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Poly (acryloyl-L-proline methyl ester) hydrogels obtained by radiation polymerization for the controlled release of drugs

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Abstract

Thermosensitive hydrogels were obtained by radiation-induced polymerization of acryloyl-L-proline methyl ester in the presence of a crosslinking agent. The measurements of equilibrium water content in the temperature range between 0° and 60°C showed that the samples swelled at low temperatures while they shrank at high temperatures. These hydrogels were used as drug delivery systems for the controlled release of insulin. In vivo studies carried out on diabetic rats ascertained a significant reduction in the hyperglycemic level in the blood which continued for about 2 months.

Keywords: Radiation polymerization; Thermosensitive hydrogel; Controlled release; Insulin

1. Introduction

Radiation processing of polymeric systems is a technology nowadays exploited for biomedical applications (Kaetsu, 1996). The main routes are represented by the radiation-induced polymerization and crosslinking to obtain hydrogels as carriers for the controlled release of drugs on the one hand and radiation modification of polymers by graft copolymerization to prepare biomaterials with an enhanced biocompatibility on the other. The reader interested in this topic can refer to an exhaustive review with almost 500 references recently published (Kabanov, 1998).

Considerable attention has been focused on hydrogels capable of changing their structure and properties in consequence of a physical or chemical stimulus such as temperature, light, pH and so on (Hoffman, 1995; Okano, 1998). These "intelligent" materials can be advantageously designed as self-regulating and targetable drug delivery systems.

Insulin, a hormone released by the pancreas, controls the glucose level in the human body and regular injections of this drug are required for the diabetic patients lacking this. It is not difficult to understand that, owing to the fundamental importance of this drug, a pressing search of administration routes other than unpleasant injections (Trehan and Ali, 1998) is

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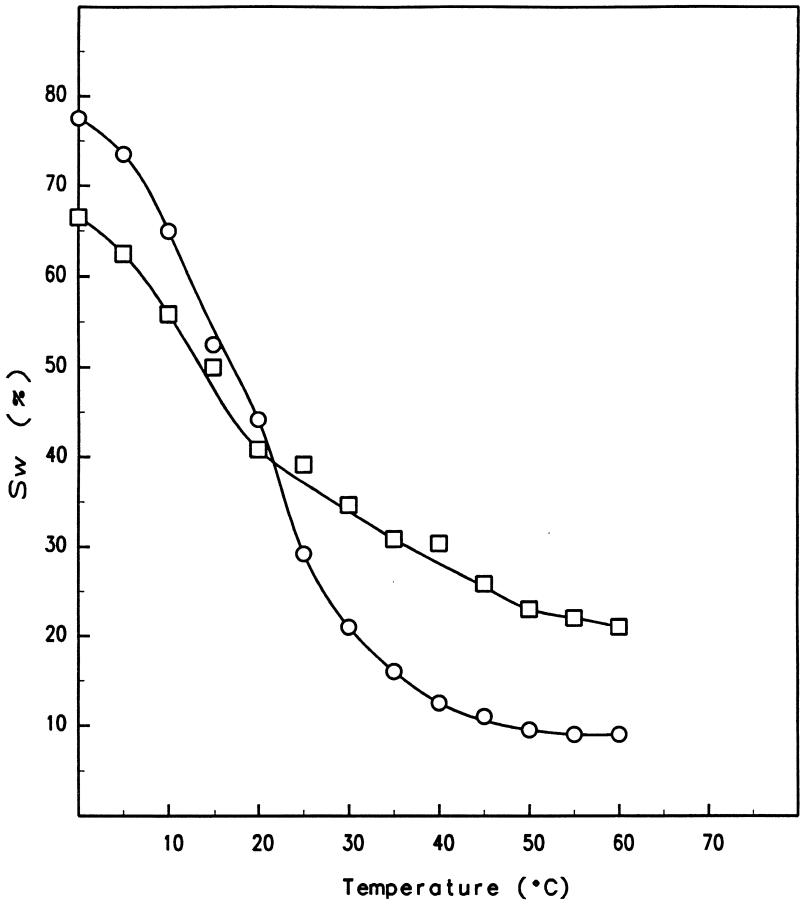

Fig. 1. Swelling percent as a function of temperature of poly(A-ProOMe) hydrogels crosslinked with TMPTMA in the ratio (w/w) 99/1 (○) and 95/5 (□).

one of the crucial challenges of the third millennium in the field of biomedicine.

A significant research interest for the treatment of diabetes is represented by self-regulating or pulsatile insulin delivery devices. A method involving the immobilization of glucose oxidase in a hydrogel membrane containing amine groups has been developed (Albin et al., 1985, 1987). When the glucose reaches such a high level to diffuse through the membrane, it is oxidized by glucose oxidase to gluconic acid thus decreasing the pH of the medium. The low pH induces the protonation of the amine groups with the consequence that the porosity of the membrane increases due to the electrostatic repulsion between the protonated amine groups. In turn, the swellability of the membrane also increases so that insulin can diffuse through it.

Hydrogels based on acryloyl-L-proline methyl ester (A-ProOMe) belong to the class of thermosensitive hydrogels. In recent years, studies have been carried out in our laboratories on the radiation polymerization, characterization and application of this material for a sustained release of drugs (Yoshida et al., 1991, 1992, 1996; Miyajima et al., 1993; Martellini et al., 1999; Carenza et al., 1999).

In vitro experiments carried out with poly(A-ProOMe) matrices showed that an analgesic and antipyretic drug was slowly released in a period of time of a couple of days (Martellini et al., 1999).

In this work it was thought of interest to use the same device for an in vivo controlled release study of insulin as a model drug whose response in the animals is easily determined.

2. Experimental

2.1. Synthesis and swelling of hydrogels

A-ProOMe was synthesized from L-proline methyl ester hydrochloride and acrylic acid as previously described (Yoshida et al., 1992). Trimethylolpropane

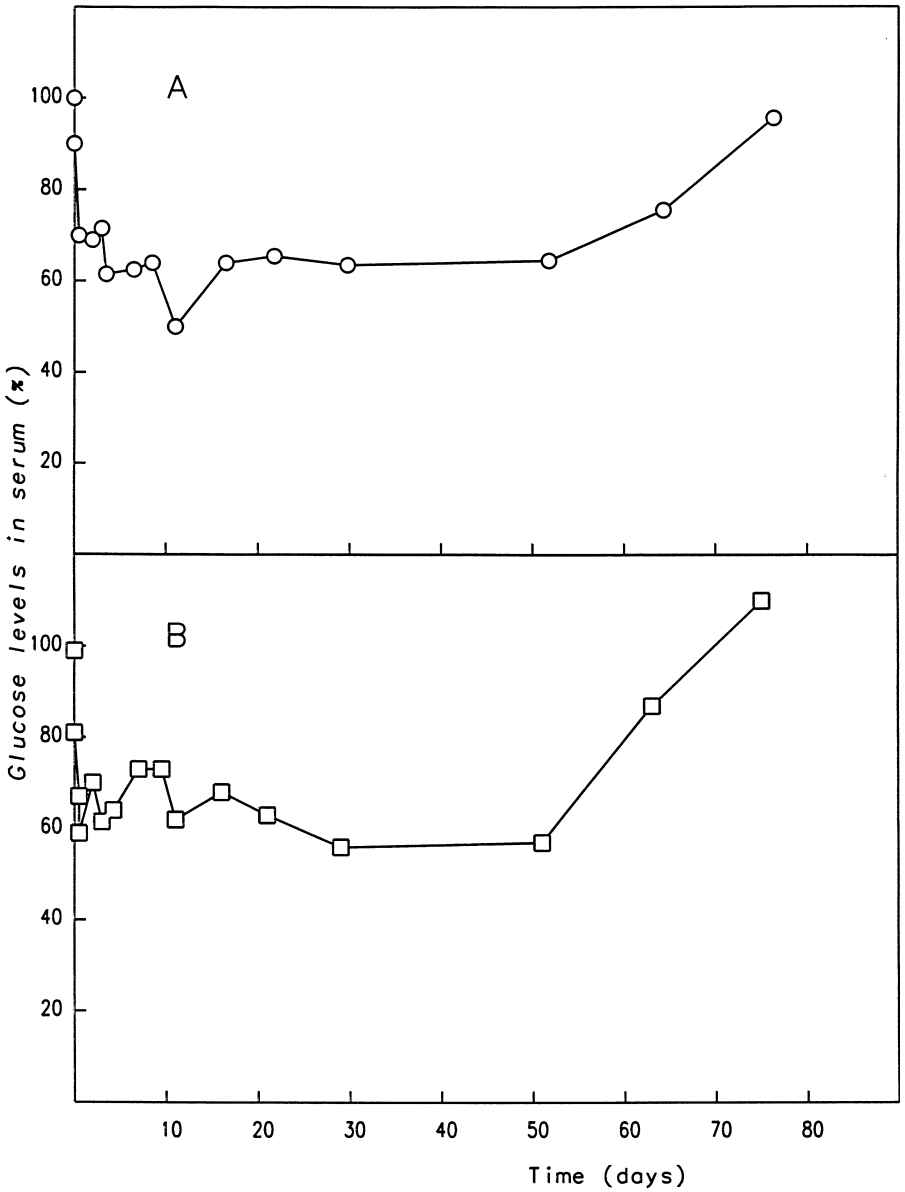

Fig. 2. Reduction of glucose concentration in the serum of diabetic rats implanted with insulin loaded matrices 1% TMPTMA (A) and 5% TMPTMA (B).

trimethacrylate (TMPTMA), an Aldrich Chemical Co. product, was used as received.

Hydrogels were obtained by radiation polymerization of A-ProOMe in the presence of 1% and 5% TMPTMA at 25°C and at the dose rate of 0.36 Gy/s (total dose = 25 kGy) under nitrogen.

Solid and transparent samples were obtained in a shape of discs 5 mm diameter and 1.5 mm height that were repeatedly washed with cold water to remove the unreacted monomer and crosslinking agent.

The swelling degree, Sw, of hydrogels was determined by the ratio of the weight of water absorbed to the weight of dry polymer.

2.2. Insulin loaded matrices preparation

Four matrices with 1% and 5% TMPTMA were maintained in 20 ml of deionized water at 4°C for 7 days. After the washing procedure, the matrices were liophilized for 2 days and weighed.

Each matrix was incubated with 0.5 ml of insulin solution (20 mg/ml in 0.2 M acetic acid) at 4°C for 7

days and then quickly washed with 0.2 M acetic acid, lyophilized and weighed. The volume of the insulin solution after incubation with the matrix was measured and the hormone concentration was evaluated by Biorad DC1 protein assay.

The matrices with 1% TMPTMA were found to contain 3.8 ± 0.7 mg of insulin while the matrices with 5% TMPTMA contained 1.3 ± 0.6 mg of drug.

2.3. In vivo studies

Six male Sprague–Dawley rats weighing 280–300 g were intraperitoneously treated with a dose of 65 mg/kg of streptozotocin to induce diabetes. After 1 week the animals were bled by an intracardiac puncture and the glucose level in serum was estimated using the glucose Trinder Kit of Sigma. The glucose level was verified for three times at alternate days.

Two matrices with 1% TMPTMA loaded with 4.1 and 4.5 mg of drug, respectively, and two matrices with 5% TMPTMA containing 1.6 and 1.8 mg of insulin, respectively, were subcutaneously implanted in four animals while two animals were maintained as controls. The glucose level in the animals was estimated at scheduled times and expressed as 100% of the starting value.

3. Results and discussion

In previous works (Yoshida et al., 1991; Martellini et al., 1999) we obtained by radiation pure poly(A-ProOMe), a polymer that shows a lower critical solution temperature (LCST) around 15°C, i.e. at temperatures below this value it is soluble in water while above it precipitation occurs. Irradiation carried out in the presence of TMPTMA, a crosslinking agent, gives rise to hydrogels whose swelling at equilibrium decreases as the temperature increases (see Fig. 1).

Hydrogels based on poly(A-ProOMe) were used as carriers for the controlled release of testosterone (Yoshida et al., 1991) and acetaminophen (Martellini et al., 1999). It was found that the diffusion of the drug depended on the swellability of the matrices, in other words the extent of release was higher the lower the temperature was.

Insulin was loaded in the polymeric matrices by incubation with a concentrated hormone solution at 4°C to obtain a high drug loading since in these conditions the swelling degree is very high (see Fig. 1). The entrapped amount of drug was found to depend on the hydrogel swellability, in particular the matrices with 1% TMPTMA entrapped three times as many as the insulin amount loaded in the matrices with 5% TMPTMA.

An advantage of using a thermosensitive hydrogel is the possibility to change the matrix porosity with the temperature. Actually, a hydrogel which exhibits both a high porosity and swelling can be not suitable for a controlled release device owing to the rapid diffusion of the drug with the consequence of a complete release in a very short time. On the contrary, a matrix allowing the maximum swelling and, hence, a maximum loading at a temperature other than that of the release, as is our case, can represent a good opportunity.

As shown in Fig. 2, in vivo studies carried out with diabetes induced rats demonstrated that the subcutaneous implantation of the matrices could significantly reduce the hyperglycemic levels in the blood. In both cases, a reduction of about 40% of the glucose levels was observed for about 2 months, thus indicating that insulin was slowly released in the active form during this period. However, a small difference can be observed in the behaviour of the two matrices. In the case of 5% TMPTMA matrices, the amount of glucose in the serum returns to the initial value of 100% about 10 days before than the 1% matrices, maybe due to the fact that in the former case the drug loading is lower that in the latter.

These preliminary results seem promising in expectation of the use of these matrices for drug delivery. A more exhaustive investigation to evaluate the effectiveness of these systems is in progress.

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