The Use of Polyglycol Succinates for the Microencapsulation of Insulin

Reyhaneh Sariri* and Ali Ghannadzadeh
Department of Chemistry, Faculty of Science, Gilan University, Rasht, I.R. Iran

Received 11 April 2001; accepted 10 September 2001

ABSTRACT

Since the discovery of insulin and establishment of its value in the treatment of diabetes mellitus, many investigators have explored the possibility of developing an effective method for its administration other than by injection. The reason is that the necessary injections of the hormone are not only inconvenient but also result in physiological complications as well. In this study, the possibility of an oral administration of insulin was investigated. A group of biodegradable polymers from polyglycol succinates family was synthesized by bulk polycondensation reaction of glycols with diethyl succinate. The polymers were purified and used for microencapsulation of insulin. The size and shape of microcapsules were studied with optical and electron scanning microscope. The rate of release of core material from the microcapsules was measured by a direct UV absorption method. It was found that pH of the environment, microcapsules wall thickness and the type of glycol used in the polymerizations are the most important factors on the controlled release.

Key Words: microencapsulation, biodegradation, insulin oral administration, control release, polyglycol succinates

INTRODUCTION

Insulin, a hormone secreted naturally by special cells in the pancreas called the Islets of Langerhans, regulates the amount of glucose in the blood [1,2]. In 1953, Frederick Sanger determined in amino acid sequence of insulin, a protein hormone (Scheme I). Insulin is the primary hormone responsible for conversion of glucose to glycogen. Secretion of insulin is a response to increased glucose levels in the blood. When blood glucose levels rise (after a meal, for example), insulin is secreted from pancreas into the pancreatic vein, which empties into portal vein system (Figure 1), so that insulin traverses the liver before it enters the systemic blood supply. Insulin acts rapidly to lower blood glucose concentration in several ways. Insulin stimulates glycogen synthesis and inhibits glycogen breakdown in liver and muscle. Several other physiological effects of insulin also serve to lower blood and tissue glucose levels (Figure 2). Insulin stimulates the active transport of glucose (and amino acids) across the plasma membranes of muscle and adipose tissue. Insulin also increases cellular utilization of glucose by including the synthesis
of several important glycolytic enzymes, namely, glucokinase, phosphofructokinase and pyruvate kinase. In addition, insulin acts to inhibit several enzymes of gluconeogenesis. These various actions enable organism to respond quickly to increases in blood glucose levels.

Diabetes mellitus is the most common endocrine disease and the third leading cause of death with approximately 6 million diagnosed cases and an estimated 4 million more borderline but undiagnosed cases in the United States only [3]. In type I diabetes or insulin-dependent diabetes mellitus (IDDM), representing 10% of all cases, elevated blood glucose results from inadequate secretion of insulin by the Islets of Langerhans in the pancreas [4, 5]. These patients, therefore, need a daily administration of insulin. Type II diabetes also called non-insulin dependent diabetes mellitus (NIDDM), representing at least 90% of all cases, results from insensitivity to insulin. Table 1 shows the number of IDDM and NIDDM cases in different parts of the world for the year 2000. These figures are expected to rise by 28% by the year 2010 [6].

Because insulin is a protein, it is readily broken-down by proteolytic enzymes in the gastrointestinal tract and must, therefore, be administrated by subcutaneous or intravenous injections. Since the discovery of insulin and establishment of its value in the treatment of diabetes mellitus, many investigators have explored the possibility of developing an effective method for its administration other than by injection. The reason is that the necessary injections of the hormone are inconvenient and result in physiological complications as well.

The alternative pathways to the administration of insulin by injection that have been investigated are: oral [7-15], nasal [16-20], by suppositories [21, 22], and a few miscellaneous routes [23-25]. It seems likely that to afford maximum protection to insulin,
Table 1. Types I and II diabetes sufferers in different regions of the world (figures are in thousands) [6].

<table>
<thead>
<tr>
<th>Region</th>
<th>IDDM</th>
<th>NIDDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td>1608.3</td>
<td>82901.9</td>
</tr>
<tr>
<td>America</td>
<td>1407.9</td>
<td>28350.9</td>
</tr>
<tr>
<td>Europe</td>
<td>1181.7</td>
<td>25325.2</td>
</tr>
<tr>
<td>Oceania</td>
<td>83.0</td>
<td>955.6</td>
</tr>
<tr>
<td>Africa</td>
<td>142.1</td>
<td>9239.7</td>
</tr>
</tbody>
</table>

the integrity of the encapsulation material should be maintained until after permeation through the intestine wall. This can be best achieved with the use of polymers to encapsulate insulin. The polymer used for this purpose must have a number of particular properties among which the most important ones are:
- Resistance to acidic conditions in the gastrointestinal tract and to the activity of intestinal enzymes.
- Capable of passing through the intestine wall.
- Capable of releasing insulin into the blood stream.
- Having non-toxic products of biodegradation and these products must be exerted easily from the body.

A number of natural and synthetic biodegradable polymers are known with a hydrolytic degradation mechanism independent of the enzymatic system, which is pH dependent. Such that in acidic environment the degradation process is insignificant compared to the dilute alkaline solution. These polymers possess a group in their backbone, which is capable of hydrolysis such as carbonyl group in polyglycol succinates.

Polyglycol succinates are polyesters of succinic acid of the general formula:

\[-(O\text{-CO-}(\text{CH}_2)_n\text{-CO-OR})_n-\]

in which R is a linear or branched alkylene radical, e.g. ethylene glycol, butane-1,4-diol and hexane-1,6-diol. Polyglycol succinates have been known for a long time for their use as the stationary phase in the gas chromatography of n-paraffins, α-n-olefins, fatty methylesters, fatty alcohols, acetates, and dicarboxylic acid methylesters [26] as well as the production of environmentally friendly disposables [27]. It has been found that, in addition to their bioabsorbable character, polyglycol succinates are particularly well tolerated by the tissues in which they are implanted. Polyglycol succinates possess very good mechanical properties and are insensitive to moisture. Their filaments are easy to handle and do not necessitate the use of special storage device.

To prepare polyglycol succinates, it is possible to use bulk polymerization of glycols with free succinic acid or diethyl succinate in the presence of usual esterification catalysts such as sulphuric acid, lead oxide, antimony trioxide and zinc acetate.

Microencapsulation is a process designed to apply a thin membrane to small particles of solids, droplets of lipids, or gases. The membrane creates an intracellular environment for the particles preventing them from leaking out or coming into direct contact with the external environment. One of the interesting uses of microcapsules is to protect drugs from their environment. Microencapsulation techniques do not provide a perfect barrier for materials, which degrade in the presence of oxygen, moisture or heat, however, a great protection against these elements can be provided. For example, vitamin A has been protected from the action of oxygen and moisture [28]. Among the other applications of microencapsulation techniques the microencapsulation of pigment particles, paints and beauty aids such as perfume coated sheets are of more interest to the industry.

EXPERIMENTAL

Materials
Table 2 gives a list of monomers, catalysts, solvents and core materials together with their supplying company.

Polymer Synthesis
In a typical experiment, a polymerization mixture consisting of 58 g (0.33 mol) of diethyl succinate, 45 g (0.5 mol) of butane-1,4-diol, 0.093 g of zinc acetate, 0.023 g of antimony trioxide were placed into a 250 mL flask equipped with a mechanical stirrer. A nitrogen inlet tube, a thermometer and a condenser
The Use of Polyglycol Succinates for the Microencapsulation of Insulin

Table 2. Sources of monomers and reagents.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Sources of chemical Co.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene glycol</td>
<td>Sigma</td>
</tr>
<tr>
<td>Propane-1,3-diol</td>
<td>Sigma</td>
</tr>
<tr>
<td>Butane-1,4-diol</td>
<td>Sigma</td>
</tr>
<tr>
<td>Hexane-1,6-diol</td>
<td>Sigma</td>
</tr>
<tr>
<td>Diethyl succinate</td>
<td>B.D.H. Ltd.</td>
</tr>
<tr>
<td>Antimony trioxide</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Zinc acetate</td>
<td>Merck</td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td>Sigma</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Sigma</td>
</tr>
<tr>
<td>Methanol</td>
<td>Sigma</td>
</tr>
<tr>
<td>Tween-20®</td>
<td>Sigma</td>
</tr>
<tr>
<td>Span-85®</td>
<td>Merck</td>
</tr>
<tr>
<td>Span-60®</td>
<td>Merck</td>
</tr>
<tr>
<td>Insulin from bovine pancreas</td>
<td>Fisons</td>
</tr>
<tr>
<td>Neutral insulin solution</td>
<td>Fisons</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>Sigma</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>Sigma</td>
</tr>
</tbody>
</table>

The contents of the flask were heated, using an isomantle, to 180 °C over a period of 45 min with stirring and under a stream of nitrogen. The conditions were maintained for 2 h. During this period, butanol was distilled over, collected in the receiver and weighed. The nitrogen bleed was then disconnected and the temperature raised to 240 °C over a period of 30 min and the apparatus was connected to a vacuum pump. The reaction mixture was left to cool down after which the solid light brown product was dissolved in 150 mL of chloroform and the resulted solution was added dropwise to 1500 mL of vigorously stirred methanol to precipitate the polymer.

The product was filtered off and dried at 45 °C in a vacuum oven.

Other members of the polyglycol succinates were also synthesized in a similar manner and the resulting polymers were purified, dried, identified and their melting points were measured using a Perkin-Elmer differential scanning calorimeter.

Microencapsulation

The microencapsulation method used in this study was a single emulsion technique, a modification of the procedure used by Chang [29] for encapsulation of enzymes and cell contents. It is a simple physical method involving no chemical reaction. The following solutions and reagents were prepared before starting microencapsulation process.

- Insulin solution (3.0 mg/mL, 0.3 % by g weight).
- Polybutane-1,4-diol succinate (10 % by g weight in chloroform).
- Emulsifier, Span-85® solution (10 % by g weight in carbon tetrachloride).
- Organic solution, 100 mL chloroform was saturated with water and 1 mL of solution 3 was then added just before use.
- Tween-20®, 50% and 1% solutions in distilled water.

To a 150 mL glass beaker with an internal diameter of 6 cm containing 2.5 mL of insulin solution, 2.5 mL of the organic solution was added. The mixture was stirred immediately with a magnetic stirrer at a speed setting 7 using a 4 cm magnetic bar. Then, 25 mL of polymer solution was added and the stirring was continued for another 60 s. The beaker was covered and placed unstirred at 4 °C for 45 min. The microcapsules, formed in the top aqueous layer, were separated carefully using a pipette and placed in a 100 mL beaker. Immediately, 30 mL of Span-85® solution was added to the beaker and stirred on a magnetic stirrer at speed setting of 5 for 30 s.

The beaker was allowed to stand unstirred and uncovered at 4 °C for 30 min. The two layers were separated using a separating funnel; the microcapsules were in the top phase. A solution of 50% Tween-20® (23 mL) was added to the separated microcapsules. The suspension was then dispersed by stirring at a speed setting of 10 for 30 s. The stirring speed was slowed down to speed 5 and then 20 mL of water was added. The stirring was continued with a speed setting of 5 for 30 s. The suspension was then diluted further.
with 200 mL of distilled water.

The suspension was centrifuged at 250 rpm for 5 min in order to remove the slightly turbid supernatant. The microcapsules containing insulin were washed repeatedly with 1% Tween-20® solution until the washing solution showed no absorptions at 280 nm ($\lambda_{\text{max}}$ for insulin, Figure 3). The microcapsules were finally centrifuged and dried in a watch glass at room temperature.

The following modifications were made to the above method to produce microcapsules with different properties for release studies.

- Polyethylene glycol succinate and polyhexane-1,6-diol were used as microencapsulating polymer.
- The microcapsule wall thickness was varied using different polymer concentrations.
- Different emulsifying agents were used, e.g. Span-60® instead of Span-85® to observe their effect on the size of microcapsules.

Release Studies

UV Spectrum of insulin showed a maximum absorption at 275 nm (typical $\lambda_{\text{max}}$ for proteins). This wavelength was used to study the effect of different factors on the release of insulin from the microcapsules. The UV instrument was calibrated at 280 nm using different concentrations of pure insulin. The UV spectrum of a 0.3 % by g weight insulin solution is represented in Figure 3.

To study the release of insulin, 5 g of polyethylene glycol succinate microcapsules (containing 10–15 mg insulin) was placed in a soxhlet extraction thimble (internal diameter 22 mm, external diameter 24 mm, length 88 mm) and the thimble was suspended in a 75 mL test tube containing 50 mL of a buffer solution such that buffer covered the surface of microcapsules.

Similar test tubes were prepared in which the microcapsules made from polybutane-1,4-diol succinate and polyhexane-1,6-diol succinate with different wall thickness were placed in thimbles and suspended in both pH 7.4 and pH 2.0 buffer solutions. The test tubes were covered and placed in water bath at 37 °C (about body's temperature) and were agitated at frequent intervals. One mL of buffer solutions was removed at suitable intervals and assayed by UV. Absorbance of some standard insulin solutions were plotted against their concentration (mg/mL). The concentration of insulin released into the buffer solutions was then determined using the calibration curve.

RESULTS AND DISCUSSION

Some typical optical and scanning electron micrographs were selected to be included in this section. Other micrographs were also taken and our conclusion is based on all of the results. Figures 4 and 5 are optical micrographs of polyethylene glycol succinate contain-
ing insulin with polymer concentrations 5 and 10%, respectively. It can be seen that all microcapsules regardless of their wall thickness are spherical in shape. By comparing the Figure 4 with Figure 5, it is evident that the concentration of polymer used in the microencapsulation process does not considerably affect the shape and size of the microcapsules. It does show, however, some effects on the wall thickness of the microcapsules that will be discussed shortly. It was also found that the type of the emulsifying agent and polymer used do not significantly affect the shape and size of microcapsules (the micrographs are not included).

Scanning electron micrographs of microcapsules also confirm these results. Figure 6 shows polybutane-1,4-diol succinate microcapsules containing insulin. Microcapsules preferably form grape-like clusters with a range of size due to stirring speed during emulsification process. Figure 6 also shows a very interesting feature, which was observed in the electron scanning micrographs of almost all of the prepared microcapsules, that there are small holes on the microcapsule surfaces. The mechanism of the formation of such holes is not clearly understood. Nozawa et al. [30] have explained that the formation of holes on the surface of polystyrene microcapsules could be controlled by the stirring speed. He showed that the speed of stirring has a positive effect on the formation and number of holes on the microcapsule surface. It, however, seems likely that they participate in the release of the core material.

Despite its relatively large molecular dimensions (about 18×18×20 Å, [31]), insulin can penetrate out through the holes (1–2 µm diameter) of the microcapsules. We have also studied the release rate of smaller molecules such as sodium fluorescein from polyethylene glycol succinate microcapsules under similar conditions (the results will be published in due course). We found that smaller molecules such as sodium fluorescein also shows similar release behaviour. It seems likely that the biodegradation of the polymer must also be an important factor in the release of core material from the microcapsules. We have shown here that the release rate of insulin in an acidic environment is slower than it is in an alkaline solution (Figure 7). This observation can lead us to the conclusion that, in acidic environment the surface of the polymeric microcapsules may change so that the hole sizes reduce significantly and penetration through the holes becomes difficult and almost negligible. Under such conditions, the release of the core material is due to the biodegradation of polymer only. We can therefore conclude that controlled release of the core material from microcapsules takes place by both penetration through the holes and biodegradation of the polymeric wall. To study the release rates and factors affecting them, the following variables were
considered:
- The type of the diol used in the polymerization process, which affects the backbone structure of polymer.
- The thickness of the wall of microcapsules, which is directly proportional to the concentration of polymer used in the microencapsulation process.
- pH of the environment in which the microcapsules would release their contents. In this case two environments were used which represent the pH of the stomach (about 2.0) and pH of the blood (about 7.4).

Figures 7–9 show the release curves in different conditions. The shape of these curves shows that the polymer does not burst suddenly to release its contents, but that it degrades gradually until the core material is released completely. By comparing the Figure 8 with Figure 9 it is shown that the polymer concentration used in the microencapsulation process and the length of the diol in the synthesis of polymer both affect the release of the core material from microcapsules in a similar way. Biodegradation of a polymer with a smaller diol is slightly faster than with a longer diol (Figure 8). Figure 9 proves that a more condense polymer solution, used for microencapsulation produces microcapsules with thicker wall that biodegrade more slowly.

The mechanism of biodegradation of polyglycol succinates could be a hydrolysis reaction which in the case of polyethylene glycol succinate, e.g., may be presented as in Scheme II, where route A represents the splitting of the glycol and route B that of succinate bonds. In both types of hydrolysis reaction the products of biodegradation are simple organic compounds which can be metabolized and exerted from the body without any harm to the patient.

**CONCLUSION**

Based on the results obtained in this study, the following conclusions were made:
- Biodegradable polyglycol succinates are suitable
The Use of Polyglycol Succinates for the Microencapsulation of Insulin

for microencapsulation of insulin and may be synthesized using bulk polycondensation reaction.

- Microcapsules produced are spherical in shape, which preferably form grape-like clusters with a range of size (5–20 μm), which is determined by the type of emulsifier and, to a higher degree, by the speed of stirring during their formation.

- All of the microcapsules contain some holes on their surface, which may play a role on the release of the core material and the mechanism of their formation is not fully known.

- Microcapsule wall thickness may be varied by changing the concentration of the polymer used in the microencapsulation process.

- Biodegradation of polyglycol succinates is a hydrolysis reaction, which may be via the splitting of the glycol or that of succinate bonds. In both cases the products of biodegradation can be exerted from the body with no toxic effect.

- The release of insulin from polyglycol succinate microcapsules depends on the following factors:
  a. The thickness of the microcapsule wall; the thicker the wall, the slower would be the release rate.
  b. The structure of the polymer used for the microcapsule wall, the higher the length of the diol in the polymer backbone; the slower would be the release of the core material.
  c. For the pH of the environment, it is shown that polyglycol succinates can be biodegraded faster in a slightly alkaline (pH 7.4) than in an acidic (pH 2.0) environment. This is a very desirable property for the microcapsules containing insulin, because the polymer can protect the hormone in the acidic condition of stomach and release it in the blood stream.

REFERENCES


