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Physico-chemical and mechanical characterization of hydrogels of poly(vinyl alcohol) and hyaluronic acid

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Hydrogels are three-dimensional polymeric networks very similar to biological tissues and potentially useful as soft tissue substitutes and drug delivery systems. Many synthetic polymers can be used to make hydrogels: poly(vinyl alcohol) is widely employed to make hydrogels for biomedical applications. Improvements in the biocompatibility characteristics of synthetic materials could be achieved by the addition of biological macromolecules. The resulting materials named "bioartificial polymeric materials" could possess the good mechanical properties of the synthetic component and adequate biocompatibility due to the biological component. We have used poly(vinyl alcohol) to make hydrogels containing various amounts of hyaluronic acid. These bioartificial materials were studied to investigate the effect of the presence of the hyaluronic acid on the structural properties of the hydrogels. Thermal, mechanical, morphological and X-ray analyses were performed. A close correspondence between the network consistency and the degree of crystallinity developed in the matrix suggested that the hyaluronic acid, when its content is about 20%, could provide heterogeneous crystallization nuclei for poly(vinyl alcohol) thus increasing the crystallization degree, and consequently, the storage modulus.

1. Introduction

Poly(vinyl alcohol) hydrogels prepared by a freezing–thawing procedure [1] have recently attracted much attention for their potential applications as biomedical materials. Repeated freezing–thawing cycles of an aqueous poly(vinyl alcohol) solution lead to the formation of crystallites which act as crosslinking sites, and a hydrogel with a high capacity to swell is obtained. The freezing–thawing technique is a physical crosslinking method that offers several advantages with respect to others, such as chemical crosslinking and radiation-induced crosslinking: it is simple, it does not require any additional chemicals and it does not require high temperature. The large content of water, the high permeability to small molecules and the good mechanical properties make hydrogels very similar to biological tissues from a physical and mechanical point of view, and potentially useful as soft tissue substitutes and drug delivery devices [2]. Problems could arise in using synthetic materials when their biological properties are considered. Improvements in the biocompatibility characteristics of synthetic materials could be achieved by the addition of biological macromolecules [3]. The resulting materials, named "bioartificial polymeric materials" [4] could possess good mechanical properties due to the synthetic component and adequate biocompatibility due to the biological component. In the last few years collagen has been used as a biological component of bioartificial materials because it is one of the major constituents of natural tissues [4, 5]. Hyaluronic acid is another important biological macromolecule that influences several cellular functions, such as migration, adhesion and proliferation [6]. In this study hydrogels of poly(vinyl alcohol) and hyaluronic acid were prepared. The aim was to investigate the effect of this biological component on the physico-chemical and mechanical properties of the hydrogels.

2. Materials and methods

2.1. Materials

All the materials used in this study are commercially available: (i) poly(vinyl alcohol) (powdered., Aldrich Chemie, Steinheim, Germany) (PVA) with an average molecular weight of 114 000 (determined by the viscosimetric method) and a hydrolysis degree of 100%; (ii) hyaluronic acid sodium salt (HA) with an average molecular weight of 250 000 (F.A.B. S.p.A Italy).

2.2. Hydrogel preparation

A 5% w/v PVA aqueous solution was prepared by adding the solid PVA to distilled water in a flask

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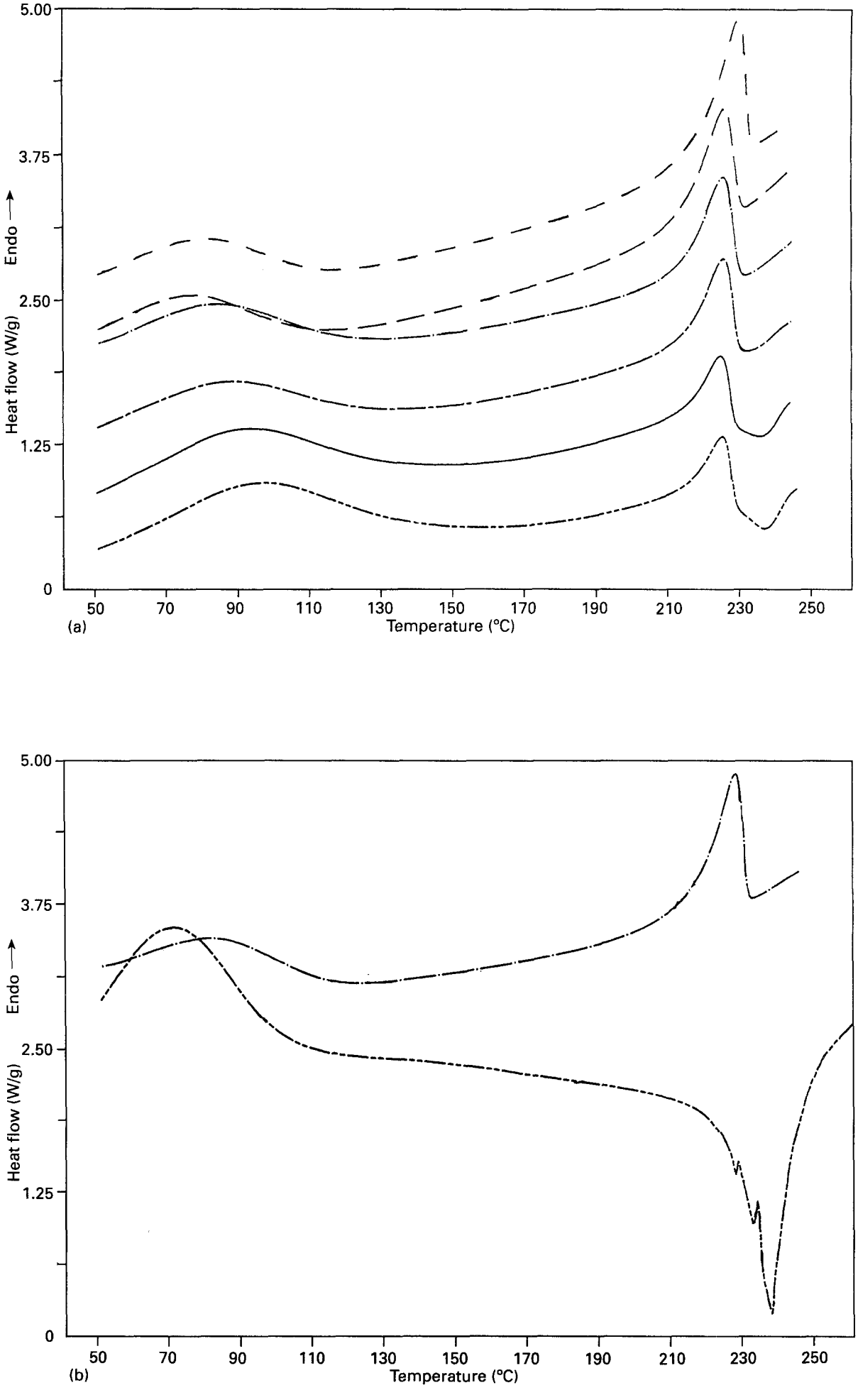

Figure 1 Normalized DSC thermograms of (a) HG1 series hydrogels (HA/PVA – – – 0/100; — — 10/90; —·— 20/80; — – — 30/70; —— 40/60; — – – — 50/50); and (b) pure PVA (—·—) and pure HA (– – –).

equipped with a reflux condenser, then gradually raising the temperature from room temperature to the boiling point of the solution by means of an oil-bath heater at 120 °C. The dissolution of PVA was complete in 2 h. A 5% w/v HA aqueous solution was prepared by adding the solid powdered HA to distilled water, and raising the temperature to 60 °C. The dissolution of HA was complete in 2 h. Mixtures of HA and PVA, with 10/90, 20/80, 30/70, 40/60, 50/50, 0/100 (w/w) polymer ratios were prepared so that the final PVA content was maintained at 2.5%. The various solutions were poured into Petri dishes and hydrogels were obtained after eight freezing–thawing cycles. With the exception of the first one, each cycle involved lowering the temperature to − 19 °C, standing at this temperature for 1 h, then raising the temperature to room temperature. The first cycle (the same for all samples) differed from the others because of a longer standing time at − 19 °C (12 h).

2.3. Characterization methods

Three series of hydrogels were prepared and labelled as follows:

HG1 series: hydrogels for thermal and X-ray analysis
HG2 series: hydrogels for mechanical tests
HG3 series: hydrogels for releasing tests

Thermal analysis was performed by a differential scanning calorimeter (DSC) Perkin-Elmer DSC-7, at a rate of 10 °C/min from 50 to 250 °C, on freeze-dried samples of HG1, HG2 and HG3 series.

Morphological analysis was carried out using a scanning electron microscope (SEM) Jeol T300. The hydrogels were dehydrated through an increasing series of ethanol solutions, critical point dried against CO_2 and sputter-coated with gold before SEM analysis.

Mechanical measurements were performed with a dynamic-mechanical analyser Perkin Elmer DMA-7, employing parallel plate geometry on samples of HG2 series 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 rate of 50 mN/min.

X-ray analysis was carried out by a SIEMENS D500 diffractometer employing CuK_α radiation, on HG1 series. For each sample two spectra were recorded: the first after freeze-drying; the second after curing the same sample at 150 °C for 20 h under vacuum. In releasing tests, each sample of HG3 series, was immersed in 30 ml of distilled water for 98 h at 37 °C. At 2, 4, 8, 24, 48 and 98 h the released amounts of HA and PVA were determined as described in detail elsewhere [7].

3. Results

The DSC thermograms of HG1 (Fig. 1a) and HG2 series showed an endothermic peak related to the

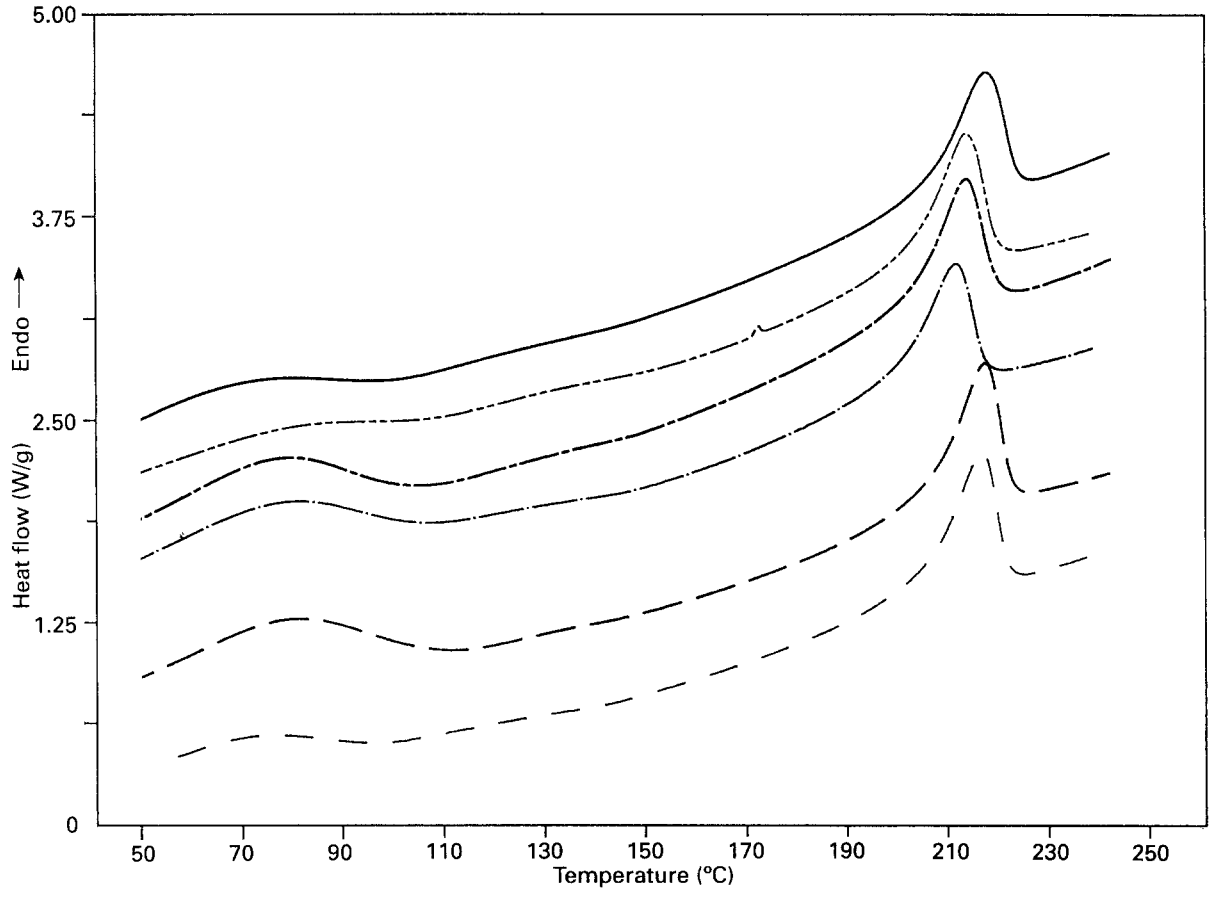

Figure 2 DSC thermograms of HG3 series hydrogels (HA/PVA —— 0/100; — - - - 10/90; — - — 20/80; —·— 30/70; — — 40/60; - - - 50/50).

melting of PVA. This transition was closely followed by, and partially superimposed by, an exothermic peak related to the degradation of HA (Fig. 1b). The exothermic peak was not present in curves of HG3 series (Fig. 2) because of the complete loss of HA from these hydrogels as was evidenced by releasing tests [7]. The PVA melting temperature (T_m) is reported as a function of the HA content (Fig. 3a). The HA/PVA hydrogels of the series HG1 and HG2 showed values of T_m slightly lower than those of the pure PVA hydrogels. A little decrease of T_m when the HA content increased was observed for the HG2 samples. The HG3 series (Fig. 2) showed a more irregular trend of T_m and all values were about 10 °C lower than those of the corresponding samples of the HG1 and HG2 series. The PVA melting enthalpy (ΔH_m) is reported as a function of the HA content (Fig. 3b). In all series (HG1, HG2 and HG3) a relative maximum of ΔH_m, corresponding to HA/PVA = 20/80, was observed. The ΔH_m values observed for 40 and 50% content of HA (asterisks in Fig. 3b) in the HG1 and HG2 series, were affected by large measurement errors because of the superimposition of exothermic degradation of HA on the melting of PVA. The ΔH_m, values for the HG3 hydrogels were lower than those of the corresponding samples of the series HG1 and HG2.

The trend of the storage modulus G' as a function of the dynamic force for HA/PVA hydrogels of the HG2 series is illustrated in Fig. 4a. The G' curves of all samples have a very similar shape and are shifted towards lower and higher values along the modulus scale with respect to the curve of the pure PVA hydrogel. In general, G' increases with increasing dynamic force, steeply at first and then more gradually. However, in no case was G' found to be independent of the dynamic force.

Accordingly, to compare homogeneous data, we fixed upon a dynamic force of 800 mN and determined the corresponding G' values, marked $G'(800)$, in the different samples. Fig. 4b reports the trend $G'(800)$ as a function of the PVA content in the hydrogel. The storage modulus of the pure PVA hydrogel is about 8.0×10^5 Pa and increases with decreasing PVA content up to a maximum value of $G'(800) = 1.2 \times 10^6$ Pa, corresponding to a composition of 80% PVA. With further decrease of the PVA content, $G'(800)$ decreases, to about 4×10^5 Pa at a composition of 50% PVA.

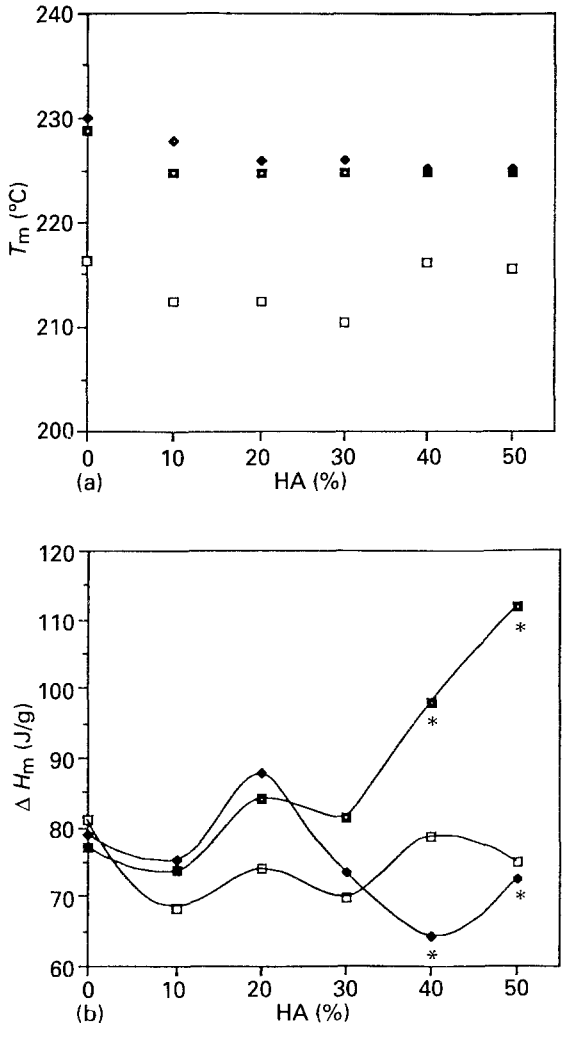

Figure 3 (a) PVA melting temperature as a function of the HA content in HG1 (■), HG2 (◆) and HG3 (□) series hydrogels. (b) PVA melting enthalpy as a function of the HA content in HG1 (■), HG2 (◆) and HG3 (□) series hydrogels.

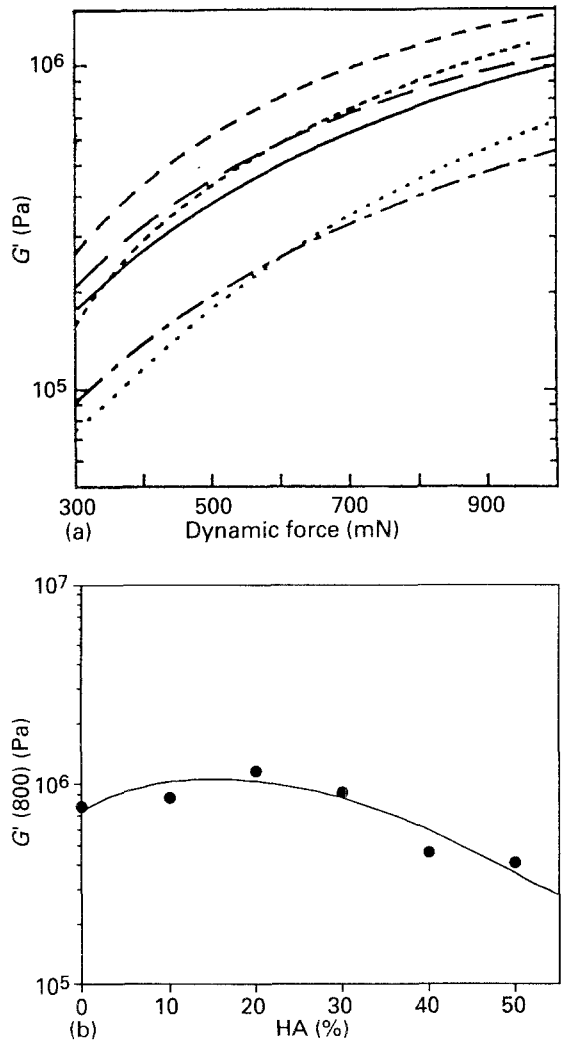

Figure 4 (a) The trend of the storage modulus G' as a function of the dynamic force for HA/PVA hydrogels. (HA/PVA —— 0/100; — — 10/90; – – 20/80; – – – 30/70; ---- 40/60; – - – 50/50). (b) The trend of G' (800) as a function of the HA content for HA/PVA hydrogels.

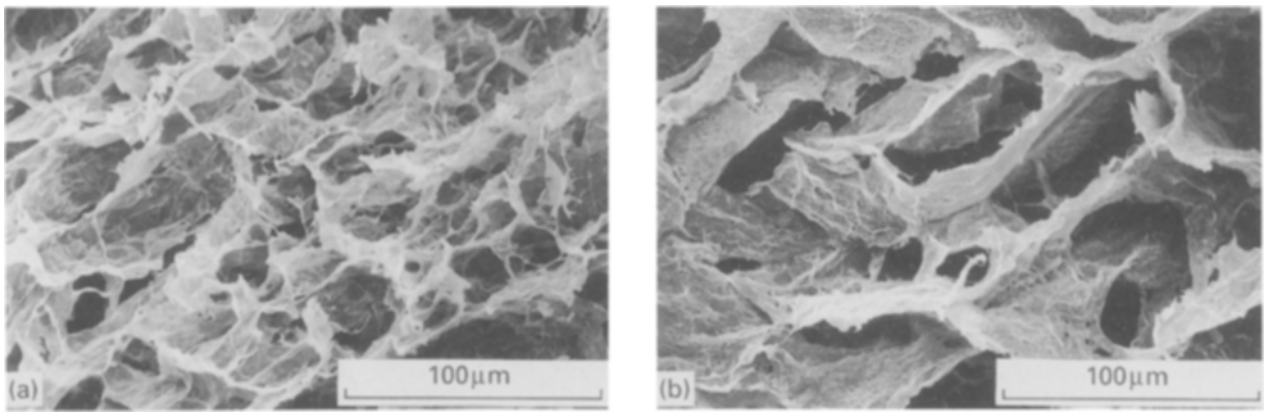

Figure 5 SEM photographs of HG1 series hydrogels with different composition: (a) HA/PVA = 0/100; (b) HA/PVA = 20/80.

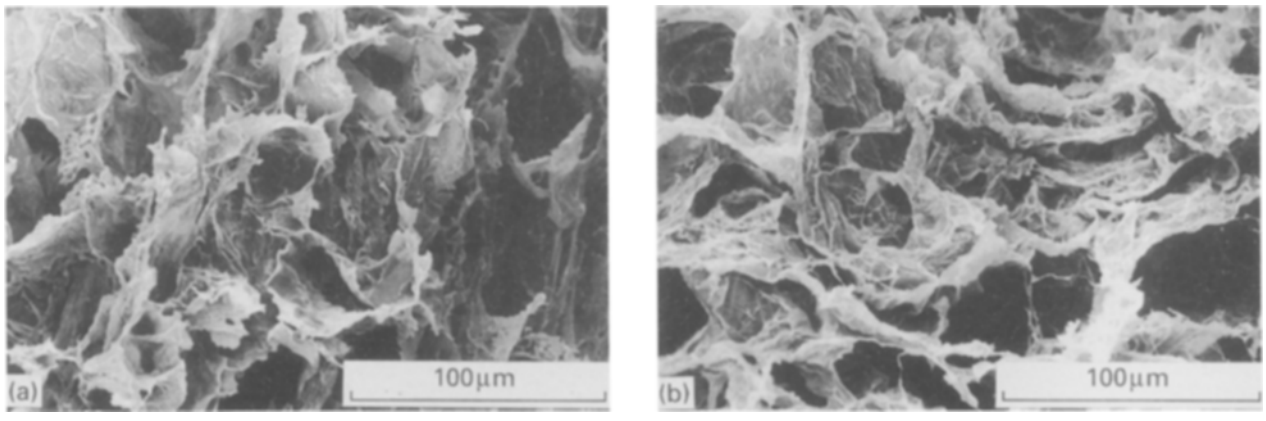

Figure 6 SEM photographs of a HA/PVA = 40/60 hydrogel: (a) before; (b) after release.

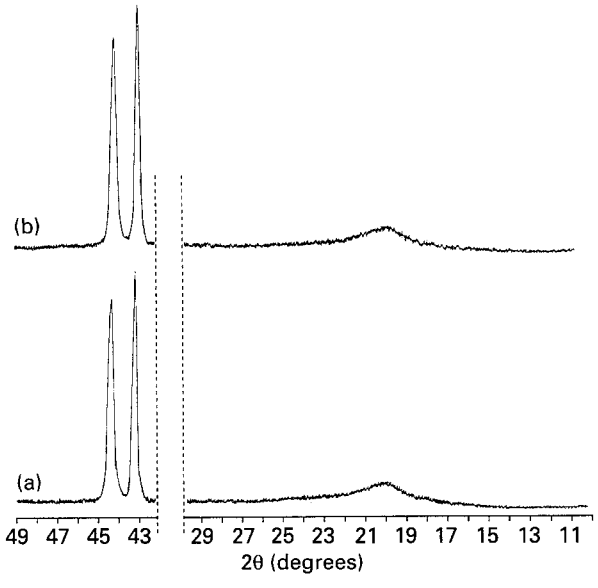

Figure 7 X-ray spectra of a HA/PVA = 20/80 hydrogel: (a) before; (b) after curing at 150 °C for 20 h under vacuum.

The morphological analysis using SEM showed porous structures in which pore size did not vary when the amount of HA changed (Fig. 5a, b). Fig. 6a, b shows that no substantial differences were observed between the HG1 series hydrogels (samples examined just as they came out from preparation) and HG3 series hydrogels (samples examined after 98 h release).

The X-ray spectra showed the presence of ordered structures revealed by sharp peaks at $2\theta = 43$ and $2\theta = 44$ degrees. The intensity of the peaks was slightly higher after curing. It was observed that the sample with 20% HA showed the highest intensity reflection peaks (Fig. 7).

4. Discussion

The very slight variation of PVA melting temperature when the HA content changes in the HG1 and HG2 hydrogels indicates that no relevant modifications in the nature of PVA crystallites occurs. The anomalous trend observed for the HG3 series could be tentatively explained by a destabilizing effect on the structure of PVA crystallites due to water molecules which, after 98 h at 37 °C, could have been able to perturb the hydrogen bonds between PVA chains. The low values of the PVA melting enthalpy of the HG3 series seems to agree with this supposition. However, more information about the influence of HA on the structure of the PVA matrix of the hydrogels can be obtained by examining the HG1 and HG2 data. As the HA chains perturb the formation of PVA crystallites, one would expect a decrease of the PVA melting enthalpy with increasing HA content. This is actually observed for

all compositions, with the exception of the sample in which the HA content is 20%. The high values of ΔH_m obtained for HA/PVA = 20/80 samples suggest that a higher degree of crystallization was reached during hydrogel formation. The DMA data seems to be in agreement with this hypotheses. In fact the hydrogel modulus variation with HA content is clearly related to the organization degree and perfection of the relevant PVA network structure. The modulus increase for the sample in which the HA content is lower than 20% suggests the presence of a mechanism leading to the formation of a more regular network structure. This behaviour can be explained by considering that the HA (at temperatures as low as $-19\,^{\circ}C$) should provide additional nucleation sites for crystallization of the PVA. The melting enthalpy displays a maximum value at a composition of 20% HA, which is very close to the composition corresponding to the maximum of $G'(800)$. The similarity between the trend of the melting enthalpy and the storage modulus as functions of the HA content in the hydrogels suggests a close correspondence between the network consistency and degree of crystallinity developed in the matrix. This in turn depends on the crystallization conditions, and in this respect we suggest that the hyaluronic acid, when its content is about 20%, provides heterogeneous crystallization nuclei thus increasing the degree of crystallization of the matrix and consequently the storage modulus value. X-ray spectra provide an additional confirmation of this hypothesis, since the maximum intensity of the reflection peak, corresponding to the structure having the higher-order degree, is showed by the sample containing 20% HA. At the moment the true nature of these ordered structures is not completely clear although the role of water molecules bound to the polymer chains could be relevant.

Acknowledgements

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