Preparation and Characterization of Inulin Ester Microspheres as Drug Carriers

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Received 16 July 1999; accepted 23 August 1999

ABSTRACT: Acetylated and succinoylated inulin were synthesized by reacting inulin with acetic anhydride and succinic anhydride. The modified inulin was characterized by FTIR, NMR, and potentiometric titration. The compositional dependence of their properties, such as solubility, pKa, and melting point, was investigated. The results reveal that the solubility of the inulin derivatives in pH 7.4 buffer solution increases with the succinyl content, varying from negligible for fully acetylated inulin to over 54% for fully succinoylated inulin, whereas the corresponding pKa of the inulin derivatives decreases with increasing succinyl content. In addition, the melting point is lowered by acetylation and/or succinoylation. The influence of pH and ionic strength on the solubility of inulin acetate succinate was also studied. The solubility increases dramatically as the pH value approaches that of the pKa. Interestingly, in pH 7.4 buffer solutions of varying ionic strength, a maximum solubility appears at an ionic strength of 0.15*M*. This is interpreted as a result of a balance of the ion exchange process and the double layer suppression. Microspheres of inulin acetate and inulin acetate succinate with and without drug were prepared by the solvent precipitation method. Cationic compounds, chlorhexidine and chymotripsin, were used as model drugs. The size and morphology of microspheres were determined by scanning electron microscope. The microspheres range in diameters from 0.5 to 4 μm for inulin acetate and inulin/chymotripsin microspheres, and from 90 to 130 μm for inulin acetate/chlorhexindine microspheres. The cross-section of the microspheres exhibits a porous interior. Preliminary results show that the microspheres are able to release the incorporated drugs for an extended period of time. J Appl Polym Sci 77: 833–840, 2000

Key words: inulin ester; microspheres; preparation and characterization; drug carrier

INTRODUCTION

Polymers suitable for human use as injectable drug carriers or implant materials must be nontoxic, biocompatible, and ideally biodegradable. The nontoxic and biocompatible properties are generally required of not only the polymer but also the degradation products. Several natural and synthetic biodegradable polymers such as serum albumin, polylactide (PLA), lactide/glycolide copolymers (PLA/GA), polyorthoesters, and poly(alkyl-cyanoacrylates) have been studied as particulate drug carriers.[1–3] Among synthetic biodegradable polymers, PLA and PLA/GA have the longest history of use in humans as suture material. These polymers undergo autocatalytic bulk erosion, and the resulting degradation products

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Contract grant sponsor: Medical Research Council of Canada (to P.I.L.); contract grant number: MT-11486.

Journal of Applied Polymer Science, Vol. 77, 833–840 (2000)

are endogenous and nontoxic.[4,5] On the other hand, polyorthoesters undergo surface-erosion mechanism; however, additives are often required to promote the erosion, and the degradation products are rather complex. In fact, allergic reactions have been reported with certain polyorthoesters.[4] Application of poly(alkyl-cyanoacrylates) for an injectable drug carrier is also limited because of the formation of toxic degradation products such as isobutanol and formaldehyde.[2] The degradation and erosion periods of these existing bioerodible polymer systems are generally in the order of weeks and months.

The aim of this work is to develop a new polymeric delivery system that can provide a shorter duration of degradation and erosion. Moreover, its degradation products would be nontoxic and biocompatible. We have chosen inulin as a backbone polymer because of its prior history of intravenous use in man as a diagnostic agent for the measurement of glomerular filtration rate.

Inulin is a nontoxic water-soluble polysaccharide with a molecular weight around 5000. It is excreted via the kidney without metabolism. Inulin derivatives containing covalently attached drug moieties have been investigated as potential macromolecular prodrugs. Schacht and coworkers[6–12] synthesized and evaluated polymeric drugs consisting of procanamide or metronidazole covalently attached to inulin and inulin monosuccinate. More recently, Williams and Taylor[13] reported the synthesis and release evaluation of conjugates of cromoglycate with inulin. However, this macromolecular prodrug approach may be limited by the relatively low drug loading achievable through covalent attachment.

Similar to other polysaccharides, the hydroxyl groups on inulin can be converted to ester or carboxyl groups by reacting with mono- or dicarboxylic acids, respectively. Depending on the specific substitution, modified inulin may display a range of different solubility and physico-chemical characteristics. Because inulin has a prior history of use in humans, and is quickly eliminated from the body, inulin esters with different solubility characteristics should be potentially useful as erodible carriers for drug delivery. In addition, inulin esters may be more advantageous over some presently used biodegradable polymers for two reasons: (a) the ester bonds can be hydrolyzed to produce inulin and acids without enzymatic catalysis; (b) the acids generated are acetic and succinic acid, which are endogenous and nontoxic.

In the present study, we have synthesized inulin acetate succinates (ILAS) with a wide range of acetyl and succinyl contents to impart different water solubilities at or near neutral pH. We have also prepared and characterized drug-loaded microspheres based on these inulin derivatives using chlorehexidine (CHX) and chymotrypsin (CTS) as model drugs.

EXPERIMENTAL

Synthesis of Inulin Acetate Succinate

The synthesis was carried out in dimethyl formamide (DMF) (Fisher Scientific) by reacting inulin (Sigma; from dahlia tubers) with acetic anhydride (Fisher Scientific) and/or succinic anhydride (Aldrich Chemical Company) in various proportions at 40°C for a duration up to 24 h. Sodium acetate (Fisher Scientific) was used as the catalyst (about 0.05% w/w). The reaction scheme is shown in Figure 1, and the reaction recipe is listed in Table I. All chemicals were reagent grade, and used as received. Typically, inulin was reacted with acetic anhydride for 1.5 h followed by the addition of succinic anhydride, and the reaction continued for an additional 22 h. The reaction product was then precipitated in an excess amount of ethyl ether (for ILS) or water (for ILA, ILAS-1 and ILAS-2). The product was subsequently purified by repeated dissolution and precipitation, and then dried in a vacuum oven at 50°C until constant weight.

Characterization of Inulin Acetate Succinate

The acetyl content of inulin acetate succinate was analyzed using USP assay for cellulose acetate phthalate (CAP). The succinyl content and the pKa values were determined by potentiometric titration with 0.1*N* NaOH in water or in ethanol/water mixture. The solubility of the inulin derivatives in aqueous media was determined at different pHs (pH 5–8) in phosphate buffers of fixed ionic strength (0.1*M*), and at a fixed pH of 7.4 in phosphate buffers of different ionic strengths (0.01–0.25*M*).

The chemical composition of these inulin esters was characterized by FTIR (Bio-Rad FTS-7) on KBr disk and ATR FTIR on polyester sheet. The

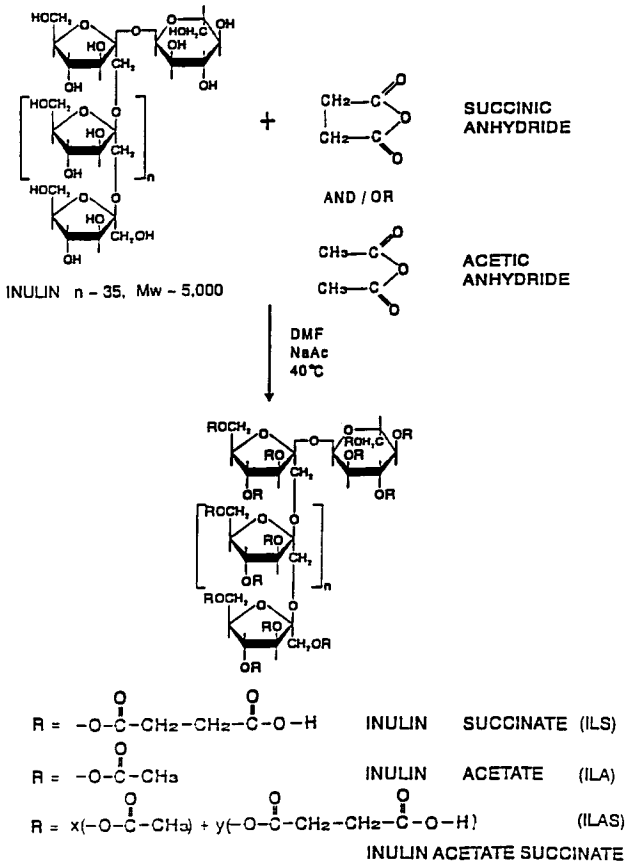

Figure 1 Reaction scheme of acetylation and succinoylation of inulin.

proton NMR spectra were measured with Gemini 200 MHZ (Varian) in deuterated DMSO using tetramethylsilane (TMS) as the reference. The melting points of inulin and inulin derivatives were measured by DSC (Perkin-Elmer DSC-2C) at a heating rate of 10°C/min, and by a capillary melting point apparatus (Thomas Hoover).

Preparation and Characterization of Microspheres

Microspheres of inulin esters, with and without the drug, were prepared by the solvent precipitation method using Tween 80 as a dispersant. Typically, a few drops of a 5% Tween 80 aqueous solution were added to a solution of 5% inulin derivative dissolved in acetone or ethanol. The solution was then titrated with deionized water under mild mixing, until the solution turned milky. Subsequently, microspheres were formed after evaporating the organic solvent. The drug-loaded microspheres were prepared using the same procedures except for the inclusion of a drug in the initial polymer solution.

Size and morphology of the microspheres were determined by scanning electron microscope (Hitachi S-570). *In vitro* drug release from the microspheres of modified inulin was studied in pH 8 phosphate buffer at 37°C in a flask with shaking. The release of chlorhexidine and chymotripsin was monitored on a UV-Vis spectrophotometer (Hewlett Packard 8452A) at wavelengths of 262 and 276 nm, respectively. The integrity of the microspheres in pH 7.4, pH 8.0 buffer solutions, and in deionized water was monitored using optical microscope, as well as scanning electron microscope (SEM).

RESULTS AND DISCUSSION

IR Spectra and NMR Spectra

Inulin esters with acetyl content 0–45% and succinyl content 0–66% (w/w) were prepared and characterized by FTIR and NMR. The IR spectra of inulin, ILA, inulin succinate (ILS) and ILAS are compared in Figure 2. It is clear that when the OH groups are substituted by acetyl and succinyl groups, the OH stretch band of inulin (~ 3353 cm^{-1}) diminishes, and the carbonyl band (C═O, ~ 1745 cm^{-1}) emerges. In addition to the carbonyl band, ILA is also characterized by the acetate C—O band (~ 1230 cm^{-1}) and —CH_3 band (~ 1370 cm^{-1}). Similarly, ILS shows an acid C—O stretching band (~ 1160 cm^{-1}) and a strong ester C═O band (~ 1750 cm^{-1}) overlapping with the acid C═O band (~ 1740 cm^{-1}). On the other hand, ILAS exhibits the characteristics of both the acetate and succinate. The absorbance ratio of C═O to C—O—C and the ratio of C—O to C—O—C are plotted in Figure 3 against the re-

Table I Reaction Recipe for Modification of Inulin

Sample	Inulin (g)	Acetic Anhydride (g)	Succinic Anhydride (g)	DMF (mL)
ILA	7.5	42.5	0	100
ILAS-1	5	8.4	6.2	40
ILAS-2	5	16.4	4.0	40
ILAS-3	10	7.8	1.9	80
ILS	10	0	25	86

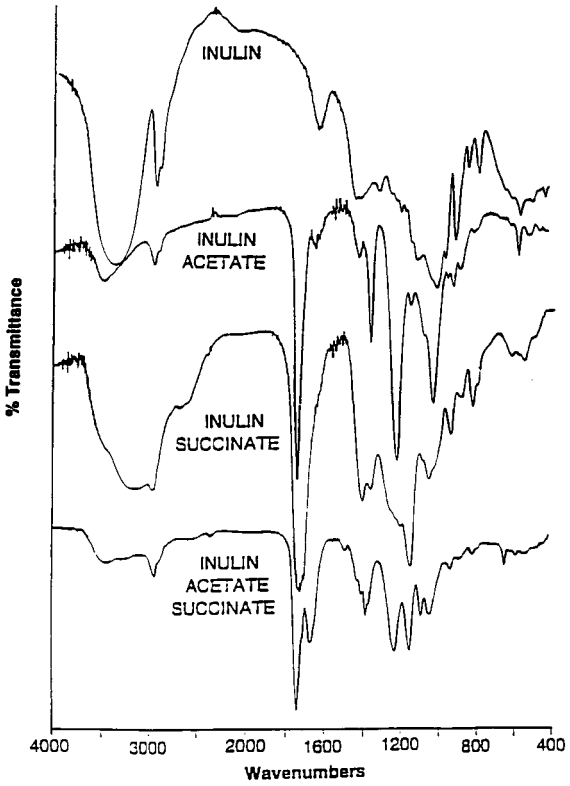

Figure 2 Comparison of IR spectra of inulin, inulin acetate, inulin succinate, and inulin acetate succinate.

action time for acetylation of inulin (ILA). It is seen that fast acetylation occurs in the first hour, followed by a slower reaction; about 65% acetylation is completed within the initial 1.5 h. This

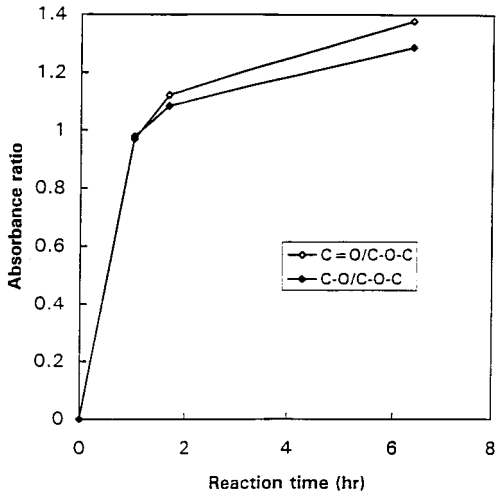

Figure 3 IR absorbance ratio vs. reaction time for acetylation of inulin.

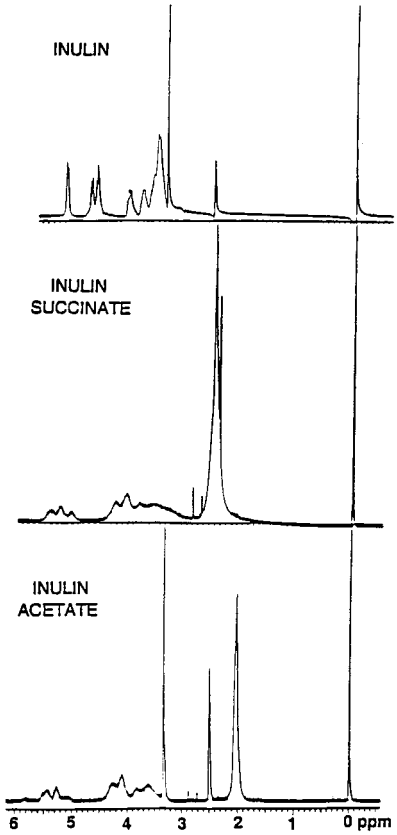

Figure 4 ^{1}H-NMR spectra of inulin, inulin acetate, and inulin succinate measured in deuterated DMSO using TMS as reference.

kinetic information was used to determine the appropriate time for addition of succinic anhydride to the reactant solution during the preparation of ILAS.

The ^{1}H-NMR spectra of inulin, ILA and ILS in deuterated DMSO are summarized in Figure 4. The spectrum of ILS is similar to that reported by Schacht et al.[7] The ^{1}H of —CH_2CH_2— in the succinyl group has a chemical shift of 2.43 ppm, overlapping with that of the trace ^{1}H in deuterated DMSO (2.52). In contrast, the ^{1}H of acetate $COCH_3$ appears at $\sim$ 1.94 ppm.

pKa, Solubility, and Melting Point

The pKa of the inulin esters in 70% ethanol and in water (for ILS only) was evaluated from the titration curve at half neutralization. The pKa of ILAS-1 and ILAS-2 in water was estimated from the plot of pKa against the ethanol volume fraction due to their low solubility in water. The pKa values, together with the composition and the

Table II Composition, pKa, and Solubility of Inulin Esters

Sample	Acetyl Content (wt %)	Succinyl Content (wt %)	pKa in 70% Ethanol	pKa in Water	Solubility in pH 7.4 Buffer (0.1*M*) (wt %)
ILA	45	0	—	—	—
ILAS-1	38	33	6.9	6.5	4.0
ILAS-2	26	41	7.0	6.1	5.4
ILS	0	66	6.7	5.0	> 54

solubility in pH 7.4 buffer (0.1*M* ionic strength), are summarized in Table II. As expected, the solubility increases with the succinyl content at the similar degree of substitution of hydroxyl groups. The dependence of ILAS solubility on the buffer pH and ionic strength was studied and the results for ILAS-1 are presented in Figure 5. The solubility of ILAS increases dramatically as the pH is increased above its pKa. In pH 7.4 buffer, the solubility reaches a maximum at an ionic strength around 0.15*M*. This peak solubility is likely a reflection of a balance between two competing processes, namely ion exchange and ionization suppression. ILAS is a weak acid which may experience the following ion exchange:

$$R\text{—}COOH + M^+ \leftrightarrow R\text{—}COO^-M^+ + H^+$$

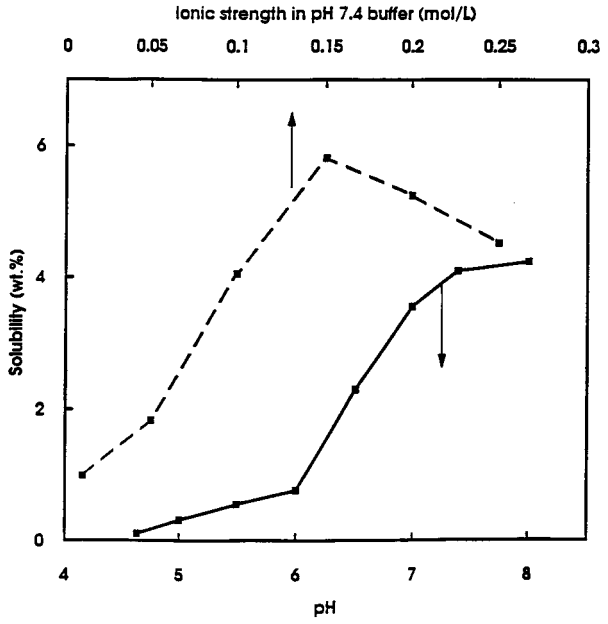

Figure 5 Solubility dependence of inulin acetate succinate (sample ILAS-1) on pH and ionic strength in phosphate buffer solutions.

where M^+ represents cationic species in the buffer such as Na^+ or K^+. At lower ionic strength, for example, $\mu < 0.15M$, ion exchange is dominant, and hence, increasing ionic strength favors the ionization of ILAS. On the other hand, increase in ionic strength also suppresses the electric double layers around the ionic polymer chains, thereby changing the polymer conformation from an expanded form to more compact one. This conformational change could cause precipitation of the polymer from solution, also known as the "salting-out effect." This suppression effect is more significant as the ionization degree increases (reflected by an elevated solubility) and becomes predominant at higher ionic strength (e.g., $\mu > 0.15M$).

The DSC spectra of inulin and its derivatives are shown in Figure 6 where the melting points are clearly visible. The melting points measured by the DSC and capillary apparatus are summarized in Table III. It is evident that the melting point significantly decreases after the esterification.

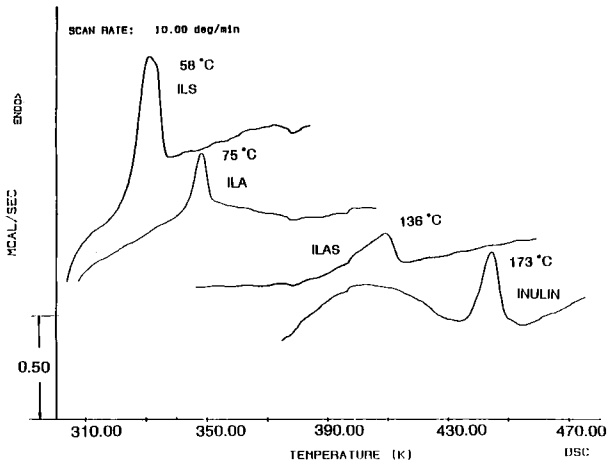

Figure 6 DSC spectra of inulin, inulin acetate, inulin succinate, and inulin acetate succinate, at a scanning rate of 10°C/min.

Table III Melting Points (T_m) of Inulin, Inulin Acetate, and Inulin Succinate

Sample	T_m (°C) by DSC	T_m (°C) by Capillary
Inulin	173	172–176
ILAS-1	136	136–139
ILA	75	87–92
ILS	58	—

Characterization of Microspheres

The particle size and morphology of inulin acetate microspheres were examined by SEM (Fig. 7). Particles of diameter 0.5–4 μm appear mostly intact after storage in deionized distilled water, but appear to be "fused" together after immersion in pH 8 phosphate buffer (ionic strength 0.1*M*) for 6 days. This may be a result of hydrolysis of the surface of the particles, which converts the inulin acetate back to the water-soluble inulin. In contrast, chymotripsin-loaded inulin acetate microspheres (ILA/CTS) show little change in pH 8 buffer, as illustrated in Figure 8. In addition, ILA microspheres loaded with chlorhexidine (ILA/CHX) are much bigger than those of ILA and ILA/CTS produced under identical conditions (90–130 μm vs. 0.5–4 μm; Figs. 7–9), presumably due to differences in surface properties of the droplets during the solvent precipitation process. It is evident from Figure 9 that ILA/CHX particles have a smooth surface and porous interior, typical of particles prepared by the solvent precipitation method.[14–16] It should be mentioned that, although bulk ILA is frangible, ILA and ILA/CTS microspheres of small sizes exhibited sufficient strength to remain intact during handling.

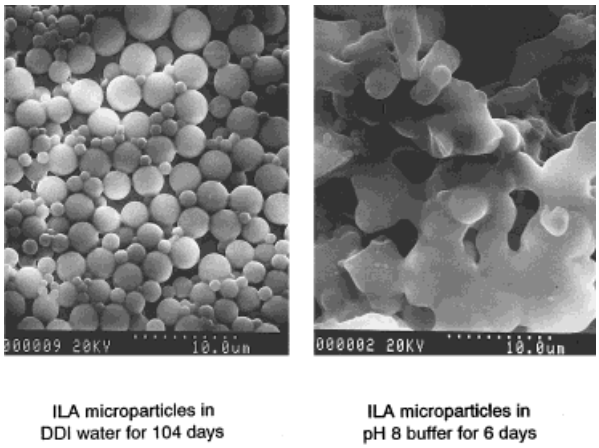

Figure 7 SEM photograph of inulin acetate microspheres after stored at room temperature (a) in deionized distilled water for 104 days; (b) in pH 8 buffer solution (ionic strength 0.1*M*) for 6 days.

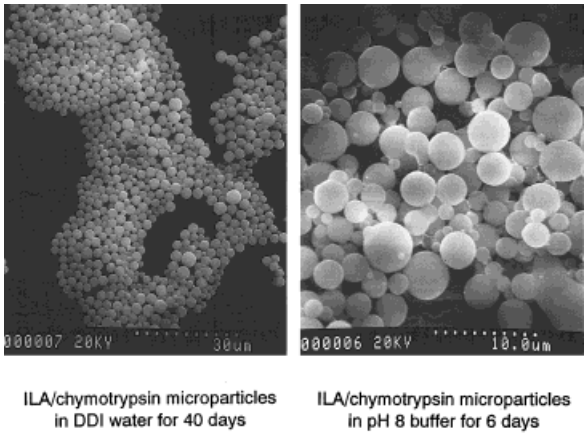

Figure 8 SEM photograph of inulin acetate microspheres loaded with chymotripsin after stored at room temperature (a) in deionized distilled water for 40 days; (b) in pH 8 buffer solution (ionic strength 0.1*M*) for 6 days.

CHX Solubility and Loading

To prepare microspheres of ILA with CHX, a solubility test was conducted on chlohexidine hydrochloride and ILA in mixtures of acetone/water and ethanol/water. The dependence of chlohexidine hydrochloride solubility on the

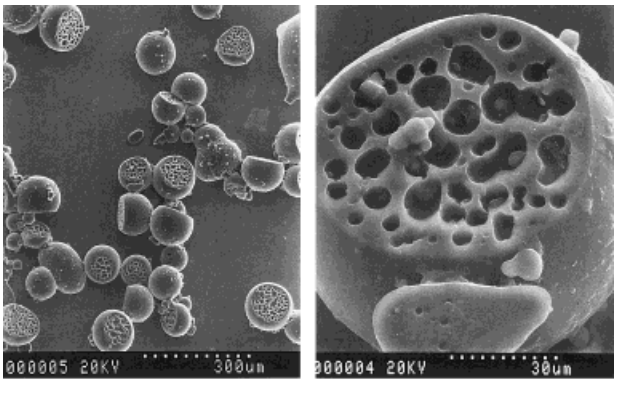

Figure 9 SEM photograph of fractured inulin acetate microspheres loaded with chlorhexidine.

volume fraction of acetone or ethanol is illustrated in Figure 10. The volume fraction at which ILA precipitates from the mixtures is also indicated in this figure. It is seen that chlohexidine hydrochloride displays maximum solubility at an acetone fraction of 0.5 and at an ethanol fraction of 0.6, respectively. When ILA solution of higher acetone or ethanol content was titrated with water, i.e., the volume fraction of the organic solvents decreases, ILA precipitates from the solution before the peak solubility of chlohexidine hydrochloride in acetone/water is reached, but beyond the peak solubility in ethanol/water. This suggests that if a CHX solution in ethanol/water (60 : 40) is used for the preparation of microspheres, some drug particles would precipitate before polymer precipitation upon the addition of water. As a result, drug particles may be entrapped during polymer precipitation resulting in microspheres with higher drug loadings. Therefore, ethanol/water was chosen as the solvent system for subsequent preparation of drug-loaded microspheres.

In Vitro Drug Release

Figure 11 shows the release profile of CHX and CTS from ILA/CHX and ILA/CTS microspheres in pH 8 buffer. It is seen that sustained release of both agents is achieved from the microspheres. Because inulin esters are biodegradable and their degradation products are nontoxic, they may be of potential application for delivery of drugs via a nonparenteral route.

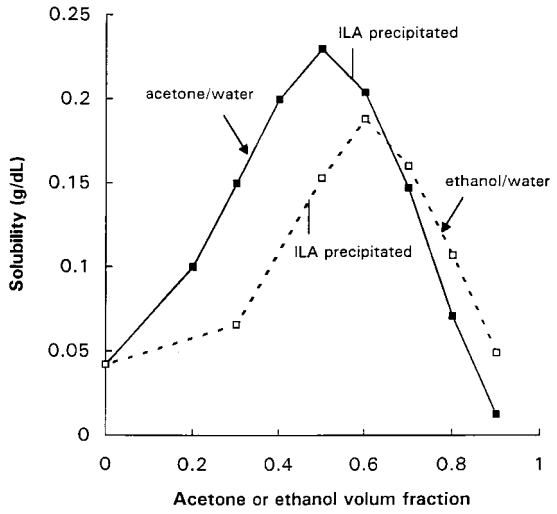

Figure 10 Solubility of chlorhexidine hydrochloride in acetone/water and ethanol/water mixtures of various compositions at room temperature. Peak solubility was observed in the both solvent mixtures. The mixture compositions at which inulin acetate (ILA) precipitated are marked by a solid line.

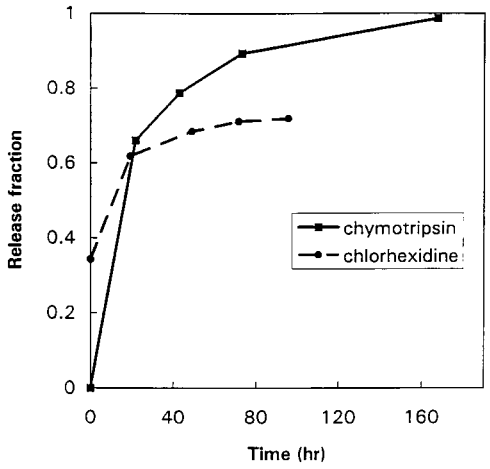

Figure 11 *In vitro* release of chymotripsin and chlorhexidine hydrochloride from inulin acetate succinate microspheres in pH 8 buffer at room temperature.

CONCLUSIONS

We have synthesized and characterized inulin acetate succinate of various acetyl and succinyl content. The solubility of the inulin esters has been found to depend on the composition, pH, and ionic strength. We have also prepared microspheres of inulin acetate loaded with model drugs, chlorhexidine, and chymotripsin. Our preliminary data suggest that these microspheres are able to release the incorporated drugs for a prolonged period of time.

This work was supported by the Medical Research Council of Canada (Grant No. MT-11486 to P.I.L.). Technical assistance from Jim Moselhy is also acknowledged.

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