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Hydrogels of poly(vinyl alcohol) and collagen as new bioartificial materials

Part I *Physical and morphological characterization*

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Poly(vinyl alcohol) was used to make hydrogels containing various amounts of collagen. These "bioartificial materials", made of synthetic and biological polymers, were studied to investigate the effect of the presence of the collagen on the structural properties of the hydrogels. A comparison between thermal and morphological properties of collagen-containing hydrogels and hydrogels of pure poly(vinyl alcohol) was made.

1. Introduction

Polymeric hydrogels have been extensively studied in the last decades for applications in the biomedical field. The network structure of such hydrogels, made of cross-linked polymer chains, allows them to hold even large amounts of fluid (water or biological fluids) without dissolving. The large content of fluid causes hydrogels to have very low interfacial tension with biological fluids. This important feature, together with a high permeability to small molecules and a viscoelastic behaviour, make hydrogels very similar to biological tissues. Effectively, hydrogels form a class of materials that are potentially useful for replacement of soft tissues or for other biomedical applications, such as drug delivery [1].

The major disadvantage of these materials is generally due to their low mechanical strength. Mechanical properties are, of course, as important as the biocompatibility charateristics. Much effort has been devoted to obtaining good mechanical properties by means of appropriate chemical or physical cross-linking procedures.

Many synthetic polymers can be used in preparing hydrogels: poly(vinyl alcohol) is widely used to make hydrogels for biomedical applications, owing to its biocompatibility charateristics, which have been extensively tested [2]. Among the various cross-linking techniques for poly(vinyl alcohol), the Nambu [3] method is one of the most interesting, because it is a simple physical method and does not require any additional chemicals as cross-linking agents. It consists of repeated freezing–thawing cycles of aqueous solutions of the polymer, which lead to a material with a high capacity to swell. More precisely, such cycles lead to the formation of crystallites that act as cross-linking sites between the poly(vinyl alcohol) chains [4, 5].

According to recent results obtained in our laboratories, improvements in the characteristics of synthetic biomaterials could be achieved by the addition of biological macromolecules. The resulting materials, named "bioartificial materials", could possess the good mechanical properties of the synthetic component and adequate biocompatibility due to the biological component [6]. For this purpose, biopolymers contained in the extracellular matrix (collagen, glycosaminoglycans, fibronectin, laminin, elastin) can be usefully employed.

As a first step, some results obtained with hydrogels of poly(vinyl alcohol) and collagen are reported. The aim of the experiment was to investigate the effect of the presence of the collagen on the structure of the hydrogels.

2. Experimental procedure

Calorimetric and morphological analyses were carried out on hydrogels of pure poly(vinyl alcohol) and hydrogels of poly(vinyl alcohol) and collagen. All the hydrogels were prepared according to the specifications described below.

Enzymatic digestion by collagenase was employed to remove collagen and to visualise changes in the hydrogels.

2.1. Materials

All the materials used in this study were commercially available: (i) poly(vinyl alcohol) (powdered, Aldrich Chemie, Steinheim, Germany) (PVA) with an average molecular weight of 114000 (determined by the viscosimetric method), a syndiotacticity degree of 62% (determined by IR analysis [7]), and a hydrolysis degree of 100%; (ii) acid soluble collagen (SC), type I

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from calf skin (C.3511, Sigma Chemical Co, St. Louis, MO, USA); (iii) collagenase (C.0773, Sigma Chemical Co, St. Louis, MO, USA) from *Clostridium histoliticum* with an activity of 1700 units/mg.

2.2. Methods

Aqueous solutions with concentrations of 2.5%, 5%, 8%, 10% and 15% w/v PVA were prepared by adding the solid PVA to distilled water into a flask 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. The various solutions were poured into Petri dishes and hydrogels were obtained after 1, 2, 4, 6, 8 and 10 freezing–thawing cycles. With the exception of the first one, each cycle involved lowering the temperature to − 30 °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 due to a longer standing time at − 30 °C (12 h). The obtained hydrogels were then placed in distilled water, left for 5 days at 37 °C and allowed to swell to their equilibrium situation. A soluble fraction of non-cross-linked PVA, corresponding to about 18% of the initial PVA content, was released by each hydrogel.

SC/PVA hydrogels were prepared by the same method. SC was dissolved in 0.1 N HCl to obtain a 1.5% solution and then mixed with a 10% PVA solution. Mixtures of SC and PVA, with 30/70, 25/75, 20/80, 15/85, 10/90 SC/PVA (w/w) ratios, were prepared so that the final PVA content was kept at 2.5%. The hydrogels were conditioned for 20 days at 37 °C by immersion into buffer solutions at pH 7.4, prepared as reported in [8]. The amounts of collagen released by the hydrogels into the buffer solutions were determined by a modified Zender method [9]. It was found that no hydrogel released more than 1.3% of the initial collagen.

In order to be morphologically examined, the hydrogels were dehydrated through an increasing series of ethanol solutions, critical point dried against CO_2, sputter-coated with gold and observed by a scanning electron microscope (SEM). The thermal analysis was carried out by a differential scanning calorimeter (DSC) on samples dried by a freeze-dryer. DSC analysis, at a rate of 10 °C min^{-1} from 20 °C to 250 °C, was performed with a Perkin-Elmer DSC-7 calorimeter.

Samples of the SC/PVA hydrogels of a few grams in weight were immersed in 1 ml of a buffer solution at pH 7.4 [8]. Each sample was incubated for 3 h at 37 °C after the addition of 450 units of collagenase.

3. Results and discussion

The DSC analysis of the pure PVA hydrogels shows a melting temperature (T_m) that remains almost constant with the number of cycles (N) and the concentration (C) (Fig. 1). This is in agreement with the results reported in the literature [10] and indicates a uniformity in the structure of the crystalline domains in the hydrogels examined. Parameters such as the rate of freezing and thawing, the molecular weight, the nature of the solvent, etc., that can affect T_m, were kept constant in these experiments. The melting enthalpy (ΔH_m) of pure PVA increases with N until it reaches a value which remains constant when $N \geq 6$ (Fig. 2).

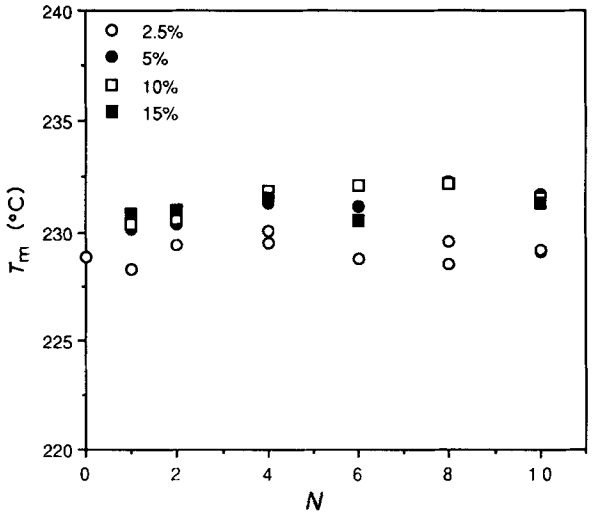


Figure 1 Dependence of the T_m of PVA on N in hydrogels of pure PVA. The symbols indicate the concentration C of PVA in the initial solution.

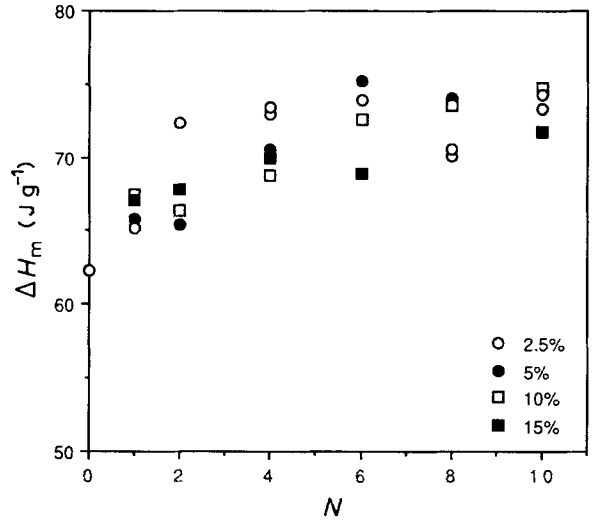
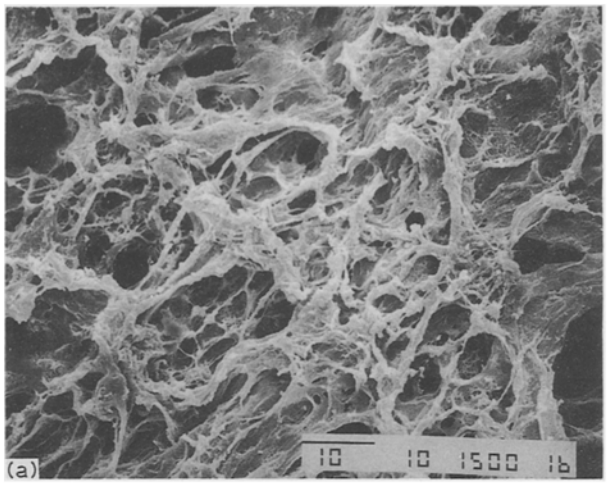
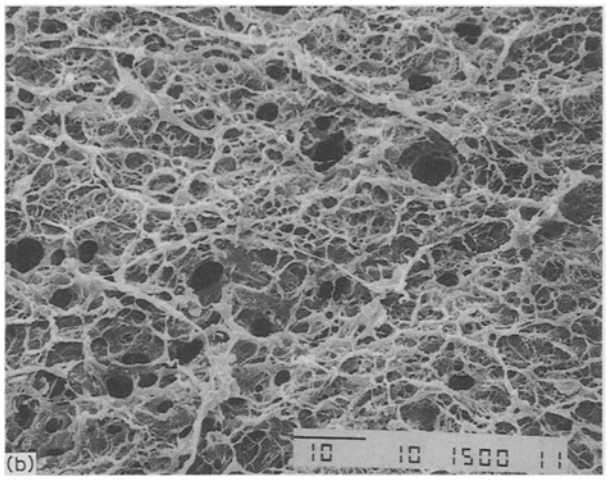


Figure 2 Dependence of the ΔH_m of PVA on N in hydrogels of pure PVA. The symbols indicate the concentration C of PVA in the initial solution.

This trend, which is confirmed by the literature [10], may be explained by the fact that the fraction of crystallised polymer increases with N until it reaches the maximum degree of crystallinity possible under the given conditions. The morphology of the hydrogels has been analysed by SEM. The analysis shows a porous structure in which pore size varies with changes in C and N. As shown in Fig. 3a–c, while N remains constant, pore size increases as C decreases. This is to be expected, since a lower concentration of PVA corresponds to lower quantities of polymer per unit volume. The hydrogel illustrated in Fig. 3a (C = 2.5%, N = 8) shows pores with diameters ranging from 10 to 30 μm. By keeping C constant, an increase





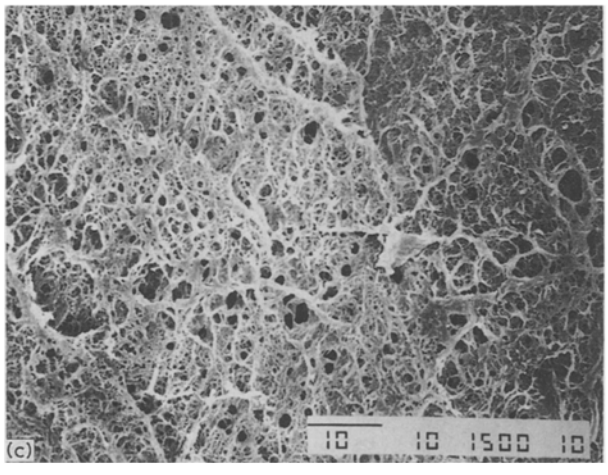
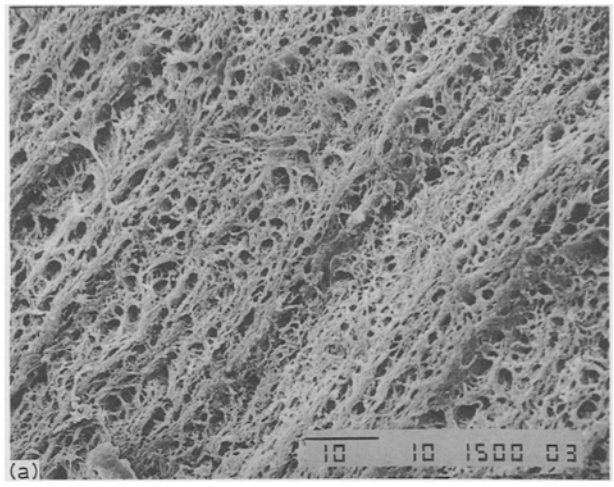
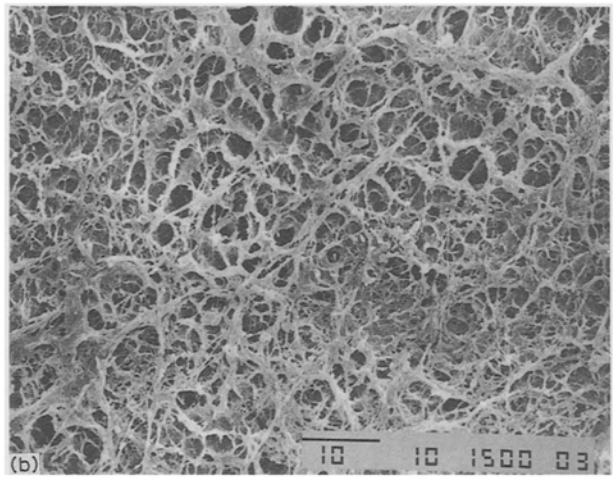


Figure 3 SEM images illustrating the structure of the surfaces of pure PVA hydrogels having a constant N (8 cycles) and different values of C: (a) 2.5%; (b) 8%; (c) 10%. (Bar = 10 μm.)





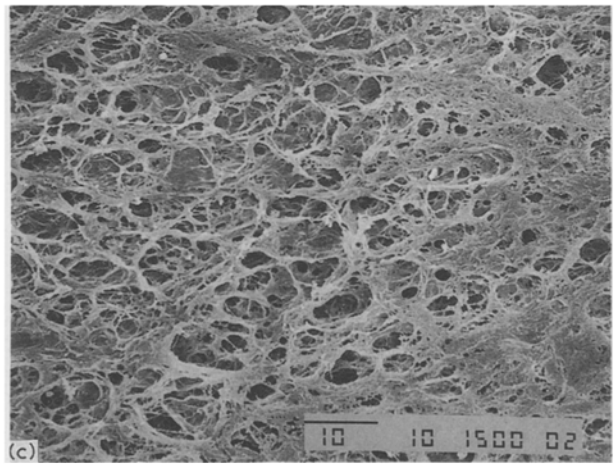


Figure 4 SEM images illustrating the structure of the surfaces of pure PVA hydrogels having a constant C (10%) and different values of N: (a) 2; (b) 6; (c) 8. (Bar = 10 μm.)

in pore size is observed when N increases, as illustrated in Fig. 4a–c.

The SC/PVA hydrogels have been obtained by submitting solutions containing a constant PVA amount of 2.5% to 8 freezing–thawing cycles. These hydrogels have been studied in relation to the relative SC content.

The SEM analysis shows that the structure and pore size (Fig. 5) of these hydrogels are almost the same as those of the pure PVA hydrogels at the same concentration (Fig. 3a). Fragments of the various hydrogels were treated with collagenase to remove collagen by enzymatic digestion. SEM images of hydrogels treated with collagenase show filaments of collagen interstitially placed in the PVA network (Fig. 6).

Quantitative determination of digested collagen was carried out both by the hydroxyproline test referred to above and by measuring the weight loss of the samples. The two methods agree quite reasonably,

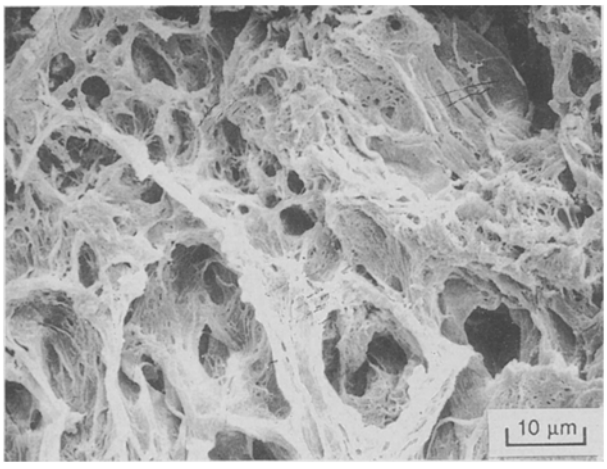


Figure 5 SEM image of a SC/PVA hydrogel having $C = 2.5\%$, $N = 8$ and SC/PVA = 10/90.

giving 16.4% and 17.4%, respectively, of digested collagen with respect to the initial collagen. It can be inferred that two distinct fractions of collagen exist inside the hydrogels: the first one (16–17%) is interstitial and can be digested by collagenase; the second, that accounts for most of the collagen, is bound to the PVA and cannot be enzymatically digested.

Fig. 7 shows the T_m of PVA as a function of the SC content. It is seen that the presence of SC lowers the T_m from about 230 °C of pure PVA to about 210 °C in all mixtures. Mixtures with a higher SC content have slightly lower melting temperatures. The ΔH_m of PVA in the SC/PVA hydrogels (Fig. 8) is less than that of the gel of pure PVA, which shows that the presence of SC disturbs the interactions between PVA chains to a certain extent. No clear dependence of ΔH_m on the SC content in different mixtures can be established from the results obtained. In effect, only slight variations of ΔH_m, apparently not related to SC content, can be seen. This would lead to the hypothesis that even very small quantities of SC are able to produce major disturbances in the PVA crystalline structure.

4. Conclusion

The results obtained in this work demonstrate the feasibility of making PVA based hydrogels containing biopolymers. The freezing–thawing technique is a physical cross-linking method that offers several advantages with respect to others, such as chemical cross-linking and radiation induced cross-linking: it is simple; it does not require any additional chemicals (such as cross-linking agents); it does not require a high temperature. These features allow the use of biopolymers in making bioartificial materials, avoiding any damage or modification of the delicate biological macromolecules.

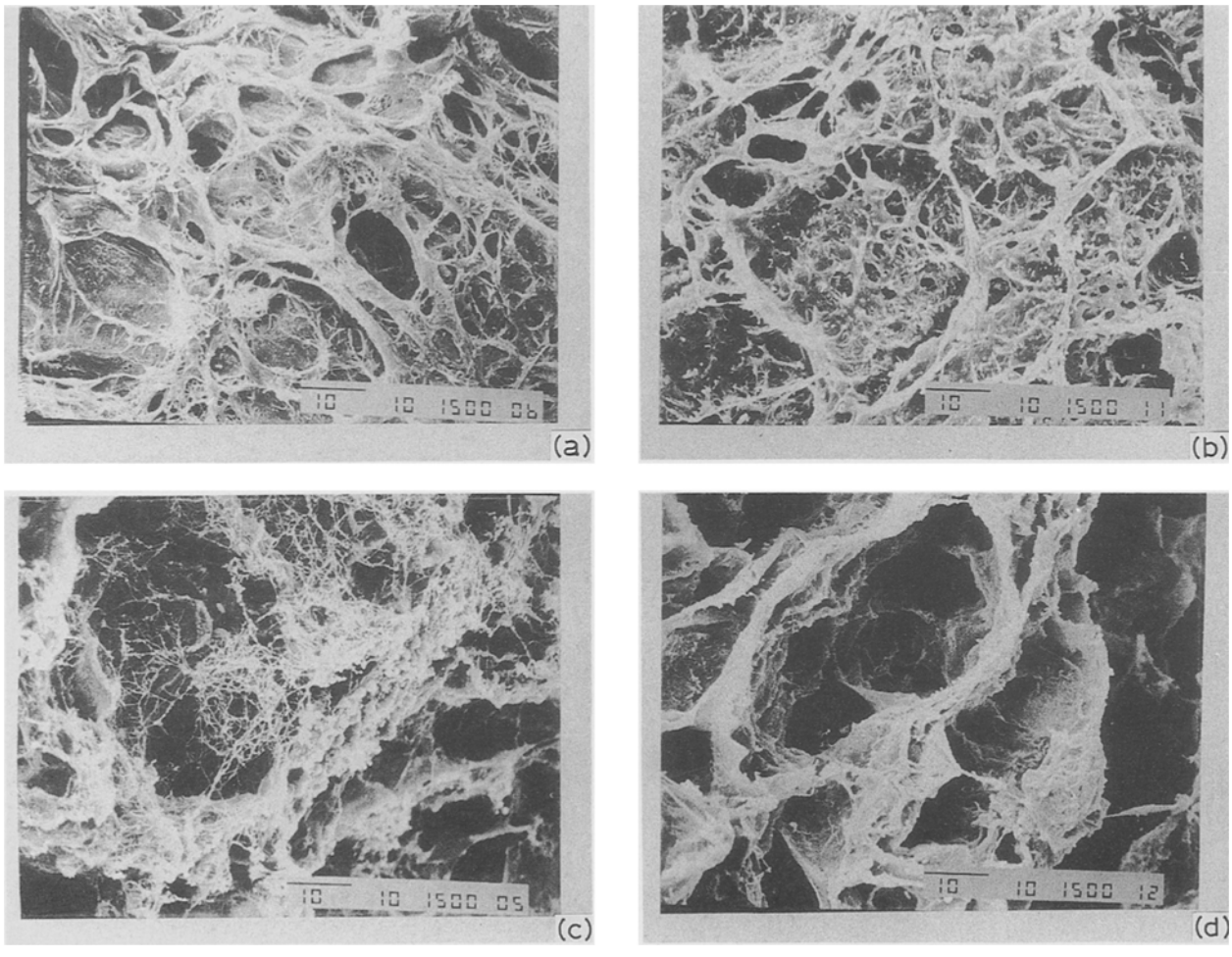


Figure 6 SEM images of a collagenase treated hydrogel (a, c) and of an untreated hydrogel (b, d) having $C = 2.5\%$, $N = 8$ and SC/PVA = 15/85. The top pair (a, b) refer to the surfaces and the bottom pair (c, d) to the sections. (Bar = 10 μm.)

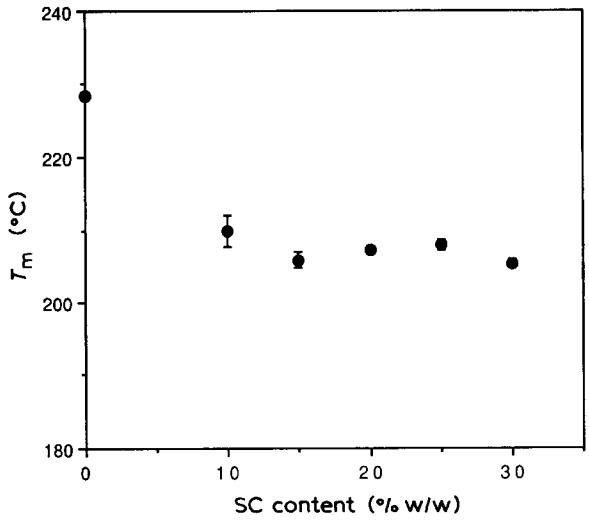


Figure 7 Dependence of the T_m of PVA on the SC content in SC/PVA hydrogels (C = 2.5%; N = 8).

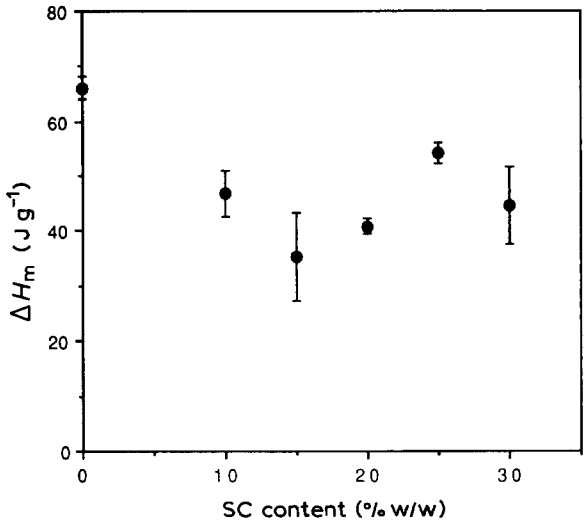


Figure 8 Dependence of the ΔH_m of PVA on the SC content in SC/PVA hydrogels (C = 2.5%; N = 8).

The interesting new ways opened by this method are now being used in our laboratories to prepare new biomaterials.

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