institute of Pharmacy, Amity University, Lucknow – 226028, Uttar Pradesh, India

⁴Department of Pharmacy, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, Rua Professor Lineu Prestes 05508–000, Brazil

⁵Department of Pharmacy and Pharmacology, The University of Bath, Claverton Down Bath, BA2 7AY, United Kingdom

⁶School of Pharmaceutical Sciences, Shoolini University, Solan, Himachal Pradesh 173229, India

⁷School of Pharmacy and Biomedical Sciences, The University of Newcastle, Newcastle, Callaghan NSW 2308, Australia

*Address correspondence to this author at the

Professor & Principal, NKBR College of Pharmacy & Research Centre,

Meerut – Hapur Road, Meerut – 245 206, Uttar Pradesh, India

Tel: +91–9459234530; E–mail: awasthi02@gmail.com

ABSTRACT

Repaglinide, an oral antidiabetic agent, has a rapid onset of action and short half-life of approximately 1 h. Developing a controlled and prolonged release delivery system is required to maintain its therapeutic plasma concentration and to eliminate its adverse effects particularly hypoglycemia. The present study aimed to develop controlled release repaglinide loaded beads using sodium alginate and pectin with dual cross-linking for effective control of drug release. The prepared beads were characterized for size, percentage drug entrapment efficiency, *in vitro* drug release and the morphological examination using scanning electron microscope. For the comparative study, the release profile of a marketed conventional tablet of repaglinide (Prandin[®] tablets 2 mg, Novo Nordisk) was determined by the same procedure as followed for beads. The particle size of beads was in the range of 698 ± 2.34 to 769 ± 1.43 μm . The drug entrapment efficiency varied between 55.24 ± 4.61 to $82.29 \pm 3.42\%$. The FTIR results suggest that there was no interaction between repaglinide and excipients. The XRD and DSC results suggest partial molecular dispersion and amorphization of the drug throughout the system. These results suggest that repaglinide did not dissolve completely in the polymer composition and seems not to be involved in the cross-linking reaction. The percent drug release was decreased with higher polymer concentrations. In conclusion, the developed beads could enhance drug entrapment efficiency, prolong the drug release and enhance bioavailability for better control of diabetes.

Keywords: Crosslinking method, crosslinked beads, repaglinide, dual crosslinking, sustained release

1. INTRODUCTION

During the last few decades, considerable efforts have been made to develop new pharmaceutically viable and therapeutically effective controlled drug delivery systems [1]. Attention has been centered particularly on orally administered controlled drug delivery systems because of the economy, ease of administration and manufacture. Bioavailability of an orally administered active moiety is the major limitation. To avoid premature drug elimination, various oral controlled release formulations have been investigated [2]. An important requisite for the successful performance of oral controlled drug delivery system is that the drug should have good absorption throughout the gastrointestinal tract [3]. The success of oral controlled release drug delivery systems is limited to drugs having site specific absorption. Moreover, the therapeutic window of many drugs is limited due to their short circulating half-life and absorption. Such pharmacokinetic limitations lead to frequent drug administration to achieve the required therapeutic effect and may cause patient non-compliance [4].

The oral antidiabetic agent repaglinide, a low water soluble (34 $\mu\text{g/ml}$ at 37°C) and highly lipophilic ($\log P = 3.97$) drug, is chemically unrelated to the oral sulfonylurea insulin secretagogues and belongs to class II of Biopharmaceutics Classification System (relatively low oral bioavailability $\approx 56\%$) (Figure 1). The elimination half-life of repaglinide is 1–1.4 h [5]. Repaglinide, a low dose drug, is administered immediately before meals to reduce the post-prandial blood glucose level by lowering blood glucose levels *via* stimulation of insulin release from the pancreas [6], an action dependent upon functioning β cells in the pancreatic islets. Repaglinide is a good choice for diabetic patients with damaged kidney function because the drug is excreted almost entirely *via* bile [7].

Developing a controlled and prolonged release delivery system is required to maintain its therapeutic plasma concentration and to eliminate its adverse effects, particularly hypoglycemia. Over the past several years, numerous attempts have been made to investigate repaglinide containing formulations such as a bioadhesive buccal drug delivery system [8], gastroretentive drug delivery systems [9, 10], transdermal system [11] and matrix systems [12, 13]. Smaller surface area and low flux results in low drug bioavailability from the buccal route. Most gastroretentive drug delivery systems are gas generating dosage forms, which have in common the risk of causing alkaline microenvironment. For the successfully traverse of drug through the stratum corneum, a drug molecule should have a molecular weight less than 500 Da and a log P (octanol/water) between 1 to 3. To overcome these limitations of the above investigations, it is hypothesized that the hydrophilic polymer based crosslinked particulate delivery system might be promising drug delivery systems. Particulate drug carriers such as beads have the advantages that they pass uniformly through the gastrointestinal tract. It is evident that particulate drug delivery systems have a more predictable release profile.

The present study aimed to develop controlled release repaglinide loaded beads using a hydrophilic polymer matrix containing a mixture of two polysaccharides. Sodium alginate, a linear polysaccharide isolated from seaweed, is composed of oxidized sugar units joined together to form an ionic polymer. The presence of negatively charged $-\text{COO}^-$ side chains and $-\text{OH}$ groups make this natural polysaccharide extremely hydrophilic (Figure 2a). Replacing the sodium ions of sodium alginate with calcium ions leads to cross-linking between the polymer chains and lead to the formation of an insoluble gel *i.e.* calcium alginate (Figure 2b) [14]. Various pharmaceutical and biomedical applications of alginate are reported due to its biodegradable, biocompatible and mucoadhesive nature, and its ability to form a hydrogel in

the presence of multivalent cations. However, single crosslinked calcium alginate beads are fragile in nature and may undergo a premature drug release [15, 16]. Similar to alginate, low methoxylated pectin (degree of esterification <50), a nontoxic linear polysaccharide extracted from citrus peels or apple, can form a rigid and stable gel upon crosslinking with calcium ions [17].

Pectin is resistant to enzymes present in the stomach and intestine, but completely degraded by the colonic bacterial enzymes. Pectin has freely available –COOH and –OH functional groups (Figure 2c) which are acting as sites to cross link with calcium ions and produce Ca²⁺ ions cross linked pectin (Figure 2d) [18]. Because of the cross-linking property of pectin with calcium ions, it is expected to increase the efficiency of drug encapsulation in an pectin–alginate matrix. However, the hydrophilic and swelling nature of pectin may also cause premature drug release from the delivery device.

To overcome the potential limitations of single crosslinked matrices, this study investigated the effects of a dual crosslinking strategy using calcium ions and epichlorhydrin (a bifunctional alkylating agent), widely used as crosslinking agent used in the preparation of drug delivery systems containing polysaccharides [19-21] in order to control the release of repaglinide for extended time periods. Theoretical schematic representations of the cross-linking reaction between pectin and epichlorohydrin is presented in Figure 3.

2. MATERIALS AND METHODS

2.1. Materials

Repaglinide and HPMC K4M were received as a kind gift sample from Dr Reddy's Laboratory, Hyderabad, India. Pectin low methoxyl (degree of esterification = 28 – 45%,

molecular weight = 86,000 and Jelly grade = 100) was purchased from Krishna Pectins Pvt. Ltd., Jalgaon, Maharashtra, India. Sodium alginate (viscosity of 1% aqueous solution = 550 mPa.s, mannuronate/glucuronate ratio = 0.7 and molecular weight = 216.121) was purchased from S.D. Fines, Mumbai, India. Epichlorhydrin and calcium chloride were purchased from Sigma Chem. Ltd., New Delhi, India.

2.2. Methods

2.2.1. Preparation of single crosslinked alginate beads

Sodium alginate based cross-linked repaglinide loaded beads (formulation RA) were prepared by the ionotropic gelation method. Briefly, sodium alginate solution was prepared in 100 ml fresh distilled water and repaglinide was added with constant stirring (Table 1). The stirring was continued for 1 h and this mixture was subjected to ultrasonication for a period of 30 min to remove the air bubbles. The resultant mixture was dropped into 100 ml calcium chloride solution (2% w/v) using 24G syringe needle from a height of 5 cm. The beads were collected, washed twice with distilled water to remove excess calcium chloride from the bead surface and air dried overnight at 30°C [23].

2.2.2. Preparation of single crosslinked pectin–alginate beads

A polymer solution containing sodium alginate in combination with low methoxylated pectin was prepared in 100 ml fresh distilled water (Table 1) [24, 25]. Pectin–alginate based single cross-linked repaglinide loaded beads (formulation RAP₁ – RAP₃) were prepared by the ionotropic gelation method as described above.

2.2.3. Preparation of dual crosslinked pectin–alginate beads

For the preparation of dual crosslinked beads (formulation RAP₄ – RAP₆), single crosslinked

beads were immediately transferred to a beaker containing 50 ml of epichlorhydrin while the beads stayed in a transitional, semisolid state (Table 1). The beads were collected and air dried overnight at 30 °C. Figure 4 represents an overview of the production process of dual crosslinked pectin–alginate beads.

2.3. Bead Characterization

2.3.1. Determination of percentage yield and percentage drug incorporation efficiency

Dried, drug loaded beads (50 mg) were powdered and dispersed into 50 ml methanol and shaken at $37 \pm 0.5^\circ\text{C}$ for 24 h. The mixture was then filtered using Whatman filter paper and the filtrate analyzed for the repaglinide content using a UV–spectrophotometer (UV 3000⁺, LabIndia Instruments, Mumbai, India) at 243 nm in comparison to a calibration curve after suitable dilution. The percentage yield and percentage drug incorporation efficiency were calculated using equations 1 and 2, respectively:

$$\text{Yield (\%)} = \frac{\text{Mass of dry beads}}{\text{Mass of drug and polymers}} \times 100 \quad (1)$$

$$\text{Drug incorporation efficiency (\%)} = \frac{\text{Actual mass of drug in beads}}{\text{Mass of drug added}} \times 100 \quad (2)$$

2.3.2. Determination of bead size and morphological characterization

The mean particle size of the developed dry beads was measured using an optical microscope (SZ-6045, Olympus, Tokyo, Japan). The mean particle size was calculated by measuring 200 beads each time. The analysis was performed in triplicate for each formulation.

Morphological examination of drug loaded beads was performed using scanning electron

microscopy (SEM) (EVO 50, ZEISS, UK). Before the examination, samples were mounted on metal grids and coated with silver under argon atmosphere using high–vacuum evaporator (Polaron SEM coating system). The SEM images were recorded at different magnifications.

2.3.3. Swelling study

For the determination of the swelling ratio of prepared beads, the dry beads were soaked in 0.1 M HCl (pH 1.2) and phosphate buffer (pH 6.8) at $37\pm 0.5^\circ\text{C}$. Hydrated beads were removed at different time intervals and weighed after removing excess water using filter paper. The swelling ratio was determined using equation 3 [26]:

$$\text{Swelling (\%)} = \frac{\text{Mass of swollen beads} - \text{Mass of dry beads}}{\text{Mass of dry beads}} \times 100 \quad (3)$$

2.3.4. Determination of mucoadhesive properties

The mucoadhesion behavior of the beads was examined by the *in vitro* wash–off method using freshly excised goat stomach mucosa obtained from a local slaughterhouse (Dehra, Himachal Pradesh, India). The mucosal membrane was transported to the laboratory in Tyrode’s solution within an hour of slaughter and cleaned using Tyrode’s solution. Thirty beads (N_a) were hydrated for 15 min with Tyrode’s solution and dispersed onto a piece of stomach mucosa (2 x 7 cm), mounted on a glass slide. After hydration, the tissue was hung to the grooves of USP tablet disintegration test apparatus (ED–2 SAPO, Electrolab, Mumbai, India) (Figure 5). The apparatus was operated to give a slow and regular up–and–down movement to the tissue specimen in Tyrode’s solution at $37\pm 0.5^\circ\text{C}$. The number of beads detached (N_b) was counted every 15 min for a period of 2 hours. The percentage mucoadhesion strength of the beads was calculated using equation 4 [25]:

$$\text{Mucoadhesion strength (\%)} = \frac{N_a - N_b}{N_a} \times 100 \quad (4)$$

2.3.5. Fourier transform infrared (FTIR) spectroscopy

Interaction between the drug and polymers was examined using FTIR spectroscopy (IR affinity-1, Shimadzu, Japan) by the KBr pellet method. The FTIR spectrum of sodium alginate, pectin, repaglinide, single and dual crosslinked pectin–alginate beads were recorded to determine the molecular interactions, if any. The samples were triturated with dry KBr powder and compacted into pellets using a KBr press at 10 tons for 2 min. The sample pellets were scanned at a resolution of 4 cm^{-1} [25].

2.3.6. X-ray diffraction study

The X-ray diffractograms of sodium alginate, pectin, pure repaglinide, single and dual crosslinked pectin–alginate beads were recorded using a X-ray powder diffractometer (PW 3040/ 60 Xpert PRO, Panalytical, Netherland) using Cu $K\alpha$ radiations ($\lambda = 1.5405980 \text{ \AA}$), a current of 30 mA and a voltage of 40 kV. The samples were analyzed over $5\text{--}60$ 2θ for sodium alginate, $5\text{--}100$ 2θ for pectin and $5\text{--}40$ 2θ range for formulations with a scan step size of 0.02 and 0.50 s per step [25].

2.3.7. Analysis of thermal behavior

The DSC thermograms of sodium alginate, pectin, pure repaglinide, single and dual crosslinked pectin–alginate beads were recorded using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT, USA). Before the observation, the instrument was calibrated using indium (156°C), tin (232°C) and zinc (419.5°C) as internal standards. The

samples were placed in a 40 μ l aluminum pan and sealed. The probes were heated from 25°C at a rate of 10 °K/min under a nitrogen atmosphere [25].

2.3.8. *In vitro* drug release study

The drug release rate from crosslinked beads was ascertained using USP type II dissolution apparatus (TDT-08L, Electrolab, Mumbai, India) in 900 ml of dissolution medium at 37 \pm 0.5°C. Considering the variations in gastrointestinal pH values and the pH dependent solubility profile of repaglinide, release studies were performed in 0.1 M HCl (first 2 h, pH 1.2) to simulate the gastric fluid and phosphate buffer (next 10 h, pH 6.8) to simulate the intestinal fluid. A weighed amount of crosslinked beads equivalent to a 40 mg dose of repaglinide was placed in the dissolution vessel and the paddle rotated at 100 rpm. Samples (5 ml) were withdrawn at every 15 min during the first hour, followed by every 30 min in the next one hour, every hour until the 4th h followed by the 6th, 8th, 10th and 12thh. Sink condition was maintained by replacing the same volume of the fresh pre-warmed dissolution media immediately after each sampling. The filtered samples were analyzed using a UV-spectrophotometer (UV 3000⁺, LabIndia Instruments, Mumbai, India) at 243 nm and the sampling was carried out in triplicate [26]. For the comparative study, the release profile of a marketed conventional tablet of repaglinide (Prandin[®] tablets 2 mg, Novo Nordisk) was determined by the same procedure as followed for beads.

2.3.9. *Kinetic analysis of drug release*

To reveal the drug release mechanism, dissolution data obtained from the *in vitro* release study was fitted to different mathematical models *viz.* zero order (percentage cumulative drug released versus time), first order (log cumulative percentage of drug remaining versus time), Higuchi's model (percentage cumulative drug released versus square root of time) and

Peppas' exponential equation (log percentage drug released versus log time). The curve plotting and simulation studies were performed using Microsoft Excel software.

3. RESULTS AND DISCUSSION

3.1. Percentage yield and percentage incorporation efficiency

Repaglinide loaded beads were prepared by the simple crosslinking method. A good percentage yield of spherical beads was observed in all the formulations (70.15 ± 2.54 to $78.24 \pm 2.51\%$) (Table 1). High drug incorporation efficiency (55.24 ± 4.61 to $82.29 \pm 3.42\%$) for all formulations might be due to the poor aqueous solubility of repaglinide and high crosslinking of the polymer matrix. The incorporation efficiency increased with increasing pectin concentration, which may be due to the increase in solution viscosity, increased degree of crosslinking and increased bead size. The pectin–alginate matrix had a higher drug incorporation efficiency when compared to alginate–matrix (formulation RA). This could be due to the higher degree of crosslinking with pectin incorporation, forming a more rigid matrix which resulted in the better drug entrapment and a lower amount of drug leaching from the beads during the transitional semisolid state. However, in case of dual crosslinked beads, no significant difference in percentage yield was observed when compared to single crosslinked beads. Dual crosslinking increased the drug incorporation efficiency up to $82.29 \pm 3.42\%$ (Table 1).

3.2. Bead size and morphological characterization

The results of particle size analysis showed that the bead size lies in the range from $698 \mu\text{m}$ to $780 \mu\text{m}$. From the data presented in table 1, it is clear that the mean size of beads was increased with the addition of pectin, which could be due to the increased system viscosity. This effect on bead size also might be due to the increased crosslinking effect of both the

polymers. It was observed that during the drying process beads shrank and a dense gel matrix was formed. The bead sphericity was increased with an increase in cross-linking duration. SEM images confirmed a spherical shape with a rough surface (Figure 6). Calcium alginate beads (formulation RA) had cracks on the surface caused by collapsing of the polymer gel network during crosslinking and dehydration process (Figure 6a and Figure 6b). From the SEM images, it is clear that the surface smoothness of the developed beads was increased with an increase in the degree of crosslinking. The results showed that the polymer nature and degree of crosslinking had a significant effect on the morphological character of the developed beads.

3.3. Swelling study

Determination of the swelling behavior of delivery systems is an important evaluation parameter as it has a direct impact on the drug release profile and release kinetics. The results of the swelling study of repaglinide-loaded crosslinked beads revealed that the degree of swelling increased with an increase in pH of the medium (Figure 7). The swelling rate was slow when beads were exposed to 0.1 M HCl, pH 1.2. This might be due to the protonization of carboxylate groups of calcium alginate in acidic medium, which resulted in decreased electrostatic repulsion among these groups, favoring shrinkage. The higher swelling in phosphate buffer (pH 6.8) could be due to the exchange of calcium ions in the beads with the sodium ions of phosphate buffer, leading to water penetration and swelling.

Equilibrium swelling depends upon the extent of crosslinking and these results suggest that the pectin concentration had a positive effect on the swelling rate. The degree of crosslinking had a negative effect on swelling property, with reduced swelling in dual crosslinked beads in comparison to single crosslinking. This could be due to the increased crosslinking density,

decreased pore volume and decreased surface cracks (as evident from SEM images). This kind of swelling behavior of pectin–alginate bead system has been reported earlier by Awasthi and Kulkarni [25].

3.4. Mucoadhesion property

The pectin–alginate beads exhibited improved mucoadhesion in comparison to simple alginate beads with bioadhesion for a period of 2 h (Figure 8). The polymer matrix allowed solvent penetration due to its hydration and swelling nature, which produced a viscous gel-like transitional state and leads to increased interpolation of polymer chains between beads and the mucous membrane. A decrease in mucoadhesion strength of dual crosslinking beads was observed when compared to the single crosslinked beads. This can be explained due to the formation of more rigid matrix, which leads to less solvent penetration in the matrix and less swelling. Crosslinking time had a negative effect on mucoadhesion of drug loaded beads.

3.5. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of pure repaglinide, pectin, sodium alginate and repaglinide loaded single and dual crosslinked pectin–alginate beads (formulation RAP₄) are presented in Figure 9. The FTIR spectrum of pure repaglinide showed characteristic peaks at 3307.2 cm⁻¹ (N–H stretching), 2934.0 cm⁻¹ (C–H stretching), 1687.2 cm⁻¹ (carbonyl group), 1635.9 cm⁻¹ (N–H bending), 1565.4 cm⁻¹ (aromatic ring), 1491.2 to 1446.9 cm⁻¹ (C – O stretching), 1215.1 cm⁻¹ (–CH₃ stretching) [27]. Symmetric stretching peaks of carboxylate salt groups were observed in the FTIR spectrum of sodium alginate. The bands at 1338 cm⁻¹ (C–O stretching), 1126 cm⁻¹ (C–C stretching), 1090 cm⁻¹ (C–O stretching), 1031 cm⁻¹ (C–O–C stretching) and 946.98 cm⁻¹ (C–O stretching) are attributed to the saccharide structure of sodium alginate. In the FTIR spectrum of pectin, the band at 1619 cm⁻¹ is attributed to the carboxyl group (C–O

stretching) [28]. Characteristic peaks of the drug were also present in the FTIR spectrum of crosslinked beads with some broadening and reduction in intensity. Some of the peaks were shifted with a very slight change in the wave number. These results suggest that there was no interaction between repaglinide and excipients.

3.6. X-ray diffraction

Drug release from a dosage form depends on the physical state of the drug *i.e.* amorphous or crystalline. In order to determine the physical state of repaglinide in the developed system, X-ray diffraction examination was conducted for sodium alginate (Figure 10a), pectin (Figure 10b), pure repaglinide (Figure 10c), single crosslinked pectin–alginate beads (formulation RAP₃; Figure 10d) and dual crosslinked pectin–alginate beads (formulation RAP₄; Figure 10e). Repaglinide (Figure 10c) exhibited characteristic intense peaks at 2θ of 7.92, 10.15, 11.24, 12.95, 13.84, 15.84, 17.34, 18.14, 19.11, 20.48, 23.41, 24.51, 25.14, 25.54, 26.17, 26.84, 27.91, 30.11 and 31.24 because of its crystallinity. Single crosslinked beads (Figure 10d) showed characteristic intense peaks at 2θ of 10.62, 11.84, 12.53, 13.68, 14.53, 15.53, 16.35, 17.26, 17.87, 18.91, 19.49, 20.22, 20.97, 21.31, 22.84, 23.45, 24.24, 25.18, 25.87, 27.24, 28.47, 29.67, 30.31, 31.10, 32.11, 34.94 and 35.92. In dual crosslinked beads (Figure 10e), the drug peaks are visible at 2θ of 10.48, 11.50, 12.44, 14.63, 15.33, 16.89, 18.22, 19.57, 22.81, 23.62, 22.84, 24.53, 26.14 and 32.54.

These results suggest that in the X-ray diffractogram of dual crosslinked beads, most of the drug peaks appeared with a decrease in intensity. The absence of some intense peaks of drugs and decrease in peak intensity in formulations indicates partial molecular dispersion and amorphization of the drug throughout the system [29].

3.7. Thermal behavior

DSC thermograms of sodium alginate, pectin, repaglinide, single and dual crosslinked pectin–alginate beads are presented in Figure 11. The DSC thermogram of sodium alginate showed a broad endotherm and exotherm at 84.0°C and 259.2°C, respectively. The endotherm at 84.0°C is due to the moisture loss, while the exotherm at 259.2°C is due to the loss of volatile components, rupture of chain and fragmentation of sodium alginate [30]. The DSC thermogram of pectin showed one endotherm at 188.7°C. The second exothermic peak is due to the degradation of pectin in the heating processing [31]. The DSC thermogram of repaglinide showed a sharp endothermic peak at 135.5°C which was consistent with its melting point [32]. In the single crosslinked pectin–alginate matrix (formulation RAP₃) the drug peak was presented, but slightly shifted from the original positions to 124.78°C, indicating absence of any interaction between the drug and the polymer in the formulated beads. A DSC thermogram of pectin–alginate dual crosslinked beads (formulation RAP₄) showed peaks at 120.78°C and a peak at 154.87°C, which might be the displaced peak of the drug and polymers. In case of both, the single crosslinked and dual crosslinked beads, the enthalpy decrease when compared with that of pure drug (93.97 J/g). This effect in peak position and enthalpy of the drug in formulations is probably due to the plasticization effect as a result of reduction in attractive forces between the polymer chains and due to the partial reduction in crystallinity. From these observations it can be concluded that repaglinide did not dissolve completely in the polymer composition and seems not to be involved in the cross-linking reaction, otherwise the drug peak would have disappeared in the formulations. These findings are also supported by the results of the X-ray diffraction study.

3.8. In vitro drug release

In vitro drug release studies were performed in 0.1 M HCl, pH 1.2, (0–2 h) and in phosphate

buffer pH 6.8 (2–12 h). The drug release was found to occur through a swelling and diffusion process. Swelling of calcium alginate scarcely occurred in the early stage of dissolution which contributed in facilitating the drug release from the surface of the beads. In the present study, an attempt was made to regulate the drug release by incorporation of methoxylated pectin of low degree of esterification (28–45%) by increasing the degree of crosslinking using a dual crosslinking approach. Figure 12 shows release profiles of repaglinide from the developed formulations. In the present study, the drug particles present on the bead surface were initially released into the surrounding media further release of drug was facilitated by bead swelling. This initial release of the antidiabetic drug is the basic requirement to prevent the sudden increase in blood glucose level. As expected, the drug release rate from the developed beads after 12 h was in the rank order of single crosslinked beads (86.23%) > dual crosslinked beads (62.96%). Further, an increase in drug release was observed with a decrease in particle size. The improved and controlled dissolution of drug from the developed matrix might be due to the increased effective surface area, swelling and hydration of the polymers. The drug release process was mainly influenced by the mechanical properties of the gel barrier that formed during the swelling of polymer. The degree of cross-linking also influences drug release. A more sustained effect on drug release was observed in the beads prepared using the dual crosslinking approach.

3.9. Kinetic analysis of drug release

The drug dissolution data were analyzed using zero and first order equations to ascertain the drug release kinetics. Further confirmation of release kinetics was performed using Higuchi's equation (equation 5).

$$Q = (D \varepsilon / \tau) (2C_{\text{tot}} - C_s) C_s t^{1/2} \quad (5)$$

where Q = amount of drug released per unit area exposed to the solvent, D = diffusion coefficient of the drug in the permeating fluid, ε = porosity of the matrix, τ = tortuosity of the matrix, t = time, C_s = saturation drug and C_{tot} = concentration of the solid drug in the dissolution medium.

It is assumed that during the dissolution study, the diffusion coefficient and other parameters remain constant and equation 5 reduces to equation 6.

$$Q = Kt^{1/2} \quad (6)$$

Thus, in case of a diffusion controlled release process, a graph of the cumulative percentage drug released against square root time should be linear. The linearity of the graph was confirmed by calculating the correlation coefficient value.

In the present study, the release was found to be diffusion controlled. Fick's first and second law of diffusion are applied to assess flux and drug concentration.

Fick's first law postulates that, under the assumption of steady state, the flux goes from a region of higher concentration to a region of lower concentration, with a magnitude that is proportional to the concentration gradient. This is a first order rate process as it depends on the concentration (Equation 7).

$$J = -D \frac{dC}{dx} \quad (7)$$

where, J is the amount of drug passing perpendicularly through a unit surface area per time, D is the diffusion coefficient, and dc/dx is the concentration gradient. The negative sign

suggests that diffusion occurs in the direction opposite to the increasing concentration i.e. it is the opposite of the concentration gradient [33].

Fick's second law of diffusion is used for unsteady state situations, *i.e.*, when we wish to predict how drug concentration changes with time. This law is valid when the amount of initial drug per unit volume is smaller than the dimensional solubility of drug particle. It predicts the drug concentration change with time during the diffusion process. This law is based on the assumptions that the entire drug dissolves during diffusion process, but that does not apply in the real situations, there are always some drug particles left behind. It says that, the rate of change in drug concentration in a volume within the diffusional field is proportional to the rate of change in spatial concentration gradient at that point. The diffusion coefficient is represented as (Equation 8):

$$\frac{dC}{dt} = D \frac{d^2C}{dx^2} \quad (8)$$

Thus, it states that the change in concentration with time in a particular region is proportional to the change in concentration gradient at that point. The concentration gradient is greatest at the beginning, as the drug amount in the matrix is at maximum at the beginning and then decreases with the time as the drug diffuses from the system [33].

To find out the exact drug release mechanism and also to determine whether diffusion was Fickian or non Fickian, *in vitro* dissolution data were treated according to Peppas' equation. According to the logarithmic form of Peppas' equation, the rate of drug release can be expressed using equation 9 [24, 34].

$$\log Q = \log K + n \log t \quad (9)$$

Peppas suggested that, if the value of (slope) $n \leq 0.5$, the mechanism of drug release is diffusion without swelling. If the value is >0.5 and <1 , the release is through diffusion with swelling and if it is > 1 , the release mechanism is anomalous diffusion, not confirming to Fick's laws (non-Fickian). The calculated slope values of Peppas' equation gave a value close to 1 but less than 1, which confirmed that the release mechanism of repaglinide from the beads was Fickian diffusion with swelling.

CONCLUSION

Repaglinide, an oral antidiabetic agent with a very short half-life, was successfully encapsulated into a dual crosslinked pectin-alginate bead matrix. There was no interaction between the drug and excipients. The developed beads had ideal physical properties, incorporation efficiency (82.29 %) and release profiles (60.96 % after 12 h) to be used as a controlled release drug delivery system for sustaining the drug release over a prolonged period of time. This system can potentially be used for drugs that are absorbed throughout the gastrointestinal tract, improving drug bioavailability by enhancing solubility and dissolution profiles.

Conflict of interest: The authors confirm that this article has no conflict of interest.

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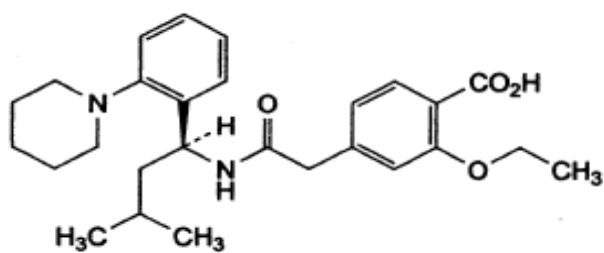


Figure 1. Chemical structure of repaglinide.

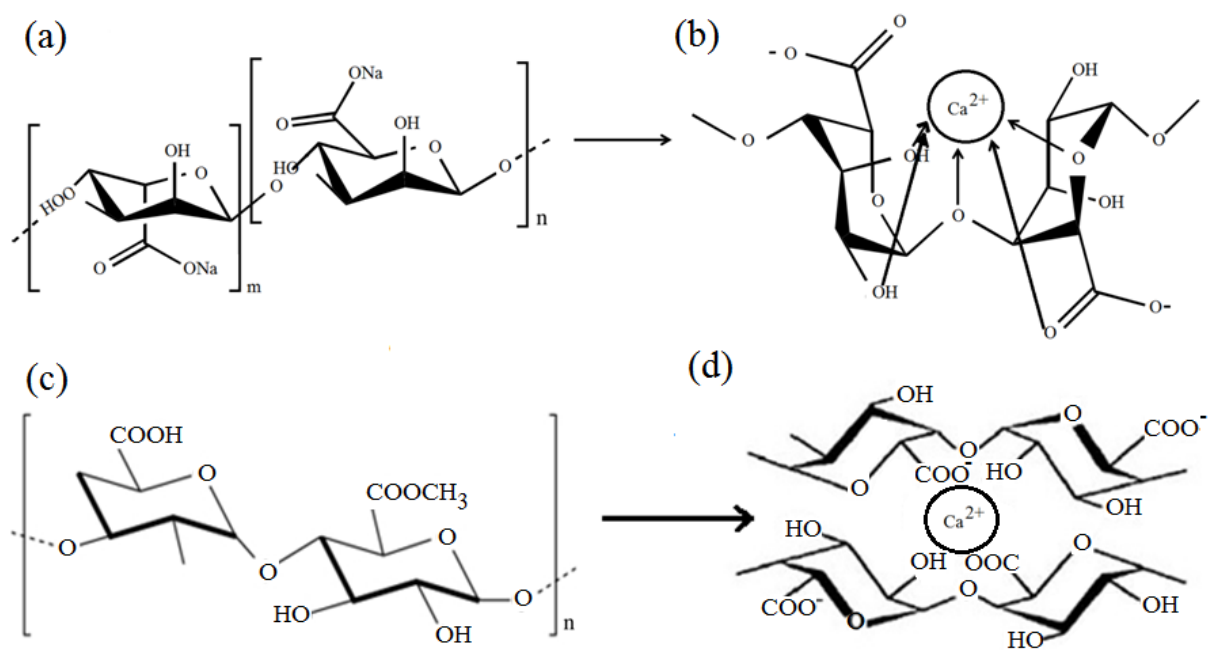


Figure 2. Chemical structure of sodium alginate (a), cross linked calcium alginate (b), pectin (c) and cross linked calcium pectinate (d).

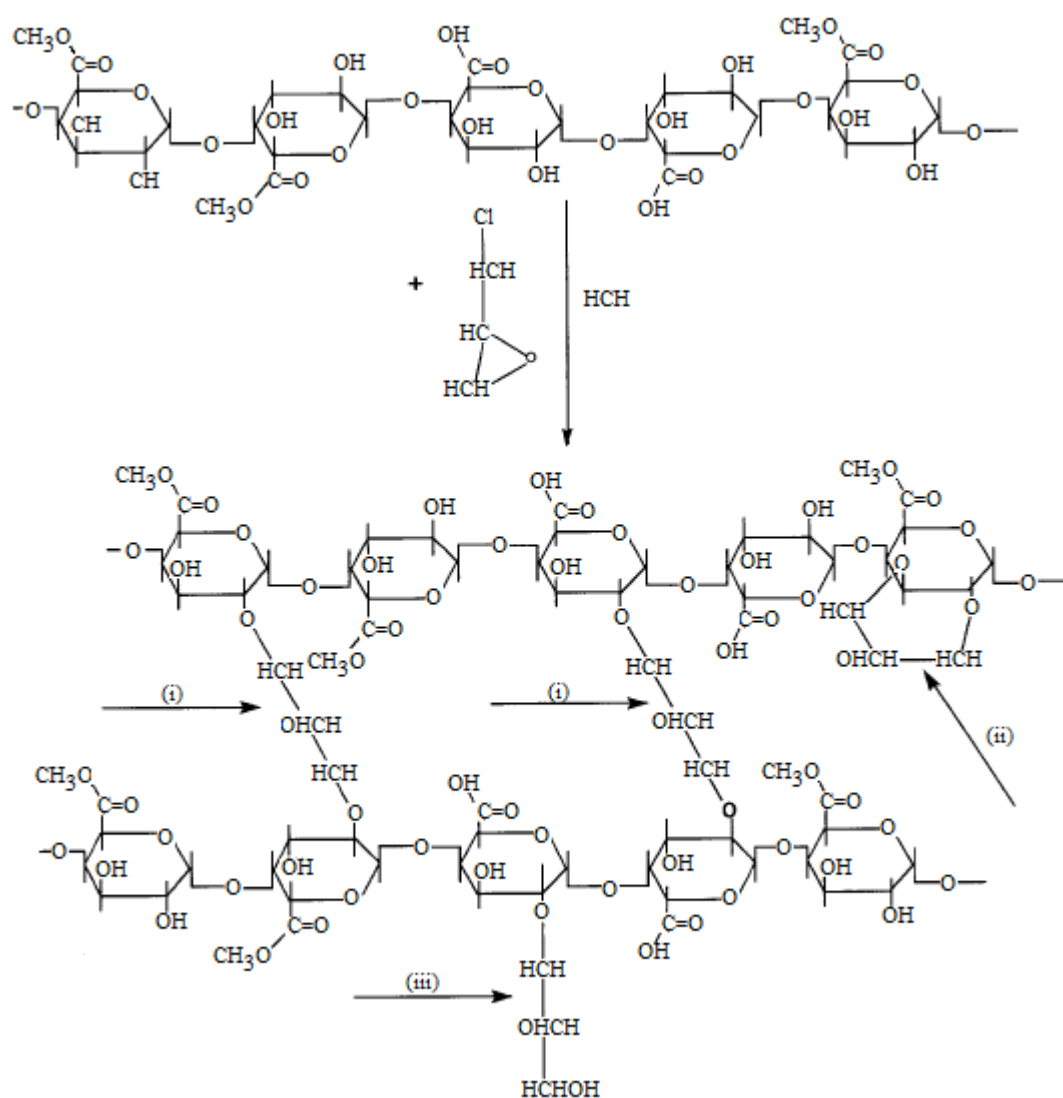


Figure 3. Theoretical schematic representations of the cross-linking reaction between pectin and epichlorohydrin. Arrows indicate the different possible modifications: (i) bridges between two polymeric chains of pectin, (ii) internal bridges on the same polymeric chain, and (iii) pendent groups. Adopted from Semde *et al.*, 2003 [22].

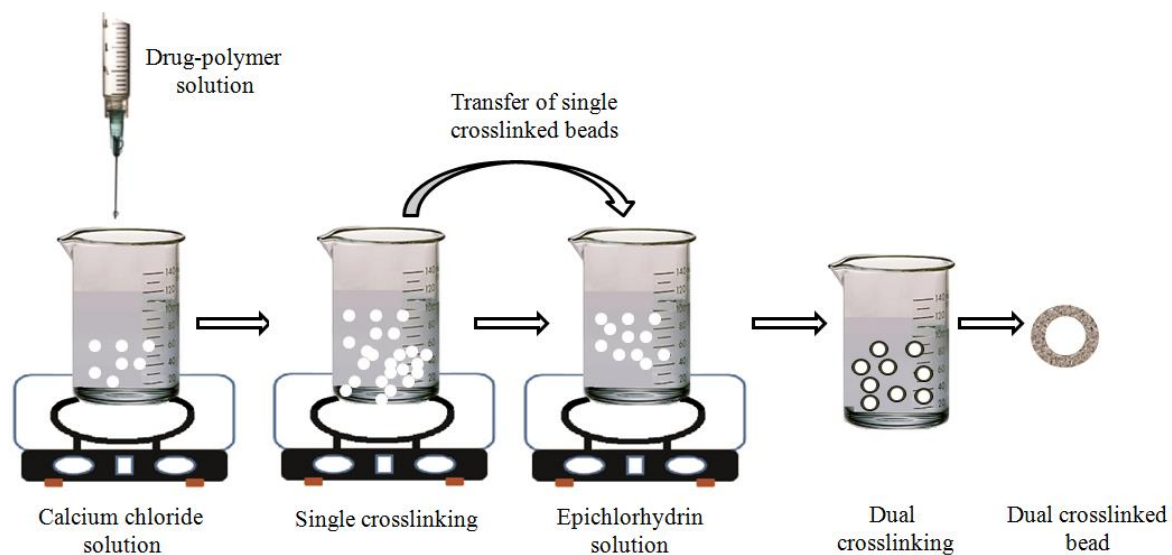


Figure 4. Schematic overview of production process of dual crosslinked pectin-alginate beads.



Figure 5. Workflow presents the mucoadhesion study of drug loaded single and dual crosslinked pectin-alginate beads using USP tablet disintegration test apparatus in Tyrode's solution at $37 \pm 0.5^\circ\text{C}$.

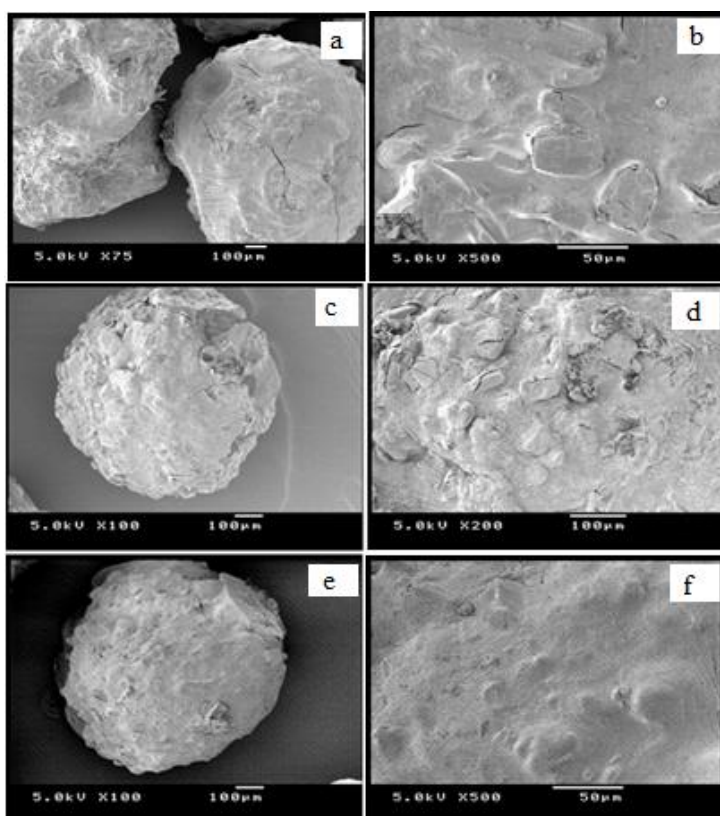


Figure 6. Scanning electron micrographs showing the general appearance and surface morphology of dried calcium alginate beads [formulation RA (a & b)], pectin–alginate single crosslinked beads [formulation RAP₃ (c & d)] and pectin–alginate dual crosslinked beads [formulation RAP₄ (e & f)].

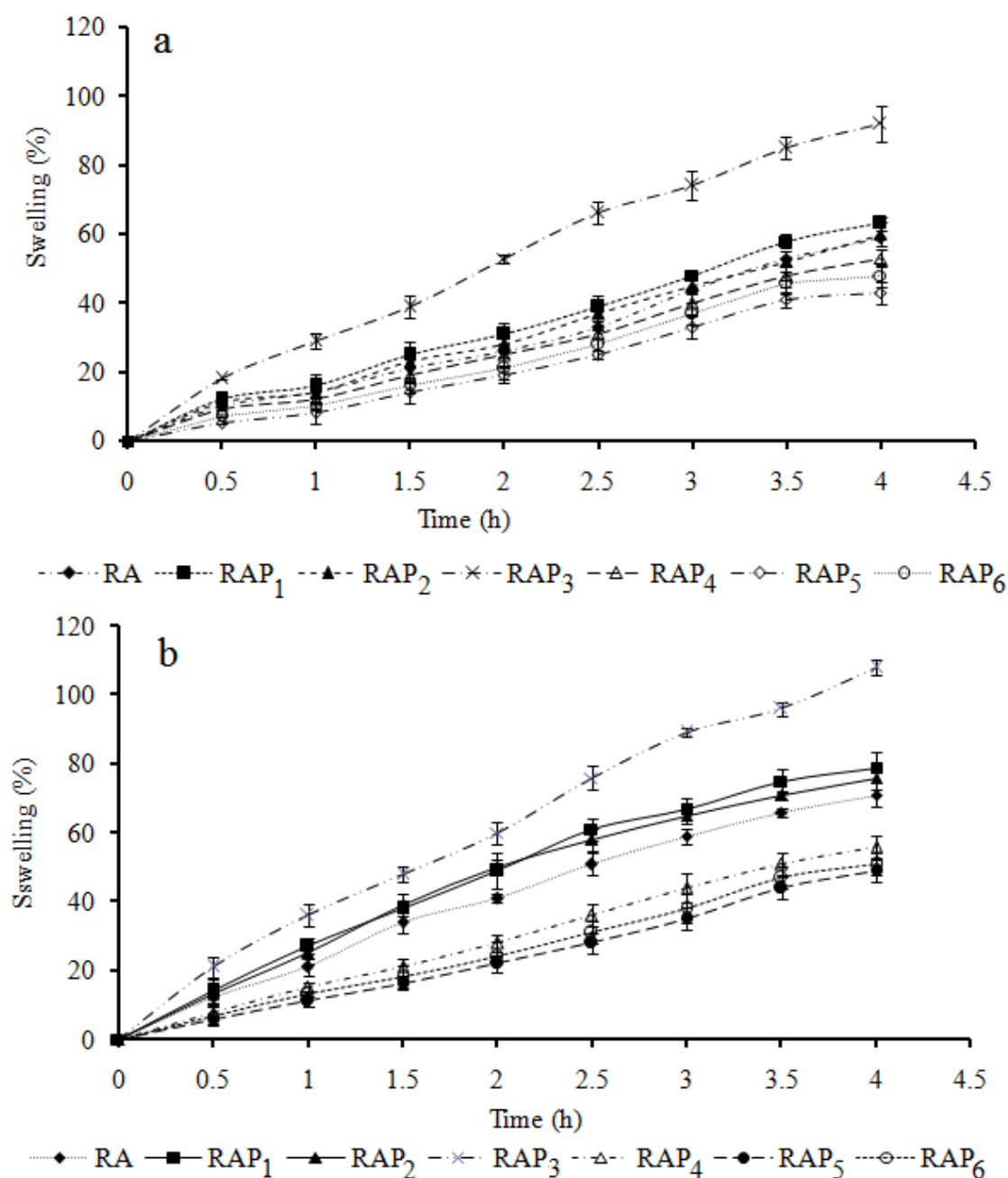


Figure 7. Swelling behavior of repaglinide loaded single and dual crosslinked pectin–alginate beads in 0.1 M HCl, pH 1.2 (a) and phosphate buffer, pH 6.8 (b) at $37\pm 0.5^\circ\text{C}$ (mean \pm SD, $n = 3$).

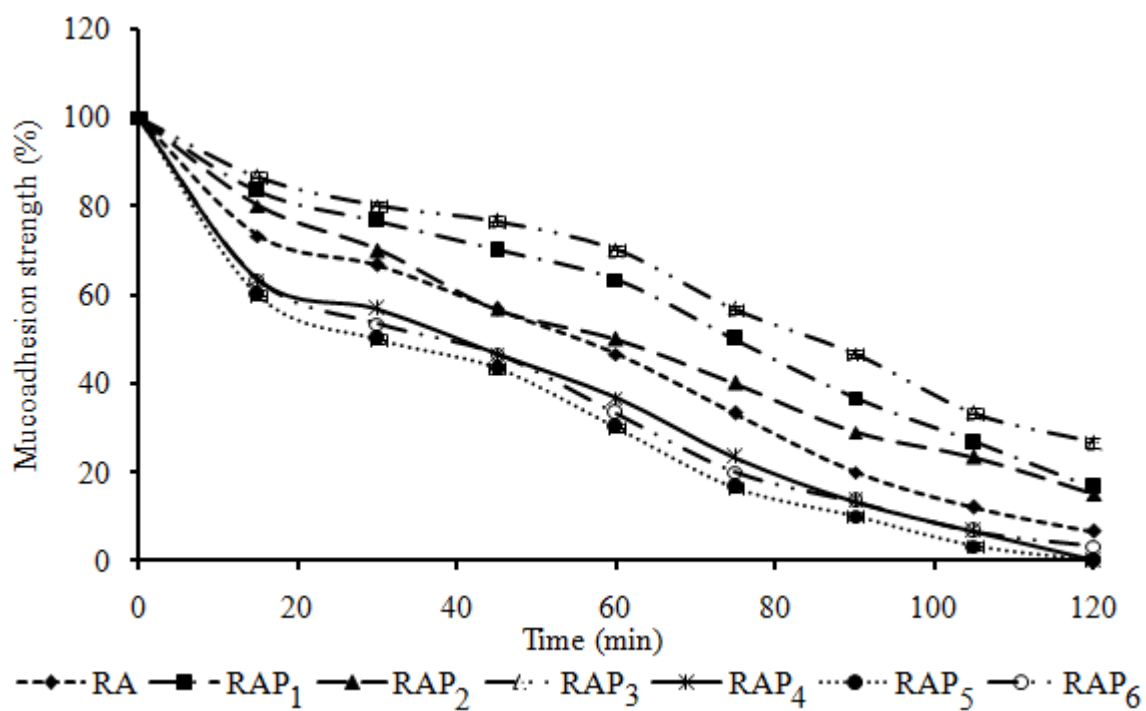


Figure 8. Results of the mucoadhesion study of repaglinide loaded single and dual crosslinked alginate pectin beads on goat stomach mucosa in Tyrode's solution at $37 \pm 0.5^\circ\text{C}$ (mean \pm SD, $n = 3$).

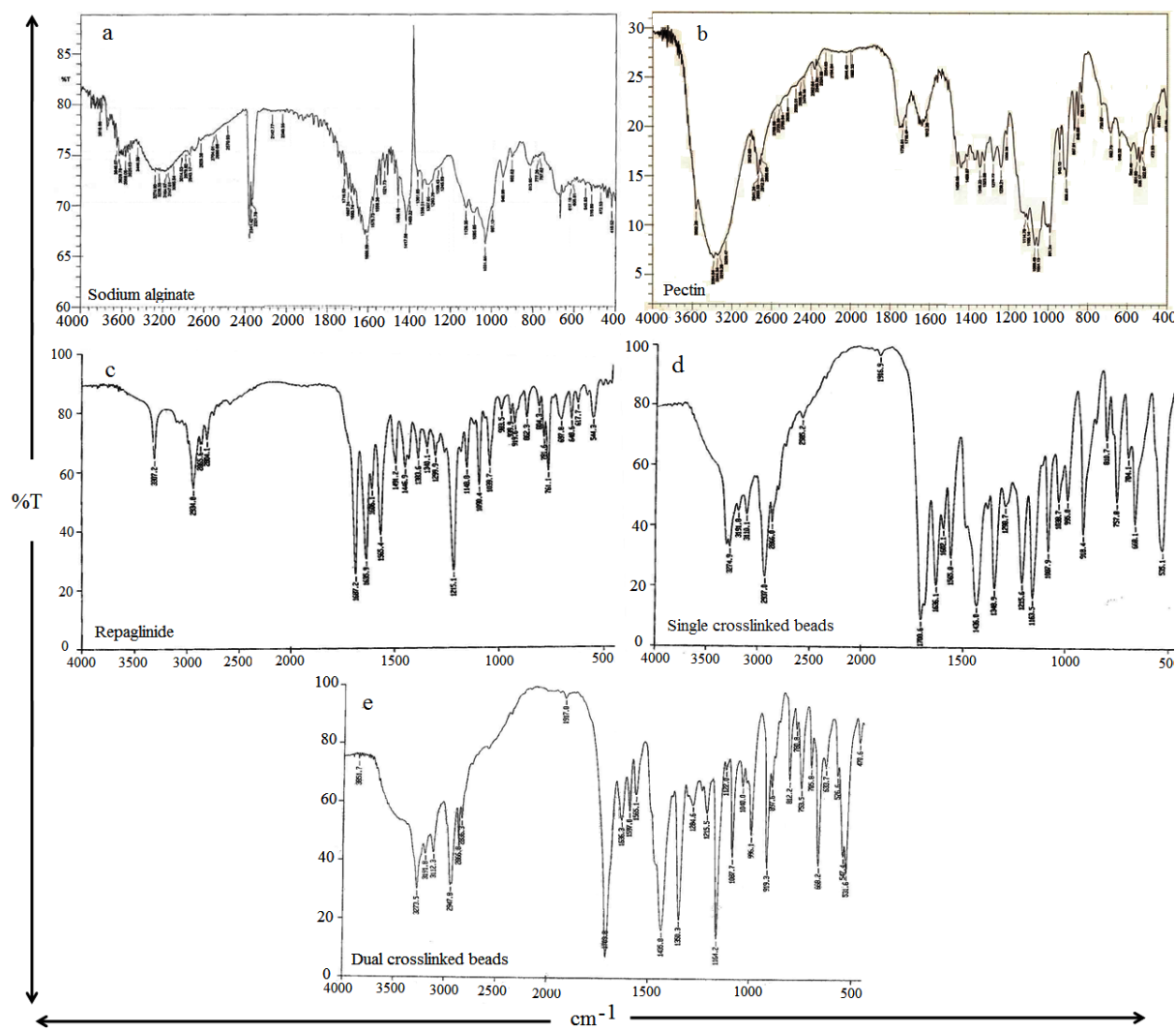


Figure 9. FTIR spectra of (a) sodium alginate, (b) pectin, (c) repaglinide, (d) single crosslinked beads (formulation RAP₃) and (e) dual crosslinked beads (formulation RAP₄).

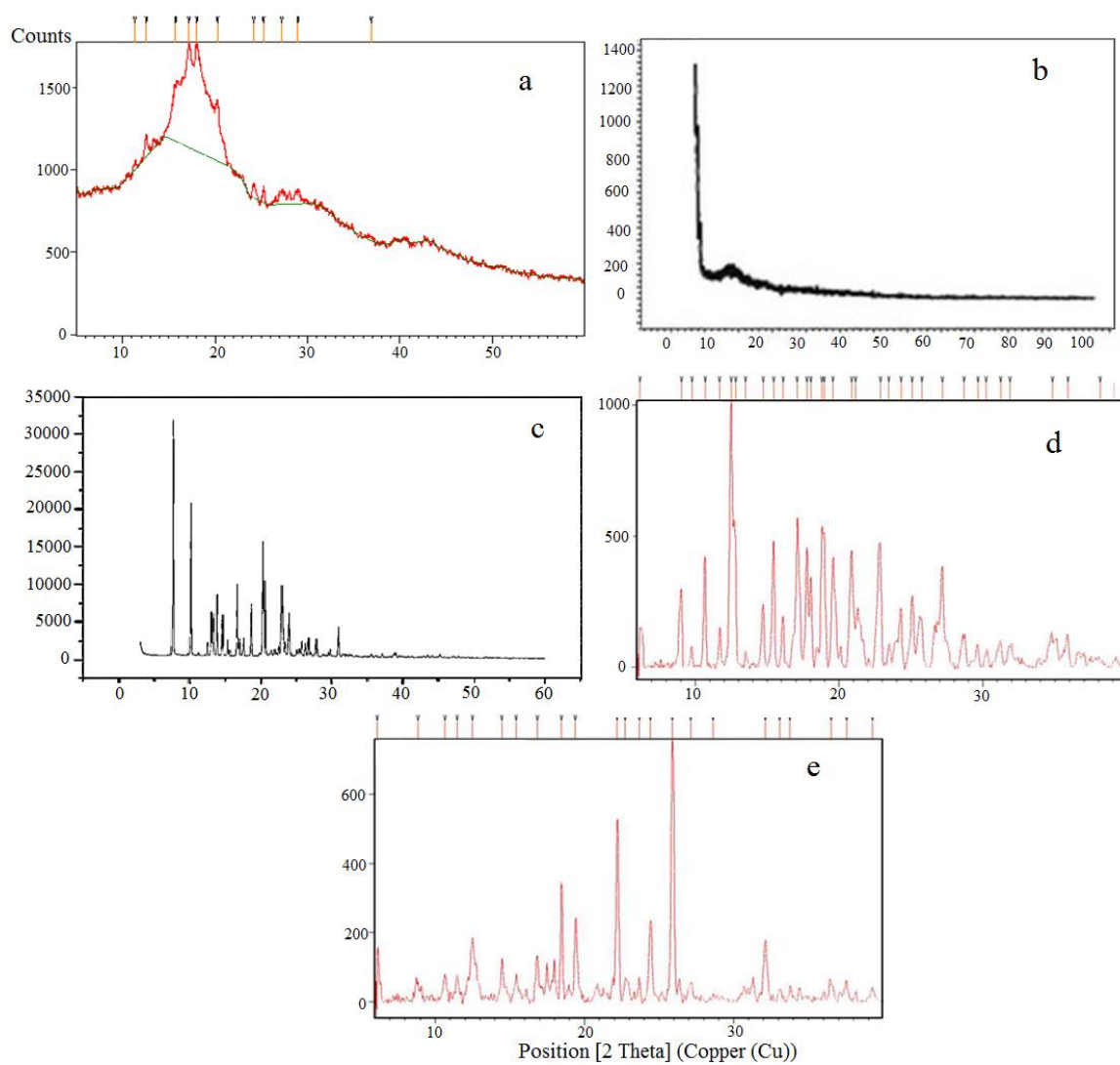


Figure 10. X-ray diffractograms of (a) sodium alginate, (b) pectin, (c) repaglinide, (d) drug loaded single crosslinked beads (formulation RAP₃) and (e) drug loaded dual crosslinked beads (formulation RAP₄).

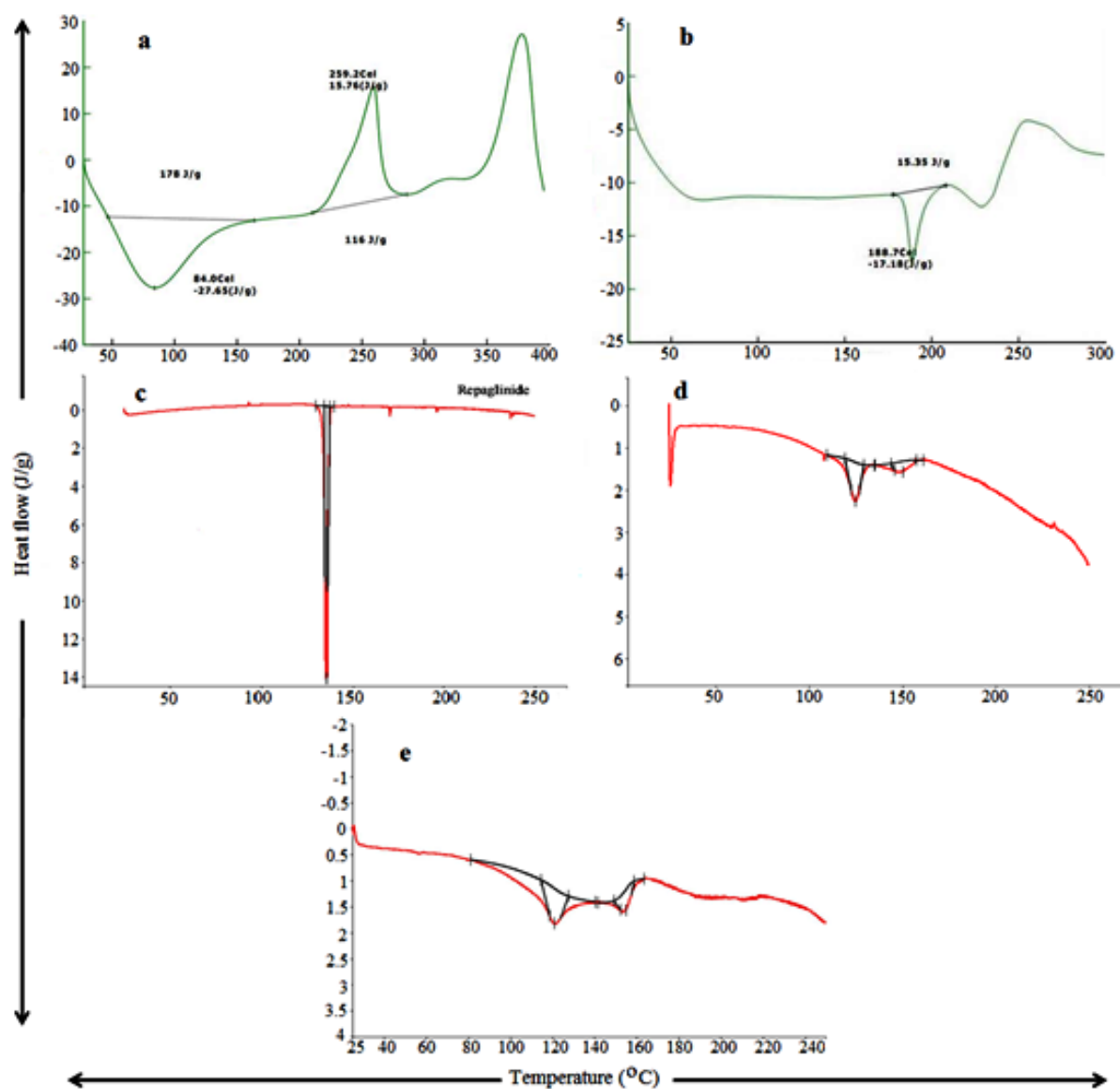


Figure 11. DSC thermograms of (a) sodium alginate, (b) pectin, (c) pure repaglinide, (d) drug loaded pectin–alginate single crosslinked beads (formulation RAP₃) and (e) drug loaded pectin–alginate dual crosslinked beads (formulation RAP₄).

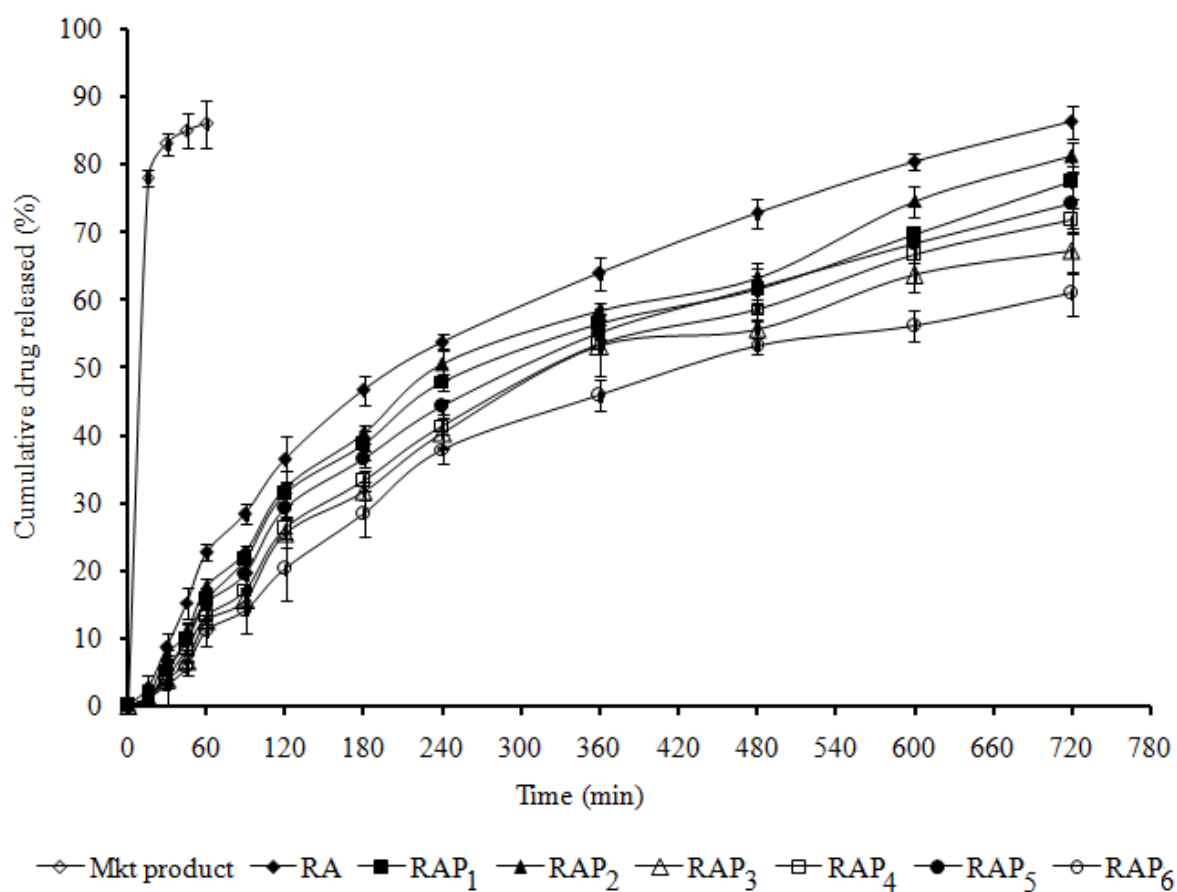


Figure 12. *In vitro* drug release profiles of different bead formulations containing repaglinide in 0.1 M HCl, pH 1.2, (0–2 h) and in phosphate buffer pH 6.8 (2–12 h) at $37\pm 0.5^\circ\text{C}$ (mean \pm SD, $n = 3$).

Table 1. Composition of various repaglinide loaded crosslinked beads and effect of formulation variables on physicochemical characteristics of developed crosslinked beads (mean \pm SD, n=3).

Formulation code	Ingredients*			Crosslinking time (min)	Yield (%) \pm SD	Drug incorporation efficiency (%) \pm SD	Mean size of beads (μ m) \pm SD
	Sodium alginate: pectin (g)	Calcium chloride (% w/v)	Epichlorhydrin (ml)				
RA	3.0:0.0	2.0	–	7	74.51 \pm 2.34	55.24 \pm 4.61	698 \pm 5.34
RAP ₁	2.0:1.0	2.0	–	5	76.28 \pm 1.27	65.41 \pm 3.34	736 \pm 10.52
RAP ₂	2.5:0.5	2.0	–	10	73.43 \pm 2.51	59.52 \pm 4.22	715 \pm 8.46
RAP ₃	1.0:1.0	2.0	–	7	70.15 \pm 2.54	71.81 \pm 2.86	749 \pm 9.22
RAP ₄	2.0:1.0	2.0	50	5	74.34 \pm 1.51	80.16 \pm 4.74	753 \pm 7.47
RAP ₅	2.5:0.5	2.0	50	10	78.24 \pm 2.51	79.26 \pm 3.41	742 \pm 10.54
RAP ₆	1.0:1.0	2.0	50	7	73.51 \pm 2.84	82.29 \pm 3.42	769 \pm 11.43

* The concentration of the components is mentioned per 100 ml of the formulation, the amount of drug was kept 20 mg in all the formulations and a polymer concentration was maintained at 3% w/v.