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Thermally reversible gels based on acryloyl-L-proline methyl ester as drug delivery systems

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Abstract

Thermally reversible hydrogels were synthesized by radiation-induced copolymerization of acryloyl-L-proline methyl ester with hydrophilic or hydrophobic monomers. The swelling behaviour was found to be affected by a proper balance of the latter. In particular, the transition temperature of the different hydrogels shifted to higher or lower values depending on the presence of hydrophilic or hydrophobic moieties in the polymer chain, respectively. Acetaminophen, an analgesic and antipyretic drug, was entrapped into some hydrogels and a wide range of release rates was obtained according to the nature of the comonomers. A novel thermoresponsive hydrogel was also prepared by radiation polymerization of acryloyl-L-proline methyl ester in the presence of 4-acryloyloxy acetanilide, an acrylic derivative of acetaminophen. Again, the swelling curves showed an inverse function of temperature. It was shown that with this hydrogel bearing the drug covalently attached to the polymer backbone, the hydrolysis process was the rate-determining process of the drug release.

Introduction

Hydrogels are polymer networks which absorb and retain significant amounts of water (Peppas, 1986; Bouwstra and Junginger, 1993) and can be used as matrices for the immobilization of enzymes and cells in biocatalysis (Marconi, 1989; Gombotz and Hoffman, 1986) and for the controlled release of drugs (Gehrke and Lee, 1990).

In recent years much effort has been directed towards "intelligent" polymers which are capable of both specifically interacting with and responding to the immediate chemical environment. Also "smart" hydrogels which reversibly swell in water in response to environmental changes such as temperature, pH, electric field, light, ionic strength are developing (Okano and Yoshida, 1993; Hoffman, 1995; Galaev, 1995). With regard to thermosensitivity, in some cases it can be negative, i.e., hydrogels shrink when the temperature increases. This behaviour is observed for hydrogels

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prepared from polymers which exhibit a lower critical solution temperature (LCST) and it has been assumed that such a temperature is a function of a suitable balance between hydrophilic and hydrophobic groups in the polymer chain (Taylor and Cerankowski, 1975).

Ionizing radiation enables hydrogels with the proper design, function and use to be obtained (Carenza, 1992; Kaetsu, 1993; Rosiak et al., 1995). By means of this technique new acrylic and methacrylic hydrogels bearing α-amino acid groups in the polymer chain have been synthesized and used as drug delivery systems (Yoshida et al., 1989).

In the present study the radiation synthesis of hydrogels based on acryloyl-L-proline methyl ester (A-ProOMe) with hydrophilic or hydrophobic moieties in the polymer chain is reported. Acetaminophen, also named paracetamol, an analgesic and antipyretic drug, was entrapped and the effect of the hydrophilic/hydrophobic balance on its release was investigated.

A great deal of work has been recently devoted to the synthesis and characterization of methacrylic esters of acetaminophen and the evaluation of their pharmacological activity and biocompatibility (San Roman et al., 1994). It was therefore thought of interest to synthesize a new thermoresponsive hydrogel by radiation copolymerization of A-ProOMe with an acrylic derivative of acetaminophen, which was synthesized in our laboratories, and to investigate the swelling and the hydrolytical degradation.

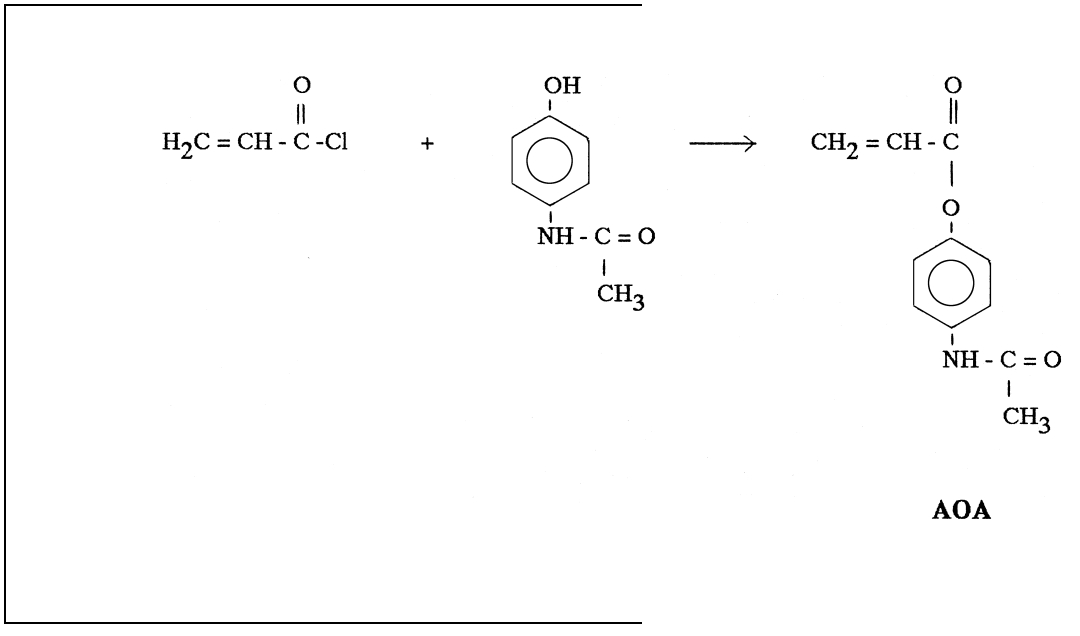
Experimental

Materials

A-ProOMe was synthesized at JAERI, Takasaki, according to the method already described (Yoshida et al., 1992). 4-acryloyloxy acetanilide (systematic name: 4-acetamidophenyl acrylate), henceforth designed as AOA, was synthesized at IPEN, Sao Paolo, as described in the following section. N,N-dimethylacrylamide (DMAA), 2-cyanoethyl acrylate (CEA), trimethylolpropane trimethylacrylate (TMPTMA) and acryloyl chloride, from Aldrich Chemical Co., and 4-hydroxyacetanilide (acetaminophen), from Sigma Chemical Co., were used as received.

Synthesis of AOA

AOA was synthesized through the reaction at 0°C of 0.2 mole acryloyl chloride and 0.1 mole 4-hydroxyacetanilide dissolved in a solution of 150 ml of 5% aqueous solution NaOH and 20 ml dioxane. AOA formed by precipitation was filtered and purified by a double precipitation with cool water and dissolution in a mixture of acetone and methanol in the ratio 4:1 (v/v). The yield was around 60%. The reaction scheme is:



AOA

Synthesis of thermoresponsive hydrogels

Poly(A-ProOMe) homopolymer was prepared by radiation-induced polymerization of the related mono-

mer using γ-rays from a ^{60}Co source at the dose rate of 0.36 Gy/s and at room temperature after flushing nitrogen. After irradiation, the polymer was repeatedly washed with cool water to remove the unreacted monomer.

In the same experimental conditions, hydrogels were obtained by irradiation of the mixtures A-ProOMe/TMPTMA, A-ProOMe/DMAA, A-ProOMe/CEA and A-ProOMe/AOA at the dose of 25 kGy which enabled the total polymerization conversion to be achieved. Solid and transparent samples were obtained in a cylindrical form by separating the product from the mould in which they were contained. The small cylinders were cut into 5 mm diameter and 1.5 mm height discs which were allowed to swell in cool water for several days to remove the unreacted monomer.

Determination of swelling

Hydrogel samples were equilibrium swollen at different temperatures and weighed after wiping the excess surface water. Subsequently, they were dried for 24 h in a vacuum heater and weighed again. The swelling ratio, *Sw*, of the hydrogels at the equilibrium was calculated as follows:

$$Sw = \frac{W - W_0}{W} \times 100 \quad (1)$$

where W and W_0 are the weights of the swollen and dried samples, respectively.

SEM analysis

Samples swollen at different temperatures were lyophilized and, after being fractured in liquid nitrogen, analysed using a Cambridge Stereoscan 250.

Drug loading

Copolymer hydrogels with entrapped 10% acetaminophen were prepared by irradiation at 25°C and at the dose rate of 0.36 Gy/s of the homogeneous solutions of A-ProOMe containing both the drug and DMAA, CEA, AOA and TMPTMA in different proportions.

Some runs were also carried out adopting a different procedure. Copolymer hydrogels in a shape of discs were prepared by irradiation of the mixtures of A-ProOMe with DMAA, CEA and TMPTMA, then lyophilized and finally immersed in a saturated aqueous solution of acetaminophen at 5°C. After three days the discs were removed from the solution, immediately transferred into liquid nitrogen and lyophilized again. The amount of the drug loaded was evaluated from the weights of the gels before and after loading.

Drug release from thermoresponsive discs

Hydrogel discs with the entrapped acetaminophen were allowed to swell in distilled water at 0°C for 24 h before determination of the drug release. Afterwards, they were quickly transferred to a tube containing 20 ml of water at 37°C and, at fixed times, 20 μl aliquots were taken and immediately replaced by pure water. After the addition of 500 μl, the concentration of the drug released was assayed by means of a UV Pharmacia LKB-Ultrospec III spectrophotometer at the wavelength of 246 nm.

Results and discussion

In recent years, radiation polymerization of homo- and co-polymers of A-ProOMe as well as their property and application as drug delivery systems have been extensively investigated (Yoshida et al., 1991,

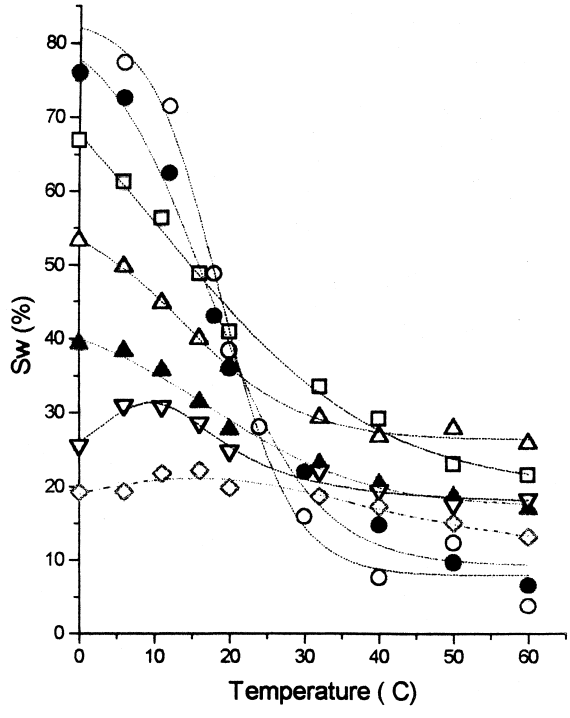


Fig. 1. Swelling ratio as a function of temperature of the hydrogels obtained by radiation-induced polymerization of the mixtures A-ProOMe/TMPTMA in the ratio (w/w):99.5/0.5 (○); 99/1 (●); 97/3 (□); 94/6 (△); 90/10 (▲); 87/13 (▽); 81/19 (◇).

1992, 1995, 1996; Safranj et al., 1993; Miyajima et al., 1994).

In this work several hydrogels based on A-ProOMe were synthesized by radiation and the hydrophilic/hydrophobic balance of the polymer chains was changed in the attempt at investigating the effects on thermosensitivity and on release of a model drug (Taylor and Cerankowski, 1975). To this purpose DMAA, a hydrophilic monomer, and CEA and TMPTMA, both hydrophobic monomers, were selected. It should be noted that the latter is a crosslinking agent while the CEA polymers are known to undergo degradation (Piskin, 1994).

To determine the LCST of pure poly(A-ProOMe), the polymer was left in water at 6°C where it was completely soluble. Every hour the temperature was increased by 1°C until the transparent polymer solution became suddenly opaque. This temperature was taken as the LCST of poly(A-ProOMe) and it was found to be 17°C, a value a little higher than 14°C previously determined (Yoshida et al., 1992). It should be stressed that the same value of 17°C was determined even when the experiment was carried out in the opposite way, i.e., by decreasing the temperature at 1°C at a time, starting from a temperature higher than 17°C, and evaluating the temperature at which transparency was again attained, in other words it can be concluded that the transition was reversible.

If A-ProOMe was irradiated in the presence of TMPTMA, the samples were not soluble any more. In Fig. 1 the swelling data of hydrogels as a function of both temperature and concentration of the crosslinking agent are reported. An inverse function of temperature is apparent and, moreover, as the concentration of TMPTMA decreases the swelling increases. The values of the latter are in the range between 82% for the hydrogel A-ProOMe/TMPTMA in the ratio 99.5/0.5 and 20% for the hydrogel in the ratio 81/19, for which the dependence of swelling on temperature is not discernible any more. Interestingly, the hydrogels containing more than 3% crosslinking agent show a continuous decrease of swelling with increasing temperature while for the concentrations lower than 3% transition temperature is observed. For the ratio 99.5/0.5 such a temperature is around 18°C and there is a tendency for it to decrease as the hydrophobic component increases (Taylor and Cerankowski, 1975).

This effect is more pronounced for hydrogels containing CEA, a hydrophobic moiety as well, whose swelling curves are shown in Fig. 2. It can be seen that for the hydrogels with a ratio A-ProOMe/CEA of 98/2

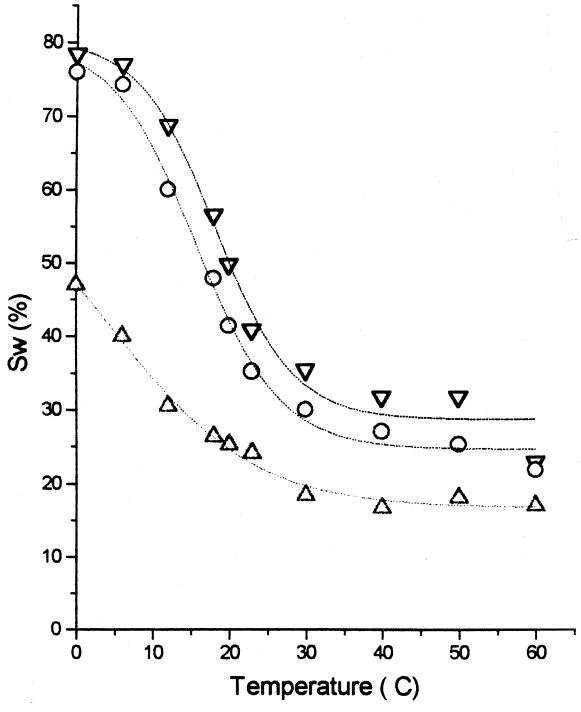


Fig. 2. Swelling ratio as a function of temperature of the hydrogels obtained by radiation-induced polymerization of the mixtures A-ProOMe/CEA/TMPTMA in the ratio (w/w):98/2/1 (▽); 95/5/1 (○); 85/15/1 (△).

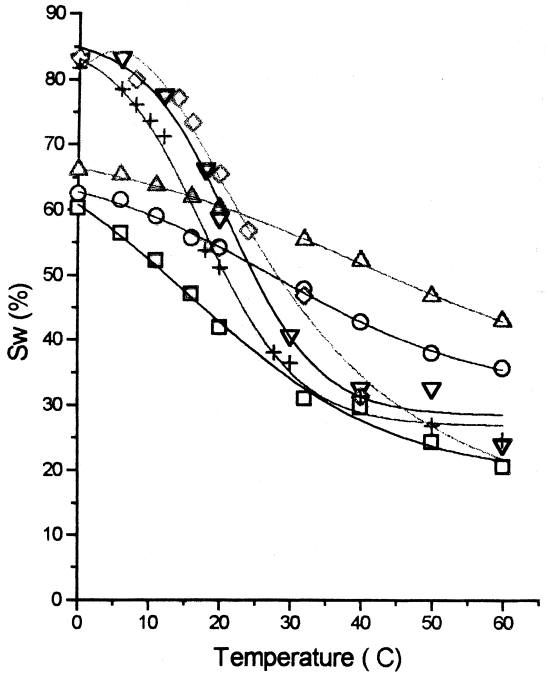


Fig. 3. Swelling ratio as a function of temperature of the hydrogels obtained by radiation-induced polymerization of the mixtures A-ProOMe/DMAA/TMPTMA in the ratio (w/w):99.5/0.5/1 (+); 98/2/1 (◇); 95/5/1 (▽); 95/5/3 △); 80/20/3 (○); 70/30/3 (□).

and 95/5 there is a shift of the transition temperature from 18° to 15°C, respectively.

When hydrogels were obtained by irradiation of A-ProOMe in the presence of hydrophilic DMAA, a pattern similar to that described in the previous figures was observed, as it appears from the data reported in Fig. 3. Again, swelling decreases with increasing temperature and a discontinuous transition is attained at low concentrations of DMAA only. However, differently from the case of hydrophobic comonomers before examined, the addition of DMAA gives rise to an increase of the transition temperature. Actually, for the hydrogels obtained by irradiation of the mixtures A-ProOMe/CEA/TMPTMA and A-ProOMe/DMAA/TMPTMA in the same ratio (95/5/1), it was found to be 15° and 20°C, respectively.

The swelling-deswelling curves when the temperatures were cycled between 6° and 35°C for 24 h at each temperature are shown in Fig. 4. The well known behaviour of the swelling at a low temperature and the shrinking at a higher temperature as well as the reversibility of the process are observed (Yoshida et al., 1991). The poly(A-ProOMe-co-DMAA-co-TMPTMA) hydrogels were allowed to swell at equilibrium at 6°C and the values obtained for the samples in the ratio 70/30/3 and 90/10/3 were 62 and 55%, respectively. After that, the temperature was raised at 35°C and every hour a measurement was carried out. A rapid loss of weight due to the shrinking was ascertained until the new equilibrium was attained after 5–6 h and the swelling values for the two samples were 50 and 30%, respectively. After 24 h the temperature was again lowered at 6°C, the hydrogel samples started to swell until the same maximum swelling values as before were reached. As expected, the samples with the highest amount of the hydrophilic moiety DMAA showed highest swelling values both at 6° and at 35°C.

In this work several hydrogels based on A-ProOMe were used for entrapping acetaminophen which was allowed to be released at 37°C, a temperature above the transition temperature and thus suitable for "squeezing" the drug.

Fig. 5 shows the release of acetaminophen from copolymer hydrogels obtained by radiation polymerization of the monomer mixtures with the dissolved drug. Despite some scattering of the data points, it can be seen that highest release rate occurred with the most swellable matrix bearing DMAA. On the contrary, for the hydrogels with the hydrophobic cross-

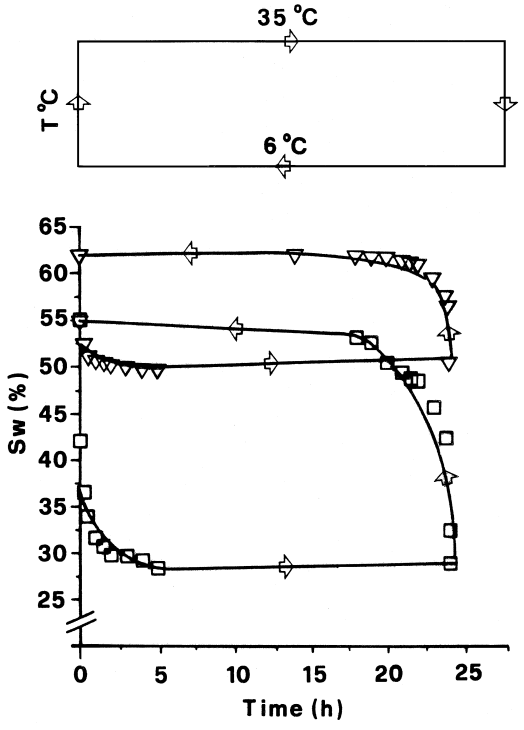


Fig. 4. Swelling–deswelling kinetics of the hydrogels obtained by radiation-induced polymerization of the mixtures A-ProOMe/DMAA/TMPTMA in the w/w ratio 70/30/3 (▽) and 90/10/3 (□) in response to the temperatures of 6° and 35°C.

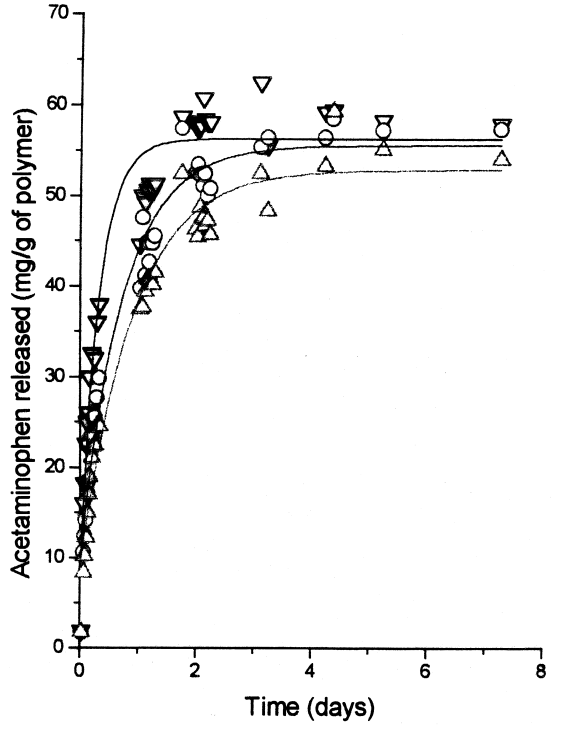


Fig. 5. Release profile at 37°C of acetaminophen from matrices obtained by radiation-induced polymerization of the mixtures in the w/w ratio: A-ProOMe/DMAA/TMPTMA 95/5/1 (▽); A-ProOMe/TMPTMA 99/1 (○); A-ProOMe/TMPTMA 97/3 (△).

linking agent, the higher the amount of the latter, the lower the extent of release was.

The hydrophilicity effect is still more evident in the release curves, reported in Fig. 6, referring to the hydrogels loaded with acetaminophen in a saturated solution of the drug at 5°C. Actually, the highly swellable hydrogel obtained with DMAA as the hydrophilic component (see Fig. 3) shows an extent of release much higher than less swellable hydrogels with the hydrophobic TMPTMA and CEA (see Figs. 1 and 2, respectively).

Recent studies dealt with the preparation of several methacrylic derivatives of acetaminophen and it was shown that both homopolymer and copolymers of 4-methacryloyloxy acetanilide underwent alkaline hydrolysis with release of acetaminophen (San Roman and Madruga, 1989; Levenfeld et al., 1991; San Roman et al., 1995). Thus, it was thought of interest to investigate if a similar behaviour occurred also in the case of copolymers of A-ProOMe with AOA.

Fig. 7 shows the swelling curves for hydrogels obtained by radiation polymerization of A-ProOMe in the presence of different proportions of the hydrophobic AOA. It can be seen that the extent of swelling decreases with increasing temperature. Moreover, the hydrogel obtained from the mixture A-ProOMe/AOA/TMPTMA in the weight ratio 95/5/1 shows that the transition temperature decreased at 12°C, in analogy with the behaviour observed for the hydrogels bearing hydrophobic moieties already depicted in the Figs. 1 and 2.

The SEM photographs of cross-section structures of poly(A-ProOMe-co-TMPTMA) hydrogels swollen at equilibrium at different temperatures are shown in Fig. 8. As expected from the swelling data before described, as the swelling temperatures increase the pore sizes decrease while their distribution becomes more and more homogeneous.

Fig. 9 shows the release of acetaminophen from hydrogels bearing AOA covalently attached to the macromolecular support through the hydrolyzable ester functional group. In this case the mechanism of drug release significantly differs from that described in the Figs. 5 and 6 in which the drug was entrapped in the polymer matrix. Actually, from the latter figures it can be seen that the time that the acetaminophen released took to reach a "plateau" was 2–3 days, i.e., approximately the same time for the hydrogel samples to reach the swelling equilibrium in water at the different temperatures, while the "plateau" time for poly(A-ProOMe-co-AOA) was much longer, about 20 days

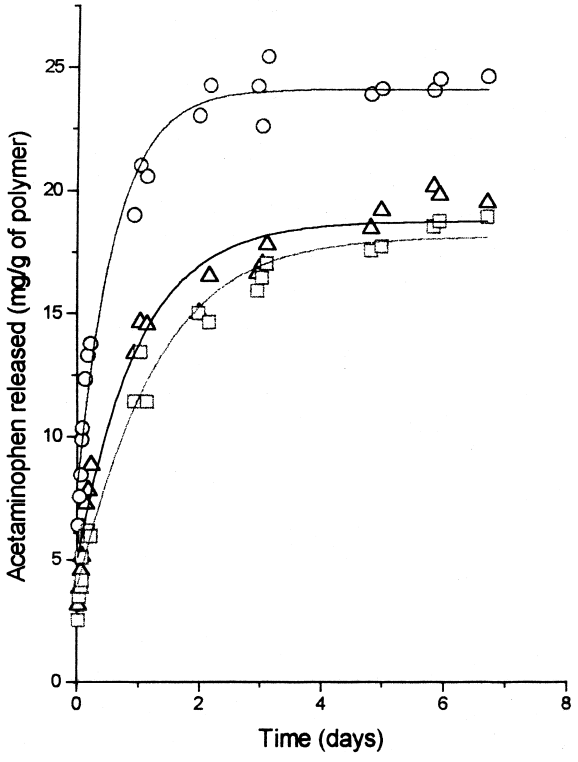


Fig. 6. Release profile at 37°C of acetaminophen from matrices obtained by radiation-induced polymerization of the mixtures in the w/w ratio: A-ProOMe/DMAA/TMPTMA 95/5/1 (○); A-ProOMe/TMPTMA 99/1 (△); A-ProOMe/CEA/TMPTMA 95/5/1 (□).

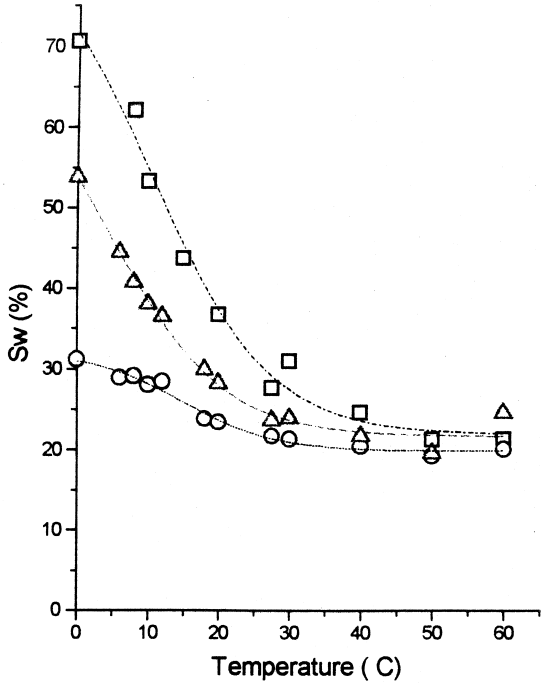


Fig. 7. Swelling ratio as a function of temperature of the hydrogels obtained by radiation-induced polymerization of the mixtures A-ProOMe/AOA/TMPTMA in the ratio (w/w): 95/5/1 (□); 90/10/1 (△); 80/20/1 (○).

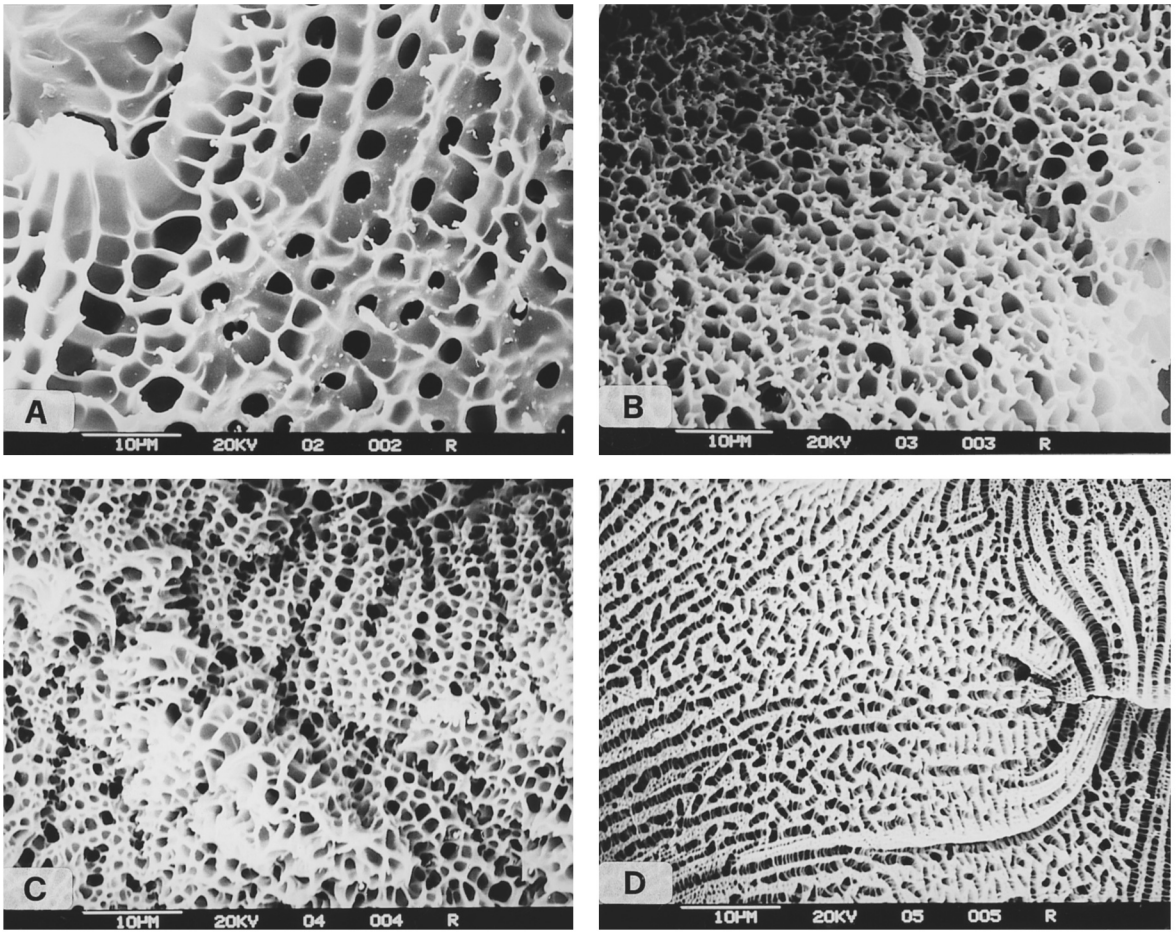


Fig. 8. SEM microphotographs of hydrogels obtained by radiation-induced polymerization of the mixture A-ProOMe/TMPTMA in the weight ratio of 99.5/0.5 and swollen at equilibrium at the temperatures of: 10°C (A); 20°C (B); 30°C (C); 50°C (D), Magnification 2000×.

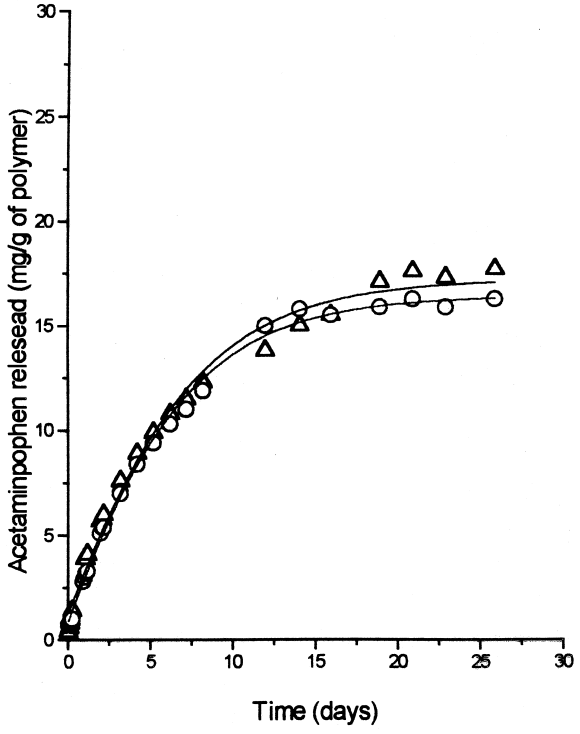


Fig. 9. Release profile at 37°C and pH = 8.5 of acetaminophen from matrices obtained by radiation-induced polymerization of the mixtures A-ProOMe/AOA/TMPTMA in the w/w ratio 80/20/1 (△) and 90/10/1 (○).

(see Fig. 9). It can therefore be concluded that in this case from the kinetic point of view the drug release is mostly determined by the hydrolysis process and not by the molecular diffusion.

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