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RESEARCH PAPER

Insulin-Loaded Calcium Pectinate Nanoparticles: Effects of Pectin Molecular Weight and Formulation pH

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ABSTRACT

Insulin-loaded calcium pectinate nanoparticles were prepared as a potential colonic delivery system by ionotropic gelation with calcium ions. The effects of pectin molecular weight (M_v) and formulation pH on the characteristics of the nanoparticles were evaluated. Commercial pectins, LM101 and LM104, with respective degrees of esterification of 36% and 28%, were depolymerized by mechanical milling to give M_v ranging from 89 to 5.6 kDa. Milled pectins did not yield nanoparticles with significantly different mean diameter and insulin association efficiency (AE) compared to nanoparticles of unmilled pectins. LM104 nanoparticles had smaller variation in mean size than the LM101 nanoparticles. Formulation pH significantly influenced the AE and stability of the nanoparticles. Increasing the pH from 2 to 3 enhanced the AE by three-fold, from 32.76% to 93.31%, at an insulin loading concentration of 80 U/mL. This increase in AE was correlated to the charge density on the pectin molecules as a function of pH. Subsequent release of associated insulin from the nanoparticles was dependent on the extent of dilution of the nanoparticle dispersion and the pH of the dissolution medium. Cross-flow filtration could be used to separate the nanoparticles from unassociated ions and molecules, without compromising the characteristics of the nanoparticles.

Key Words: Pectin nanoparticles; Insulin; pH; Molecular weight; Association efficiency; Cross-flow filtration.

INTRODUCTION

Insulin, a 51-amino acid peptide, is generally administered as injections for the treatment of diabetes mellitus. Among the noninvasive routes of administration that have been evaluated for the delivery of insulin,[1–3] the oral route remains the most convenient. It is also physiologically desirable, since the

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exogenous insulin imitates the pathway of physiological insulin in undergoing first hepatic bypass, which warrants a primary effect by inhibiting hepatic glucose output.[1,2] Nonetheless, the development of oral therapeutic insulin formulations is fraught with difficulties, mainly because of the extensive proteolysis of insulin in the gastrointestinal tract and the poor permeability of the insulin molecules across the intestinal epithelium.[1,2,4] Among the novel approaches reported, colon-specific delivery appears to have some advantages owing to the lower level of peptidases in the colon compared to the gastric and small intestine tract.[3,5]

Pectins are anionic, soluble nonstarch polysaccharides extracted from the primary cell walls of plants. Comprising a heterogeneous complex polysaccharide of linear 1,4-linked α-D-galacturonic acid, pectins are used as gelling and thickening agents in food industries.[6] In recent years, the polymer has also been explored as pharmaceutical excipient.[7] The functional properties of pectin are determined by the percentage of carboxyl groups that have been esterified or amidated, denoted as the degree of esterification (DE) and degree of amidation (DA), respectively.[5,6,8] Pectins with low DE form gels by controlled calcium-mediated interchain association to give an extended, uniformly regular junction zone, possibly similar to that depicted in the eggbox model proposed for calcium alginate.[8] Calcium pectinate has been reported to facilitate the colonic delivery of several model drugs.[9–11] Drug release in these systems is retarded in the upper gastrointestinal tract (GIT) because of the insolubility of pectin, but is initiated upon the degradation of the pectin carrier by pectinolytic enzymes in the colon.

We hypothesized that the association of insulin to calcium pectinate nanoparticles could enhance the oral bioavailability of insulin. Not only would nano-sized particles have a longer residence time in the colon compared with larger dosage units, but they would degrade faster, thereby allowing the associated insulin to be released in the upper colon. Because the blood supply in the upper colon drains into the hepatic portal circulation, the delivery of insulin to the upper colon would resemble somewhat the natural delivery route of physiological insulin.[12,13]

The formulation of insulin-loaded pectin nanoparticles has not been reported. In this study, we prepared insulin-loaded calcium pectinate nanoparticles by adapting an ionotropic gelation method reported for the manufacture of calcium pectinate beads.[5] This method is advantageous in being a simple method that does not involve expensive equipment, harsh processing conditions, or organic solvent systems. Ionotropic gelation is also a common approach for the preparation of chitosan nanoparticles.[14] In adapting the method to prepare calcium pectinate nanoparticles, we employed techniques common to those adopted for the preparation of chitosan nanoparticles, specifically a high agitation speed of 1000 rpm, as well as dilute solutions of polymer and counterions.[14] In this paper, we focused on the effects of pectin molecular weight and formulation pH on the characteristics of the nanoparticles. In addition, we also explored the viability of using cross-flow filtration[15,16] to separate the calcium pectinate nanoparticles from unassociated ions and molecules in the dispersion.

MATERIALS AND METHODS

Materials

Amidated low methoxy (LM) pectins with degree of esterification (DE) of 36% and degree of amidation (DA) of 14% (GENU® pectin type LM-101 AS, abbreviated as LM101) and with DE of 28% and DA of 20% (GENU pectin type LM-104 AS-FS, abbreviated as LM104) were the generous gifts of Copenhagen Pectin (Lille Skensved, Denmark). Porcine insulin and 5(6)-carboxyfluorescein (CF) were purchased from the Sigma Chemical Co. (MO). Calcium chloride ($CaCl_2$) and potassium dihydrogen orthophosphate were obtained from BDH (Poole, England), citric acid monohydrate and hydrochloric acid from Merck (Darmstadt, Germany), and phosphoric acid from Mallinckrodt Chemical (KY). All other chemicals were of the highest commercial grade.

Depolymerization of Pectin

Pectins of different molecular weights were prepared by mechanical milling of 10-g batches of LM101 and LM104 in a ball mill (PASCALL 9VS, The Pascall Engineering Co. Ltd., Crawley, England) for 12, 24, and 48 hours according to the manufacturer's instructions. Molecular weight was determined by dilute solution viscometry at 30°C in an Ubbelohde-type capillary viscometer using solutions of concentrations 0.1% to 0.4% w/v (solvent comprised of 0.44 M CH_3COOH, 0.06 M CH_3COONa, and 0.10 M $NaSO_4$). The viscosity average molecular weight (M_v) was calculated from the intrinsic viscosity using the Mark-Houwink equation: $[\eta]=KM^{\alpha}$, and the Mark-Houwink constants of 0.166×10^{-3} (K) and 0.853 (α).[17]

Preparation and Characterization of Insulin-Loaded Calcium Pectinate Nanoparticles

The effects of pectin M_v and formulation pH on the characteristics of the insulin-loaded calcium pectinate nanoparticles were evaluated. To prepare nanoparticles using pectin of different M_v, 0.25 mL of insulin solution (30 U/mL in 0.01 M HCl) was premixed with 5 mL of pectin solution (0.1% w/v in 0.01 M HCl) before adding dropwise to 2.5 mL of $CaCl_2$ solution (1% w/v in water) under 1000 rpm agitation (Thermolyne, Dubuque, IA) at ambient conditions. The final pH of the formulation was 2. Formulations with final pH ranging from 2.2 to 3.4 were prepared with several modifications of this method. The desired formulation pH was obtained by adding 0.25 M HCl (20 to 100 μL) or 0.25 M NaOH (50 μL) to the pectin solution (0.1% in water) prior to mixing with the insulin solution. In addition, the concentration of the insulin solution was increased to 80 U/mL to enhance insulin association efficiency, while that of the $CaCl_2$ solution was lowered to 0.1% to reduce particle agglomeration.

Nanoparticles in a formulation were separated from free ions and unassociated insulin by cross-flow filtration using a MicroKros™ cross-flow filtration unit (Spectrum, Laguna Hills, CA) fitted with a hollow fiber filter (1000 kDa pore size). Particles smaller than the pore size passed through the membrane and emerged as permeate, while larger particles were retained and concentrated during the filtration process.[15,16]

Nanoparticle size and zeta potential (ζ) were determined in triplicates in a particle size analyzer (Zetasizer 3000, MicroKros Instruments Ltd., UK) after the nanoparticle dispersion was diluted to four times its volume with 0.05 M NaCl. Nanoparticle morphology was observed under a transmission electron microscope (TEM) (JEOL JEM 100 CX II, Japan) after staining with 2% of tungstophosphoric acid solution.

The association efficiency (AE) was determined by pelletizing a nanoparticle dispersion at 25,000 rpm for 60 min at 20°C (Beckman Avanti™ J-25 Centrifuge, Beckman Instruments, Fullerton, CA) and measuring the insulin content in the supernatant. Association efficiency (%) was calculated as $[(W - W_s)/W] \times 100\%$, where W_s was the insulin content in the supernatant and W, the initial insulin load in a formulation. Triplicate samples were measured for each formulation and the results expressed as mean ± SD.

In vitro drug release experiments were conducted by agitating 2-mL aliquots of each nanoparticle dispersion with 8 mL of United States Pharmacopoeia (USP) simulated gastric fluid or intestinal fluid (without enzymes) at 100 rpm at 37°C. Triplicate samples were removed at predetermined times, centrifuged at 25,000 rpm for 40 min and the supernatant assayed for insulin content. The cumulative amount of insulin released as a percent of the insulin load in the nanoparticles was plotted as a function of time to give the drug release profile.

Insulin was quantified by reversed-phase high-performance liquid chromatography (HPLC) (Shimadzu LC-10 AT, Kyoto, Japan) in a Waters column (Spherisorb S10 ODS1, 4.6×250 mm). Gradient elution was employed using a mobile phase of phosphate buffer (pH 2.0, 0.1N) and acetonitrile, the proportion of acetonitrile increasing linearly from 26% to 32% in 35 min. The flow rate was 1.5 mL/min and the eluant was detected at 214 nm. A linear calibration curve ($R^2 = 0.999$) based on peak area was obtained for insulin standards in the concentration range of 0.06 to 2.2 IU/mL.

Preparation and Characterization of Carboxyfluorescein-Loaded Calcium Pectinate Nanoparticles

The carboxyfluorescein (CF) solution (1 mL, 10 μg/mL in pH 5.6 buffer) was acidified with 1 mL of 0.1 M HCl before it was premixed with 10 mL of LM104 solution (0.1% in water). To initiate the formation of nanoparticles, 11 mL of 0.1% $CaCl_2$ was added with agitation at 1000 rpm. Particle diameter, ζ, and AE of the nanoparticles were measured using methods similar to those described for the insulin-loaded calcium pectinate nanoparticles. Carboxyfluorescein was assayed by fluorometric spectroscopy (PerkinElmer LS-5B, Beaconsfield, Buckinghamshire, UK, λ_{exc} 490 nm, λ_{emi} 520 nm). A linear calibration curve ($R^2 = 0.999$) was obtained with CF solutions in the concentration range of 1.95 to 250 ng/mL.

To evaluate the efficiency of the cross-flow filter to remove free ions and molecules in the nanoparticles, 5 mL of a CF nanoparticle sample was concentrated to 1 mL by passing it through the filter. The filtrate was collected for CF analysis by fluorimetry, and the concentrate reconstituted to 5 mL with water. The filtration process was repeated twice. The efficiency of purification (%) was calculated as $[W_f - W_c]/W_f \times 100$, where W_f was the free CF content in the nanoparticle sample as determined from its AE, and W_c was the cumulative CF content in the filtrates after three rounds of filtration. The experiment was carried out in triplicate and the results expressed as means ± SD.

Statistical Analyses

Data are expressed as means ± SD, and the means were analyzed by one-way analysis of variance (ANOVA) followed by posthoc unpaired t-tests (SPSS 10.0) at a p value of 0.05.

RESULTS

M_v for the LM101 sample decreased significantly from 59.3 ± 1.2 kDa to 40.5 ± 1.5 kDa after 12 h of milling, the decrease in M_v being proportional to increasing milling time (R^2=0.98) (Fig. 1) to reach a value of 5.6 ± 3.9 kDa after 48 h of milling. In contrast, the higher molecular weight LM104 sample (89.1 ± 3.1 kDa) exhibited a significant loss in M_v only after 24 h of milling, although the decrease in M_v thereafter was also proportional to increasing milling time. M_v of LM104 after 48 h of milling was 33.6 ± 1.5 kDa. Mechanical milling was employed for the depolymerization of LM101 and LM104 because it has been shown to reduce pectin molecular weight without modifying its degree of esterification, possibly by random scissions of the main chain by mechanical attrition.[17] To ease discussion, the following notation is adopted for the milled pectin samples: LMX-Y where X is 101 or 104 to represent the two pectin samples, and Y represents the milling time of 12, 24, or 48 h.

Considerable batch-to-batch variation in particle size were observed in the three batches of insulin-loaded LM101 nanoparticles prepared, the mean particle diameter of each batch ranging from 589 to 1043 nm [coefficient of variation (CV) of 30%] (Fig. 2). The samples were observed under the TEM (Fig. 3) to contain significant particle agglomeration. Milled samples, LM101-12 and LM101-24, did not produce nanoparticles of statistically different mean size from those of the LM101 (Fig. 2). The LM101-48 nanoparticles were not only of larger mean size, but showed much higher CV of 60% with respect to particle size. These nanoparticles exhibited extensive particle agglomeration (Fig. 3A).

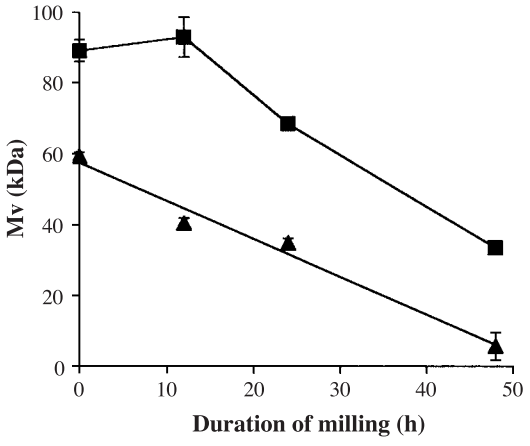

Figure 1. Viscosity-average molecular weight of two pectin samples, LM101 (▲) and LM104 (■), subjected to ball milling for various periods of time (mean ± SD, n=3).

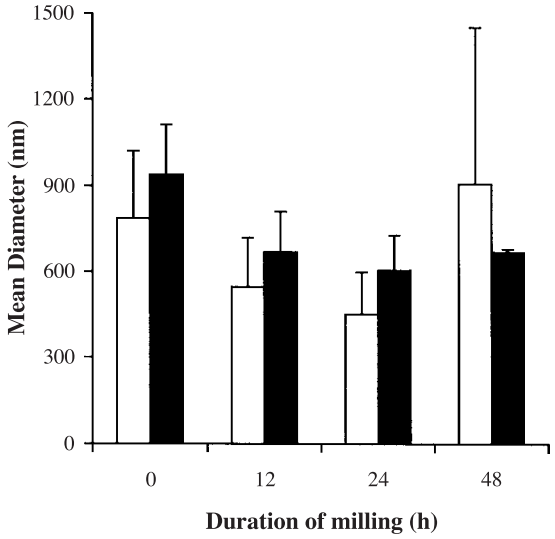

Figure 2. Mean particle size of insulin-loaded pectin nanoparticles prepared with unmilled and milled LM101 (□) and LM104 (■) samples (mean ± SD, n = 3).

Nanoparticles of LM104, before and after milling, did not exhibit significant differences in mean diameter compared with equivalent nanoparticles of LM101. However, the LM104 produced nanoparticles of smaller polydispersity, the mean diameters for three batches of samples ranging from 741 to 1064 nm (CV of 18%) (Fig. 2). In addition, the LM104-48 yielded smaller nanoparticles with a relatively small CV of 1%, unlike the LM101-48 sample. Milling also did not significantly modify the mean diameter of nanoparticles produced with the LM104.

The LM101 and LM104 nanoparticles showed low insulin association efficiency in the range of 10–15% (Fig. 4) when formulated at pH 2. Milling of the pectin samples prior to nanoparticle manufacturing did not produce significant differences in AE (p=0.49 and 0.69 for LM101 and LM104 series, respectively). Nor were there significant differences in AE between the LM101 and corresponding LM104 formulations.

Since the M_v and DE of pectin did not significantly affect the AE and mean size of the nanoparticles, the

unmilled pectin was selected as the starting material for preparing subsequent batches of nanoparticles to save cost and time. Of the two pectin samples, the LM104, which had lower DE than the LM101, was preferred because pectins with lower DE have been reported to yield more rigid matrices with Ca^{2+} ions. This is reflected in the smaller variation in mean diameter of the LM104 nanoparticles.

Dissolved pectin is negatively charged at neutral pH and approaches zero charge at low pH.[6] The pH of the formulation could therefore affect the degree of ionization of the pectin molecule and its electrostatic interaction with Ca^{2+} and insulin. The effect of solution pH on the polyelectrolyte properties of pectin was examined using the zeta potential (ζ) as a measure of the density of surface charges present on the polymer molecule.

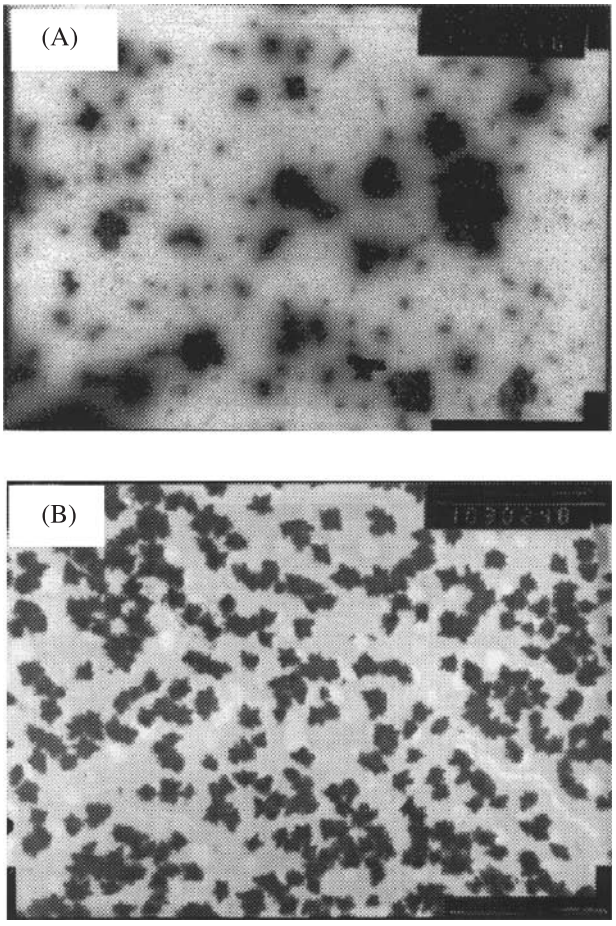

Figure 3. TEM micrographs of insulin-loaded nanoparticles prepared with (A) 0.1% LM101-48, 30 IU/mL insulin, and 1% $CaCl_2$ at pH 2; and (B) 0.1% LM104, 80 IU/mL insulin, and 0.1% $CaCl_2$ at pH 3 (Magnification 10,000×).

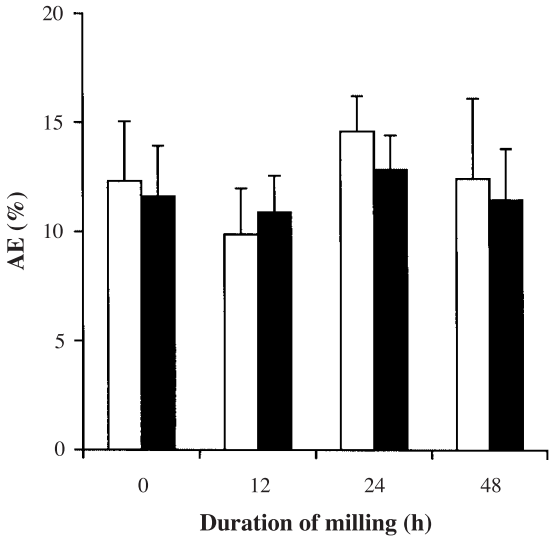

Figure 4. Association efficiency of insulin (AE) for pectin nanoparticles prepared with unmilled and milled LM101 (□) and LM104 (■) samples (mean ± SD, n = 3).

The zeta potential (ζ) for an LM104 solution (0.1% in water adjusted to the desired pH with 0.25 M HCl or NaOH) increased in magnitude from −4.4 to −54.5 mV with increasing pH from 2 to 5.5, but leveled at values between −52 and −56 mV in the pH range of 5.5 to 7.6 (Fig. 5). Although the highest ζ was obtained at pH>5.5, the polymer is known to rapidly degrade at high pH; even at pH 5, depolymerization is considerable.[6,18] For these reasons, the characteristics of the insulin-loaded calcium pectinate nanoparticles were evaluated in the pH range of 2.2 to 3.4. To prepare nanoparticles at different pH, we used insulin

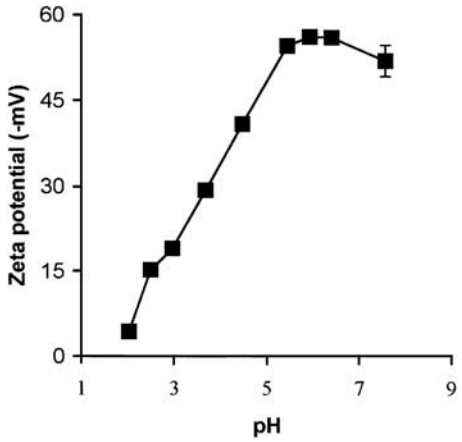

Figure 5. Effect of pH on the zeta potential of a 0.1% pectin (LM104) solution (mean ± SD, n = 3).

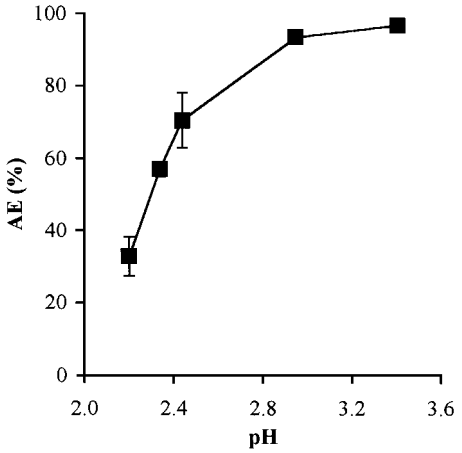

Figure 6. Effect of formulation pH on the AE of insulin-loaded LM104 nanoparticles prepared with 80 IU/mL insulin loading concentration (mean ± SD, n = 3).

solutions of a higher concentration (80 U/mL) and $CaCl_2$ solutions of lower concentration (0.1%) because these conditions have been shown to, respectively, increase the insulin AE to about 30% and reduce particle agglomeration at pH 2.

Insulin-loaded LM104 nanoparticles thus prepared had similar mean diameters in the narrow range of 450 to 500 nm, while the zeta potential increased from −2.3 to −12.3 mV when the formulation pH was raised from 2.2 to 3.4. There was a dramatic change in AE within this pH range, the AE increasing from 32.76% to 93.31% when the pH was increased from 2.2 to 3.0. At pH 3 and above, AE was consistently above 90% (Fig. 6), and particle agglomeration was significantly reduced (Fig. 3B).

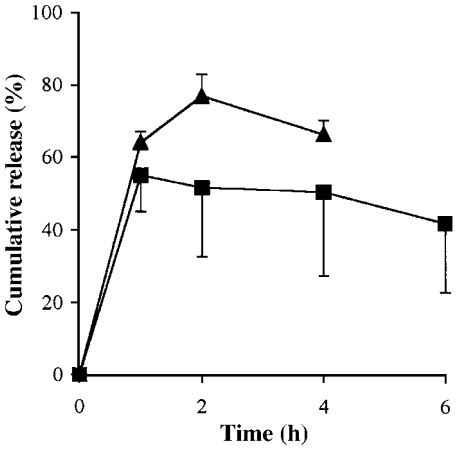

Figure 7. In vitro release of insulin from insulin-loaded LM104 nanoparticles into simulated gastric fluid (▲) and intestinal fluid (■) (mean ± SD, n = 3).

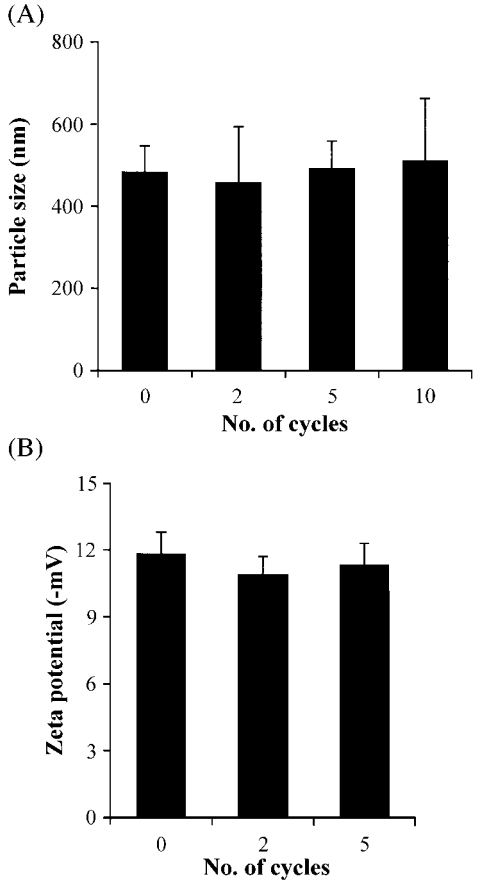

Figure 8. Mean particle diameter and zeta potential of insulin-loaded LM104 nanoparticles following several cycles of cross-flow filtration (mean ± SD, n = 3).

Figure 7 shows the in vitro release profiles of insulin from the nanoparticles formulated at pH 3. Up to 75% of the associated insulin was released into the simulated gastric fluid over a 4-h period. There was an initial burst effect in the first hour followed by little insulin released thereafter. Similarly, about 50% of the associated insulin was released into the intestinal fluid within the first hour, with insignificant amounts of insulin released over the next 5 hours.

Figure 8 indicates that the mean diameter (A) and zeta potential (B) of the insulin-loaded LM104 nanoparticles formulated at pH 3 were not significantly modified by several cycles of cross-flow filtration. The filtration process concomitantly reduced the sample to 1/2, 1/5, and 1/10 of its original volume after 2, 5, and 10 cycles, respectively. To assess the efficiency of the cross-flow filter in removing ions and soluble molecules from calcium pectinate nanoparticle samples, we

used CF-loaded calcium pectinate nanoparticles instead of the insulin-loaded LM104 nanoparticles. The high AE of the latter made it difficult to accurately quantify by HPLC the small amount of free insulin permeating across the cross-flow filter. In contrast, CF lends itself to a quick and sensitive quantification by fluorescence spectroscopy. Moreover, the CF-loaded LM104 nanoparticles had a substantially lower AE of 25.70±1.02% (n=3). In terms of size and zeta potential, the CF-loaded calcium pectinate nanoparticles resembled the insulin-loaded nanoparticles prepared under similar conditions. The free CF (74.3%) in the CF-loaded LM104 nanoparticle samples was removed with 97.61±2.50% efficiency after three cycles of cross-flow filtration.

DISCUSSION

We have established that calcium pectinate particles of less than 1 μm could be obtained by simply mixing dilute solutions of pectin and Ca^{2+} at a high agitation speed of 1000 rpm. This straightforward method, which does not involve expensive equipment and noxious organic solvents, is also suitable for the loading of labile insulin molecules into the calcium pectinate nanoparticles. Coacervation of pectin is postulated to occur through the divalent Ca^{2+} ions, which interlink the negatively charged carboxyl groups of neighboring pectin molecules into an ''egg-box'' conformation.[5,18,19]

Pectin M_v in the range of 89 kDa to 5.6 kDa did not have a significant impact on the mean diameter of the insulin-loaded calcium pectinate nanoparticles. This could be attributed partially to the large batch-to-batch variation in the mean diameter of the nanoparticles prepared with a pectin sample. The variation in size was related to the conditions used to prepare the nanoparticles. The relatively high Ca^{2+} content combined with the low pH of 2 resulted in small ζ (magnitude less than 6 mV) values for the nanoparticles, leading to an unstable formulation prone to particle agglomeration. Particle agglomeration was particularly extensive for nanoparticles prepared with the LM101-48 sample, probably because the short and flexible chains of this pectin sample were more amenable to coacervation by the Ca^{2+} ions.

The data also suggest that pectin M_v in the range of 89 kDa to 5.6 kDa, and DE in the range of 28% to 36% did not influence the association of insulin with the pectin nanoparticles at pH 2. Insulin association with the pectin nanoparticles was likely to involve an electrostatic interaction between the positively charged insulin (isoelectric pH of 5.3)[20] and the negatively charged pectin molecules. In which case, the AE would be a function of the density of accessible charged groups on the two molecular species. Since the same quantities of pectin and insulin were used to prepare the nanoparticles, it is not surprising that the milled samples did not yield nanoparticles with significantly different AE from those prepared with the corresponding unmilled LM101 and LM104 samples.

In contrast, formulation pH was an important moderator of the AE of the insulin-loaded calcium pectinate nanoparticles, the AE increasing three-fold when pH was increased from 2 to 3. Within the pH range of 2.2 to 3.4, which was below the isoelectric point of the peptide (5.30 to 5.35), the peptide molecules were positively charged. However, the carboxylate function of the pectin molecules, which have pK_a of about 3.5–4.5[6,21–23] would show varying degrees of ionization over the pH range of 2.2 to 3.4. Figure 4 confirmed this, showing a four-fold increase in the magnitude of the polymer ζ when pH was increased from 2 to 3.4. A higher degree of ionization of the pectin molecules at pH 3 and above allowed for a greater extent of binding between the negatively charged pectin and the positively charged insulin, thereby accounting for the high AE.

The data also explained the tendency of the insulin-loaded calcium pectinate nanoparticles towards agglomeration at different pH. Colloidal dispersions are stabilized by the particles possessing a lyophilic surface or having ζ above a critical value, so that particle attraction and agglomeration are discouraged. Particles with larger zeta potential have higher charge density and are kept apart by mutual repulsive forces. Stable systems are those in which the repulsive forces exceed the attractive London forces at zeta potential of 25 mV or more.[23] Insulin-loaded calcium pectinate nanoparticles formulated at pH 2 had $\zeta < -6$ mV, which was inadequate to prevent particle agglomeration. Although it might be desirable to prepare the nanoparticles at pH>5.5 where the pectin molecules exhibited maximum ζ values, the upward adjustment of the formulation pH is limited by the chemical instability of the pectin molecules at high pH. As a compromise, a maximum pH of 3.5 was selected as this would give the pectin solution ζ values of about −25 mV without inducing considerable depolymerization of the pectin molecules.

Formulation of the pectin into insulin-loaded calcium pectinate nanoparticles at pH 3, however, lowered the ζ value to −12 mV. Electrostatic interactions with Ca^{2+} ions and insulin molecules might have shielded the charge density on the pectin molecules,

resulting in lower ζ for the nanoparticles. This ζ value was, however, sufficient to reduce to acceptable levels the particle agglomeration and batch-to-batch variation in size. Almost complete (> 90%) association of the insulin load occurred at pH 3 despite the incomplete dissociation of the pectin molecules, which might be related to the low molar ratio of [insulin]:[pectin] (= 2.2) used for the formulation.

In vitro release experiments conducted with simulated gastric and intestinal fluids showed a burst release of insulin from these nanoparticles within the first hour followed by little insulin released over the next 3–5 h. The burst effect probably resulted from a shift in the equilibrium between associated and free insulin upon dilution of the nanoparticle dispersion with the dissolution media. Dilution promoted the dissociation of insulin from the nanoparticles, but once the new equilibrium was established, no further rise in free insulin concentration was observed. Compared with the intestinal fluid, more insulin was released into the gastric fluid, possibly due to the displacement of insulin by the competitive binding of H^+ to the pectin molecules in the acidic medium. Although these phenomena are predicted to occur in vivo, the percent release of associated insulin will depend on the degree of dilution of the nanoparticle dispersion by the GI fluids.

Conventional methods for purifying and concentrating polymer nanoparticles are pelletization by centrifugation followed by lyophilization.[24,25] The insulin-loaded calcium pectinate nanoparticles were, however, unstable to centrifugation, agglomerating into a pellet that could not be redispersed into nanoparticles before and after lyophilization. In this project, we evaluated the viability of using cross-flow filtration to purify and concentrate the insulin-loaded calcium pectinate nanoparticles. Unlike dead-end filtration mode where the liquid flow is perpendicular to the filter surface, the cross-flow technique employed a liquid flow tangential to the filter surface, thereby minimizing filter clogging and blockage.[15,16] Where filtration is performed in several cycles without pure solvent feed, the sample volume decreased and became enriched in the nanoparticles that could not pass through the filter. We have shown that cross-flow filtration was efficient in removing free CF from the CF-loaded calcium pectinate molecules. Cross-flow filtration also did not modify the size distribution and zeta potential of the insulin-loaded calcium pectinate nanoparticles. The collective data suggest that it is a viable method for the purification and concentration of drug-loaded calcium pectinate nanoparticles.

CONCLUSION

Insulin-loaded calcium pectinate nanoparticles with narrow size distribution and high association efficiency can be formulated by a simple ionotropic gelation method in which dilute solutions of pectin and calcium chloride were mixed at high agitation of 1000 rpm. The association efficiency of the nanoparticles was not dependent on pectin molecular weight, but was sensitive to formulation pH. Association efficiency greater than 90% was obtained at pH 3 for a molar ratio of [insulin]:[pectin] = 2.2. In vitro insulin release profiles showed a burst effect within the first hour with little insulin released thereafter in simulated gastric and intestinal fluids. The burst effect was pH-dependent. Cross-flow filtration could be used to remove free molecules and ions from the nanoparticle dispersion without compromising on the size and surface characteristics of the nanoparticles.

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