Effect of chitosan and dextran on the properties of poly(vinyl alcohol) hydrogels

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Hydrogels are three-dimensional polymeric networks very similar to biological tissues and potentially useful as drug delivery systems. Poly(vinyl alcohol)-based hydrogels containing different amounts of dextran or chitosan were prepared using the freezing–thawing method. Repeated freezing–thawing cycles of a poly(vinyl alcohol) (PVA) aqueous solution lead to the formation of crystallites which act as cross-linking sites, and a hydrogel with a high capacity to swell is obtained.

The effects of the two different polysaccharides on the properties of the obtained materials were investigated by differential scanning calorimetry, dynamic mechanical analysis and scanning electron microscopy. In addition the release with time of poly(vinyl alcohol) in aqueous medium, was monitored and evaluated.

On the basis of the obtained results it seems that the presence of dextran favors the crystallization process of PVA, allowing the formation of a more ordered and homogeneous structure. Instead, chitosan seems to perturb the formation of PVA crystallites leading to a material with a less regular structure.
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1. Introduction

Hydrogels can be defined as aqueous gel networks, that are able to swell rapidly and retain large volumes of water. They have some unique properties that make them highly biocompatible. Firstly, they have a low interfacial tension with surrounding biological fluids and tissues. This minimizes the driving force for protein adsorption and cell adhesion [1–2]. Secondly, because of its very high water content, the hydrogel surface is highly hydrophilic and able to simulate some properties of natural tissues, thus making them biocompatible [3–5]. Thirdly, the soft nature of the hydrogels minimizes mechanical and frictional irritation of the surrounding tissues [6].

Biodegradable polymeric systems have been used frequently in the development of drug delivery systems. Biodegradable hydrogels could find wider application in the improvement of existing dosage forms and the development of better drug delivery systems [7].

Among the many synthetic polymers available for biomedical applications, poly(vinyl alcohol) (PVA) is one of the most widely used. Various cross-linking methods can be employed to obtain PVA hydrogels. We prepare PVA hydrogels using a physical method consisting of repeated freezing and thawing cycles of aqueous solutions of the polymer [8]. Improvements in the characteristics of synthetic biomaterials could be achieved by the addition of biological macromolecules [9–11], therefore, PVA hydrogels containing various percentages of biological macromolecules such as soluble collagen (SC) and hyaluronic acid (HA) were previously prepared in our laboratories and tested for physicochemical, mechanical and biological properties [12–14]. In addition, these hydrogels were evaluated as systems for the controlled release of human growth hormone (GH) [15–16]. Both SC/PVA and HA/PVA hydrogels were found to be biocompatible and in the case of HA/PVA samples we found an increase in the elastic modulus value and in the thermal stability for a specific HA/PVA composition. Both types of hydrogels proved to be useful for the release of GH which was dependent on the content of the biological component.

In order to find a less expensive alternative to collagen and hyaluronic acid, PVA was blended with two different polysaccharides, chitosan and dextran, respectively, and these blends were used to prepare hydrogels by freeze–thawing. The aim of the present work was to investigate the effects of the two biopolymers on the structure and the stability of the obtained materials.

Chitosan is a cationic glucosamine composed of 1–4 β-linked glucosamine and *N*-acetylglucosamine residues. This polysaccharide has been investigated for many

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interesting applications in the pharmaceutical and medical field such as contact lenses, suture wires [17], tablet binder [18–20], wound healing [21–23] or carrier material for the sustained release of drugs [24–27].

Dextran is a high molecular weight polymer of D-glucose, produced by different bacterial strains. Dextran and its derivatives are used as plasma-expanders, blood substitutes, bone healing promoters and for dermal and subcutaneous augmentation and for drug delivery.

In order to investigate the material behavior, differential scanning calorimetry (DSC), dynamic-mechanical analysis (DMA) and scanning electron microscopy (SEM) were performed. In addition, the release with time of PVA, in aqueous medium, was monitored and evaluated.

2. Materials and methods

2.1. Materials

Dextran (average molecular weight 78 000, produced by *Leuconostoc mesenteroides*), chitosan (from crab shells, average molecular weight 190 000–375 000, deacetylation degree 87.4%) and PVA, (average molecular weight 85 000–146 000), were supplied by Sigma-Aldrich, Milano, Italy.

2.2. Hydrogel preparation

A 3% chitosan (Ch) solution was obtained by dissolving in 0.5 M acetic acid at 40 °C. A 3% dextran (Dx) solution was prepared in distilled water. Ten grams of PVA was added to 100 ml of distilled water and dissolved in an autoclave for 1 h at 120 °C to obtain a final concentration of 10% PVA. Ch/PVA and Dx/PVA mixtures with: 10/90, 20/80, 30/70 and 40/60 (w/w) polymer ratios were prepared so that the final PVA content was maintained at 2.5%.

The various blends were dispensed into 12-well plates (2.5 ml/well) and hydrogels were obtained after eight cycles of freeze–thawing. Each cycle, with the exception of the first one, consisted of 1 h at − 20 °C and 30 min at room temperature. The first cycle differed from the others by having a longer standing time (12 h) at − 20 °C.

2.3. Differential scanning calorimetry (DSC)

The hydrogel thermal behavior was investigated by a Perkin Elmer differential scanning calorimeter DSC 7. The scans were performed at a rate of 10 °C min^{-1}, from 40 to 250 °C on samples dehydrated by lyophilization.

2.4. Dynamic-mechanical analysis

Mechanical measurements were performed with a dynamic-mechanical analyzer (Perkin-Elmer DMA-7), employing the parallel plate geometry on samples in their wet state. The plate diameter was 10 mm. Stress scans in the 10–1000 mN range were performed using a static to dynamic stress ratio of 150% at 1 Hz frequency and at a stress rate of 50 mN min^{-1}.

2.5. Scanning electron microscopy

The internal structure of the hydrogels was analyzed using a scanning electron microscope (SEM, Jeol T300). The materials were dehydrated by lyophilization, sputter coated with gold and then observed.

2.6. PVA releasing test

A releasing test was carried out to evaluate the amount of PVA released from both Ch/PVA and Dx/PVA hydrogels in aqueous solution. Each hydrogel was immersed in 30 ml of distilled water, for 144 h at 37 °C.

At 1, 2, 3, 5, 8, 24, 48, 72, 96, 120 and 144 h the releasing solution was removed and replaced with 30 ml of fresh distilled water. The determination of released PVA was carried out spectrophotometrically according to the method of Bujanda and Rudin [19].

3. Results and discussion

The DSC thermograms of Ch/PVA and Dx/PVA hydrogels are reported in Figs 1 and 2, respectively. An endothermic peak can be observed, related to the melting of PVA, whose intensity decreases as Ch content increases but increases as Dx content increases.

Ch and Dx do not affect the PVA melting temperature which does not show any relevant variation when the Ch or Dx content of the blends are varied. This indicates that no substantial modification occurs in the nature of PVA crystallites because of the presence of the biological components. The percentage of PVA crystallinity as a function of Ch or Dx content is reported in Fig. 3a, b. Both polysaccharides affect the PVA crystalline degree, which increases as Dx content increases whereas decreases as Ch content increases.

The PVA glass transition temperature (T_g) shifts towards higher values, with respect to that of pure PVA hydrogel, due to the presence of either Ch or Dx (Fig. 4a, b). A small amount (10%) of Ch or Dx is sufficient to increase the T_g value in the PVA blends. The T_g value remains constant by further increasing the Ch or Dx content of the hydrogels.

The trend of the storage modulus E' as a function of the dynamic force is illustrated in Figs 5 and 6. The E' curves of all the samples have a very similar shape. In

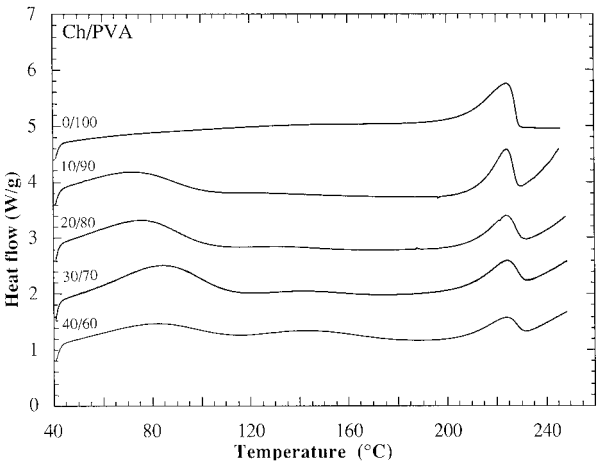


Figure 1 DSC thermograms of Ch/PVA hydrogels (scan rate: 10 °C min^{-1}).

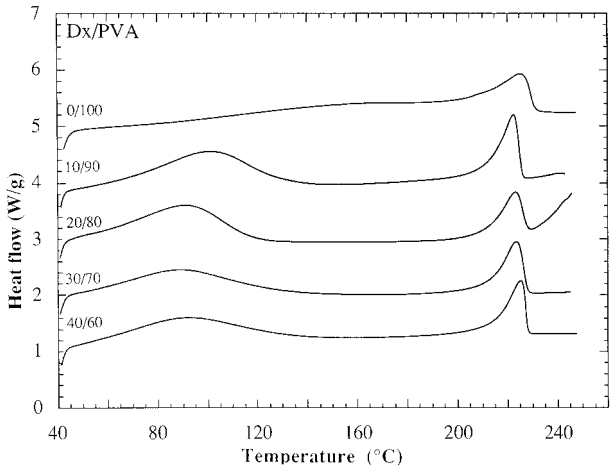
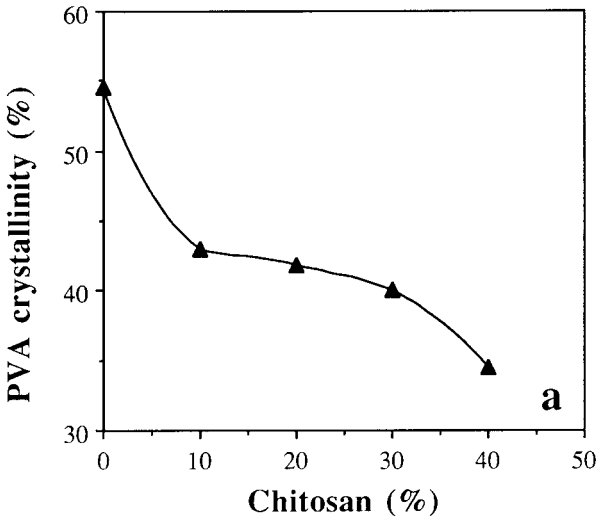


Figure 2 DSC thermograms of Dx/PVA hydrogels (scan rate: 10 °C min^{-1}).

general the modulus value increases with increasing dynamic force. In the case of Ch/PVA samples (Fig. 5), the E' curves are shifted towards lower values, while in the case of Dx/PVA samples (Fig. 6) the modulus curves are shifted toward higher values, along the modulus scale, with respect to the curve of the pure PVA



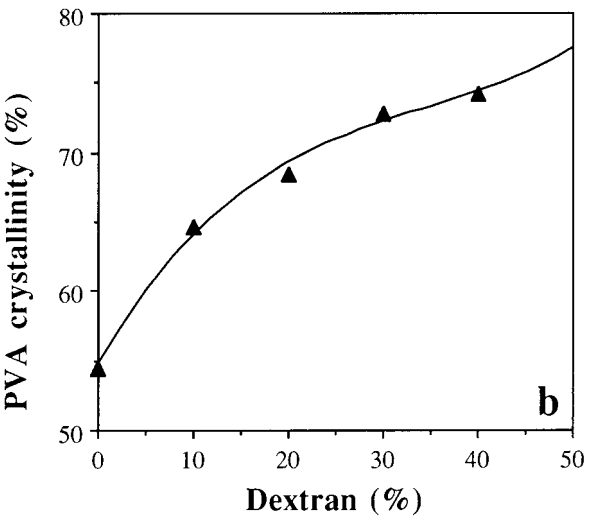
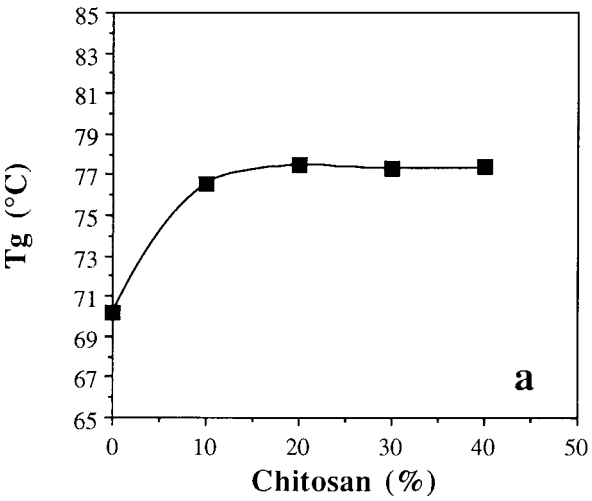


Figure 3 Percentage of PVA crystallinity reported as a function of Ch (a) or Dx (b) content.



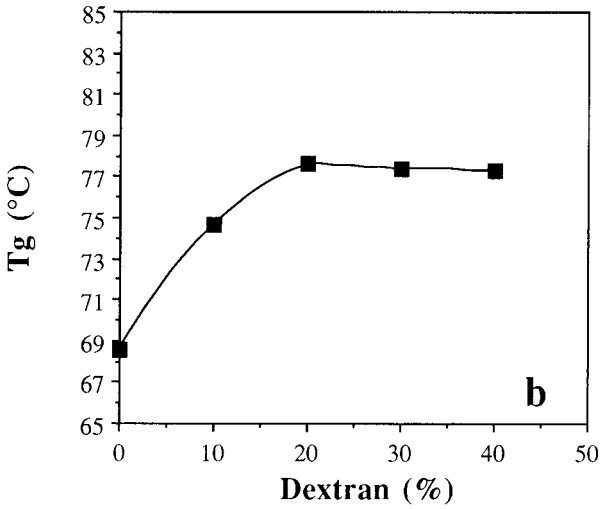


Figure 4 PVA glass transition temperature reported as a function of Ch (a) or Dx (b) content.

hydrogels. The modulus value increases with increasing Dx content but decreases with increasing Ch content in the blends.

The hydrogel modulus variation with Ch or Dx content is clearly related to the organization degree of the PVA network structure. The modulus increase with increasing Dx content suggests the formation of a more regular structure. On the other hand, the modulus decrease with increasing Ch content indicates that the presence of this

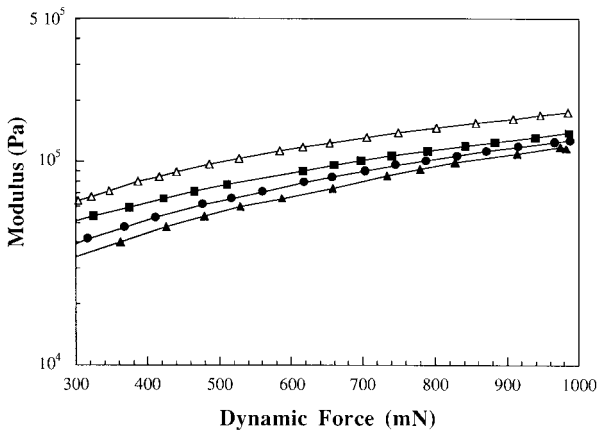


Figure 5 The trend of the storage modulus E' as a function of the dynamic force in Ch/PVA hydrogels (Ch/PVA: Δ 0/100; ■ 10/90; • 20/80; ▲ 30/70).

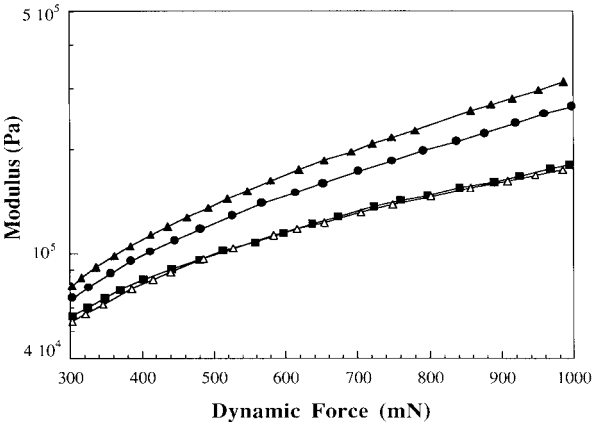


Figure 6 The trend of the storage modulus E' as a function of the dynamic force in Dx/PVA hydrogels (Dx/PVA: Δ 0/100; ■ 10/90; • 20/80; ▲ 30/70).

biological polymer perturbs the formation of a regular PVA network.

The results of the PVA releasing test, confirming the DSC and DMA data, indicate that Ch-containing hydrogels (Fig. 7a) release a higher amount of PVA with respect to pure PVA hydrogels, whilst Dx-containing hydrogels (Fig. 7b) release a smaller amount of PVA than the pure PVA hydrogels.

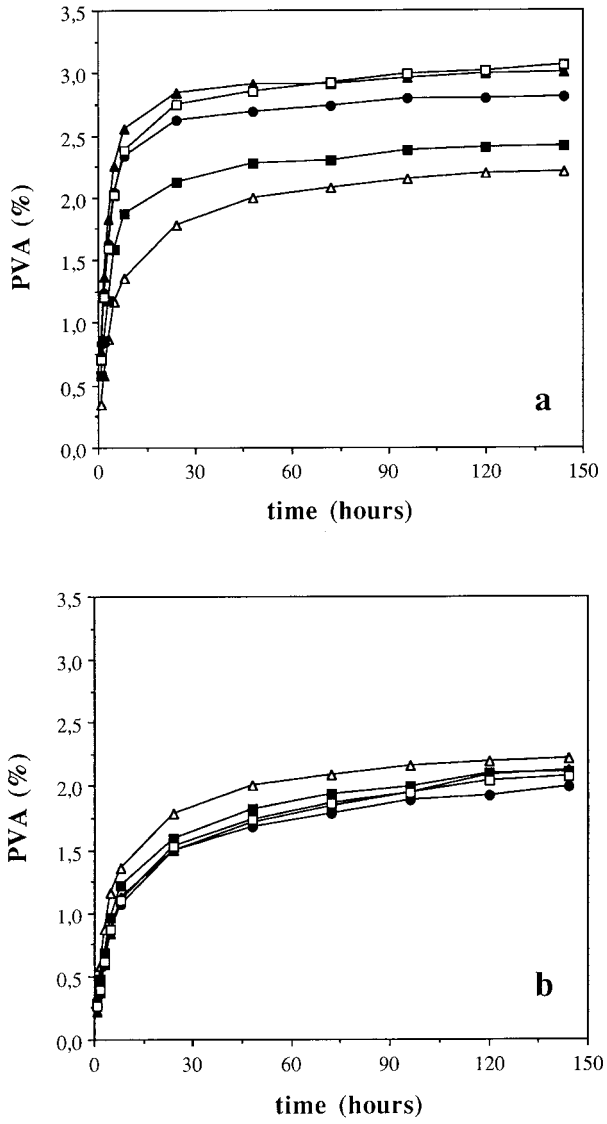


Figure 7 Percentage of PVA released from Dx/PVA (a) and Ch/PVA (b) normalized to the initial PVA content of the hydrogels, reported as a function of time. Each point is the mean of two determinations (Dx/PVA or Ch/PVA: Δ 0/100; ■ 10/90; • 20/80; ▲ 30/70, □ 40/60).

SEM micrographs of the internal hydrogel structure show a porous filamentous matrix which could allow the transport of additives through the matrix. In the case of Ch/PVA hydrogels (Fig. 8a–c) the structure becomes less ordered and more porous increasing Ch content, while in the case of Dx/PVA hydrogels (Fig. 9a–c) it becomes more homogeneous with increasing Dx content.

On the basis of the obtained results it seems that the presence of Dx favors the crystallization process of PVA, allowing the formation of a more ordered and homogeneous structure. Instead, Ch seems to perturb the formation of PVA crystallites leading to a material with a less regular structure.

It can be concluded that both the macromolecules used in this study are able to affect the material structure:

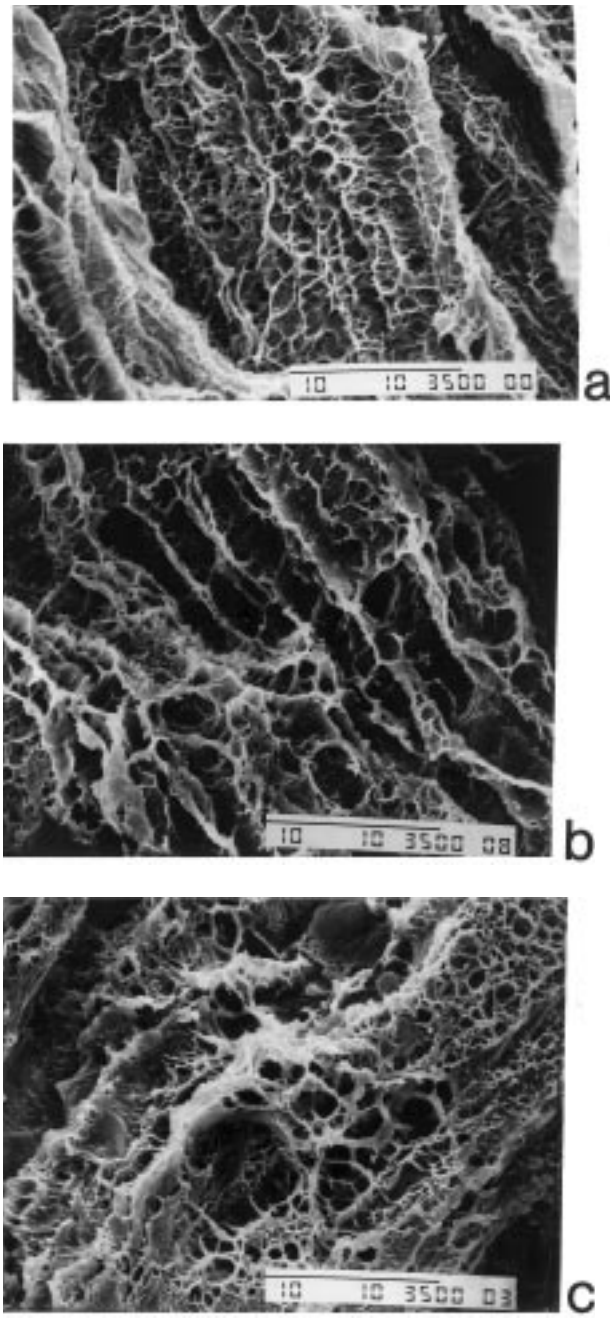


Figure 8 SEM image of the internal structure of Ch/PVA hydrogels: (a) 10/90, (b) 20/80, (c) 30/70. Bar = 10 μm.

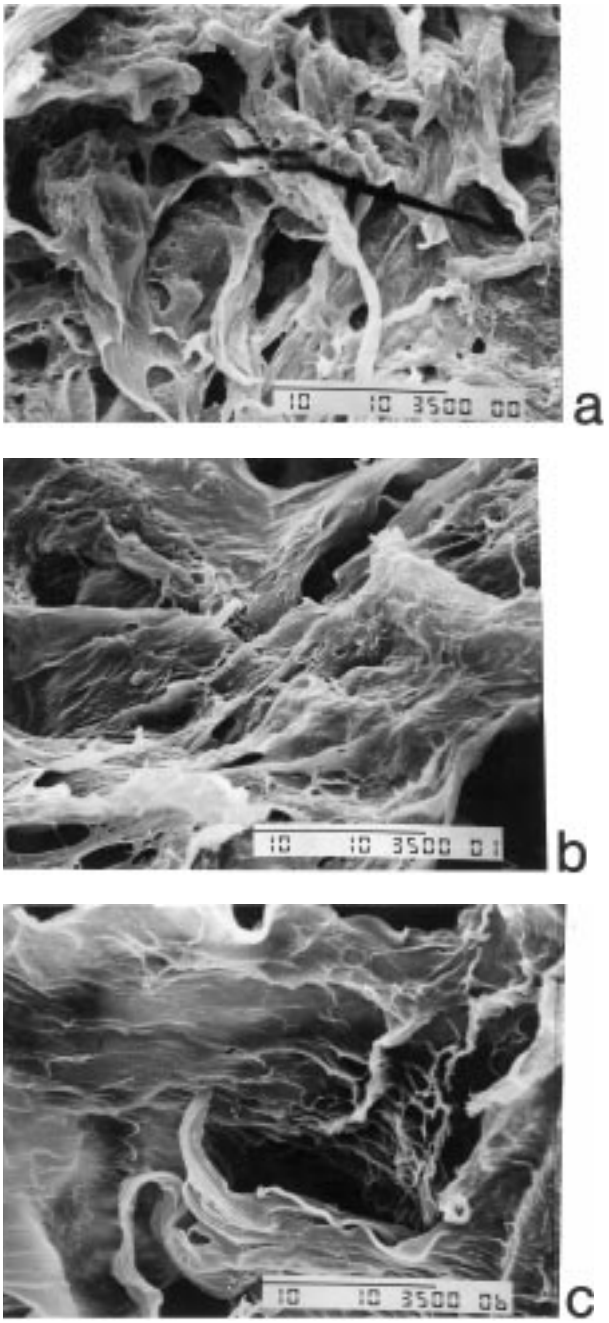


Figure 9 SEM image of the internal structure of Dx/PVA hydrogels: (a) 10/90, (b) 20/80, (c) 30/70. Bar = 10 μm.

dextran exerting a stabilizing effect and chitosan a perturbing effect on PVA crystallization. At the moment it is not clear why these biopolymers, both belonging to the class of polysaccharides, affect PVA crystallization and morphology differently. It could be hypothesized that there is a different hindrance exerted by the two macromolecules because of the different chemical structures and molecular weights.

Further investigation will be addressed to this opposite behavior, possibly in connection with the potential use of chitosan and dextran to modulate the PVA-based hydrogels in the exploitation of novel drug release systems [34].

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Received 8 January
and accepted 31 August 1998