Polyvinyl alcohol cross-linked macroporous polymeric hydrogels: Structure formation and regularity investigation

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A B S T R A C T

A series of novel polyvinyl alcohol (PVA) hydrogels were synthesized by cross-linking of acrylate-modified PVA in aqueous solutions. Hydrogels were prepared at a temperature range —7.5 to —25 °C, macromer concentration 4–12 wt.%, and initiator concentration 0.4 to 1.6 mg/ml. The swelling behavior of polymeric hydrogels in aqueous media with different pH and ionic strength values was investigated. It was shown that they possess a high level of water absorption. The influence of different factors (porosity, pore size, and pore size distribution) and reaction conditions on the hydrogel structure was studied. The interior morphology of the hydrogel networks exhibits a complicated structure filled by fibrillar, lamellar and dendritic formations consisting of cross-linked polymer. The dispersed pores which are randomly distributed can be observed inside these formations and between them.

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1. Introduction

Hydrogels are cross-linked networks of water-soluble polymers. To obtain macroporous hydrogels, a phase separation must occur during the course of the network formation process so that the two-phase structure formed is fixed by the formation of additional cross-links [1]. Polymeric hydrogels have found applications in many different areas of medicine and biotechnology including their use as controlled release system components, carriers for the immobilization and cultivation of molecules and cells, matrices for electrophoresis and immunodiffusion, materials for surgery and as a gel basis for solid culture media [1–6]. A variety of problems associated with their use, as well as the broad range of biological objects encountered, lead to new, often contradictory, requirements for the gels. These requirements stimulate the development and commercialization of novel hydrogel materials for medical and biological applications.

Recently, great attention is being paid to macroporous polymeric hydrogels which are hydrogels containing systems of connected pores of micrometer size. Due to their porous structure such systems have several advantages compared to the usual isotropic hydrogels, in particular considerable osmotic stability, utterly developed specific surface and high permeability [7–10]. Moreover, the existence of connected pores provides effective transport of nutrients to growing cells and tissues and elimination of their metabolism products during their application as paddings for cells and tissue cultivation [8]. Several methods of preparation of porous polymeric hydrogels have been developed: hydrogel freeze-drying after their swelling in water [11]; monomer radical polymerization in the presence of substances capable to emit gas (CO2, N2) during the reaction process [11,12]; polymerization in the presence of reagents soluble in monomer but insoluble in polymer [13,14]; polymerization in the presence of heterophase of insoluble reagent (glucose, sucrose, NaCl), washed out after completing the polymerization process [8,15,16]; and formation of a three-dimensional polymeric matrix in the presence of a heterophase of frozen solvent (water, formamide) by successive defrosting of the system [9,10,17].

Currently, the most widely used and studied macroporous systems are physical cryogels of polyvinyl alcohol (PVA) [18]. At the same time such systems have several essential disadvantages. They are thermally reversible and transfer to solution under heating [19,20] or demand complicated methods of structure stabilization using bifunctional linking agents [21–24] or hard radiation [25–28].

In the present study we propose to use polyvinyl alcohol acrylic derivatives for the creation of cross-linked polymeric macroporous systems. In this case unsaturated groups introduced in the polymer structure are able to form covalent cross-links in the presence of radical polymerization initiators. Hydrogels prepared using this method do not need additional structure fixing and are characterized by high thermal stability sustaining even heating to more than 100 °C [29]. Earlier we have studied the influence of process conditions on the yield of prepared hydrogels [29]. In this work we continue our study of the influence of reaction conditions on the three-dimensional structure of the obtained porous hydrogels and present results of equilibrium swelling.

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2. Experimental

2.1. Materials

Polyvinyl alcohol, BF-03 sort with molecular weight M = 12,000 (CCP, Taiwan) was additionally purified by methanol extraction. Glycidylmethacrylate, N,N,N,N′-tetramethylethylenediamine, and potassium persulphate (Sigma, USA) were used as received. All other chemicals used were of reagent grade and used as purchased without further purification. All solvents and components of buffer solutions were of analytical grade preparations. Distilled-deionized water was prepared with a Milli-Q Plus System (Millipore, USA).

2.2. Synthesis of polymeric hydrogels

The modification of polyvinyl alcohol was carried out following a procedure described previously [26] using a macromer containing 3.5 mol.% of substituted links. The cross-linking of the modified macromers was carried out in thin-walled forms with a size of 100 × 50 × 3 mm. The general scheme of this process is presented in Fig. 1. The accurate amounts of macromer (prepared using methods described earlier [26]) with a substitution degree of about 3.5 mol.% were diluted in distilled water while heating at 70 °C. The obtained solution was cooled to 0–5 °C and then the initiating system (potassium persulphate and N,N,N,N′-tetramethylethylenediamine) was added. The reaction mixture was flooded in forms that were placed in a cryostat (Julabo F-32, USA) where the temperature was adjusted between −5 and −20 °C. At the end of the cross-linking macroporous hydrogel formation process, the reaction system was quickly thawed and the obtained hydrogels were rinsed in boiling water until the complete disappearance of the unreacted components in rinsing water. The quality of washing was controlled by monitoring the absorption intensity in the wave-length range 200–300 nm by using a spectrophotometer (Beckman DU-65, Germany). The rinsed hydrogels were frozen in liquid nitrogen and lyophilized by an Alpha I-4LD freeze dryer system (Martin Christ GmbH, Germany).

2.3. Hydrogel equilibrium swelling degree measurements

The volume equilibrium swelling of the synthesized cross-linked hydrogels was investigated by using the weight method. Hydrogel samples were weighted on an analytical balance (Shimadzu, Japan), and then placed in distilled water or, for the case of osmotic stability studies, in different solutions (pH 1–13) and incubated during the preset period of time. Swollen hydrogels were retrieved from the solutions and the weight of swollen gel was determined as a difference between the weight of hydrogel on the balance plate and the weight of water residues left on the balance plate after the hydrogel was removed. Measurements for each hydrogel sample were carried out at least five times and the average weight of the swollen hydrogels was estimated. The value of equilibrium swelling was determined using the following equation:

\[ S = \frac{m_w}{m_d} - 1 \]

where

- \( m_w \) weight of swollen gel;
- \( m_d \) weight of dry gel;
- \( \rho \) density of water or buffer solution.

In order to measure the swelling of the polymeric part of macroporous hydrogels, swollen samples were wringed between several layers of the filter paper to the constant weight, and when all free water was removed from the hydrogel pores, swelling was estimated using the equation given above.

2.4. Hydrogel texture determination

Scanning electron microscopy runs were carried out at magnifications of 100×, 300× and 1000× on a scanning microscope equipped with a WinEDS system at an accelerating voltage of 15 kV and electron ray current \( 1 \times 10^{-10} \) A (JSM U3, Japan).

3. Results and discussion

The present investigation of the surface of the synthesized polymeric macroporous hydrogels (Fig. 1) showed a rather complicated structural and morphological organization (Fig. 2). As it is clearly demonstrated on the microphotographs the major pattern is filled by fibrillar, lamellar and dendritic formations consisting of cross-linked polymer. The dispersed pores which are randomly distributed on the sample surface can be observed inside these formations and between them.

3.1. Hydrogel total porosity changes investigation

As formation of polymeric macroporous hydrogels was carried out in frozen aqueous solutions it was expected that great influence on the structure of the formed polymeric systems will be exerted by the...
reactive system concentration. As we can see from presented
dependences (Fig. 3) the increase of macromer concentration in
the reactive system leads to a decrease of hydrogel total porosity. This
can be explained by an increase of the polymer part in the volume
unit. Moreover it is well-known that pure water always crystallizes in
the form of hexagonal dendrites whereas water from solutions of
different substances in water crystallizes in a way that is dependent
on solution concentration, freezing rate and temperature [30].
Crystals of irregular form can be observed as a transition stage
between hexagonal crystals and spherulites. Disappearing spherulites
are formed at higher solution concentrations and at higher tempera-
tures. A diverse class of irregular and transition ice crystals forms is
obtained depending on solution concentrations and freezing rates. On
the other hand, the regular dendrites and spherulites can be prepared
only at certain limited conditions [30]. During the increase of solution
concentration the conditions of structural supercooling can take place
which lead to irregular ice crystallization and formation of domains
with a very high concentrated solution. As a result these domains can
be captured in the form of drops during the promotion of the
crystallization front which will cause a decrease of system total
porosity. Besides this, an increase of reactive system concentration
leads to an increase of probability of polyvinyl alcohol physical cryogel
gel-fraction formation with a relatively low porosity. The increase of
cross-linking reactions rates caused by the increase of reactive system
concentration [29] can also influence the hydrogel porosity. The
dependence of hydrogel total porosity on temperature is presented on
Fig. 4. Using Method A (simple freezing) in the temperature range −15
to −25 °C a porosity decrease takes place with a decrease of
temperature which can be explained by the formation of more small
pores, by conditions of structural supercooling and by more active
freezing of the solvent accompanied by an increase of physical cryogel
fraction share. The dramatic decrease of hydrogel porosity for synthesis

Fig. 2. Typical SEM scans of macroporous cryo-formed hydrogels.

Fig. 3. Influence of macromer concentration in the reactive mixture on total porosity
○ and average pore diameter ▲. (Macromer substitution degree — 3.5 molar %,
reaction temperature — −15 °C, and initiator concentration — 1.2 mg/ml).

Fig. 4. Influence of synthesis process temperature on total porosity and average pore
diameter. (●) — porosity, Method A; (▲) — average pore diameter, Method A;
(○) — porosity, Method B; and (□) — average pore diameter, Method A.
(Macromer substitution degree — 3.5 molar %, macromer concentration — 6.0 wt.%,
and initiator concentration — 1.2 mg/ml).
at temperatures higher than $-10 \, ^{\circ}\mathrm{C}$ is probably explained by the formation of isotropic gel due to the long-term placement of the system in the liquid state caused by the low crystallization rate of the solvent. During the use of Method B (temperature hardening) (Fig. 4) hydrogel total porosity changed insignificantly and was much lower than for simple freezing. Obviously it is connected with the higher rate of ice crystal formation and with formation of polyvinyl alcohol cryogel before beginning of the cross-linking reaction by radical mechanism.

The investigation of surface morphology of the hydrogel samples prepared at different initiator concentrations showed that the total porosity decreases slightly with the increase of initiator concentration up to $1.2 \, \text{mg/ml}$ and further increase of initiator concentration leads to a dramatic decrease of total porosity (Fig. 5). This dependence can be explained by the decrease of pore size due to more rapid formation of the three-dimensional structure of the polymeric matrix. In the case of high initiator concentration (more than $1.2 \, \text{mg/ml}$) the decrease of total porosity can be explained by the formation of a considerable fraction of isotropic polymeric gel before the end of solvent crystallization.

### 3.2. Pore size and size distribution studies.

The investigations carried out showed that the experimental conditions of the polymeric macroporous hydrogel synthesis exert a significant influence on hydrogel pore size and size distribution. As we can see in Fig. 3, the average pore size noticeably decreases with the increase of reactive mixture concentration which can be explained by the same as in the case of total porosity changes by the increase of polymer part share in volume unit, and consequently by low possibility for large crystal growth, formation of isotropic gel before the beginning of crystal structure formation, physical cryogel forming and possibility of structural super cooling. The diagram of pore size distribution in Fig. 6 shows that at a higher concentration of reactive system, the size distribution is narrower and most of the pores have sizes less than $20 \, \mu\text{m}$. During the reactive mixture concentration decrease the possibility of formation of larger crystals increases, the pore size distribution becomes wider and the share of large pores increases. By observing the influence of hydrogel synthesis process temperature on hydrogel pore size we can assume that decreasing the temperature in the range between $-10 \, ^{\circ}\mathrm{C}$ and $-25 \, ^{\circ}\mathrm{C}$ for the case of simple freezing (Method A), leads to a monotonous decrease of pore average size (Fig. 4), narrowing of pores size distribution and increase of small pore share (Fig. 7). This can be probably explained by an increase on the number of crystallization centers, while decreasing the temperature, reflected formation of large numbers of smaller pores.

Such a suggestion was also confirmed by a pattern observed for hydrogel synthesis using temperature hardening (Method B) at a very rapid freezing of the system at a liquid nitrogen temperature. In this case the polymeric hydrogels contained predominantly small pores and their size and size distribution did not depend on the process realization temperature (Fig. 8). Figs. 5 and 9 show the influence of initiator concentration on pore average size and diagrams of pore size distributions for hydrogel samples synthesized at different initiator concentrations. From the demonstrated dependence as well as for the case of total porosity, the increase of initiator concentration up to $1.2 \, \text{mg/ml}$ was slightly influenced on pore size, and further increase of

![Fig. 5. Influence of initiator concentration in the reactive mixture on total porosity (○) and average pore diameter (▲). (Macromer substitution degree — 3.5 molar %, reaction temperature — $-15 \, ^{\circ}\mathrm{C}$, and macromer concentration — 6.0 wt.%).](image1)

![Fig. 6. Pore size distribution diagram for hydrogel-samples prepared at different concentrations of macromer in the reactive mixture. (Macromer substitution degree — 3.5 molar %, reaction temperature — $-15 \, ^{\circ}\mathrm{C}$, and initiator concentration — 1.2 mg/ml).](image2)
initiator concentration over 1.2 mg/ml led to a dramatic decrease of pore average diameter. As we have mentioned above, such a pattern can be explained by rapid formation of cross-linked isotropic gel before finalization of the water crystallization process. From the diagram of pore size distribution on Fig. 9 it follows that samples prepared at high initiator concentrations are enriched by small pores which are defects that arise from the water crystallization in the volume of the isotropic hydrogel.

**Fig. 7.** Pore size distribution diagram for hydrogel samples prepared at different reaction temperatures using simple freezing method (Method A). (Macromer substitution degree — 3.5 molar %, macromer concentration — 6.0 wt.%, and initiator concentration — 1.2 mg/ml).

**Fig. 8.** Pore size distribution diagram for hydrogel samples prepared at different reaction temperatures using temperature hardening method (Method B). (Macromer substitution degree — 3.5 molar %, macromer concentration — 6.0 wt.%, and initiator concentration — 1.2 mg/ml).
3.3. Hydrogel equilibrium swelling and osmotic properties study

As it was demonstrated above, hydrogels prepared in cryoconditions represent systems consisting of a large amount of reach-through pores which occupy a considerable part of the sample volume and are divided by zones of cross-linked polymer (Fig. 2). That is why the total volumetric swelling of such hydrogels results from the swelling of the cross-linked polymeric network which is very small value and of the volume of the non-linked solvent filling the macropore space which will mainly determine the total volumetric swelling of the gel. Therefore, we can expect that the total equilibrium swelling of the synthesized polymeric systems will strongly depend on their porosity. The experimental data shown in Table 1 confirm that for all investigated hydrogels their swelling is in direct relation to their total porosity.

Table 1
Total porosity and equilibrium swelling of macroporous hydrogels.

<table>
<thead>
<tr>
<th>Macromer concentration, weight %</th>
<th>Temperature, °C</th>
<th>Initiator concentration, mg/ml</th>
<th>Total porosity, %</th>
<th>Equilibrium swelling, ml/g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>−15</td>
<td>1.2</td>
<td>83.8</td>
<td>16.4</td>
</tr>
<tr>
<td>4</td>
<td>−15</td>
<td>1.2</td>
<td>84.3</td>
<td>15.7</td>
</tr>
<tr>
<td>6</td>
<td>−15</td>
<td>1.2</td>
<td>79</td>
<td>14.3</td>
</tr>
<tr>
<td>8</td>
<td>−15</td>
<td>1.2</td>
<td>51.1</td>
<td>11.6</td>
</tr>
<tr>
<td>10</td>
<td>−15</td>
<td>1.2</td>
<td>82</td>
<td>15.7</td>
</tr>
<tr>
<td>12</td>
<td>−15</td>
<td>1.2</td>
<td>82</td>
<td>15.7</td>
</tr>
<tr>
<td>14</td>
<td>−15</td>
<td>1.2</td>
<td>72.5</td>
<td>13.8</td>
</tr>
<tr>
<td>16</td>
<td>−15</td>
<td>0.4</td>
<td>68.5</td>
<td>13.5</td>
</tr>
<tr>
<td>18</td>
<td>−15</td>
<td>0.6</td>
<td>86.3</td>
<td>16.4</td>
</tr>
<tr>
<td>20</td>
<td>−15</td>
<td>0.8</td>
<td>84.3</td>
<td>16</td>
</tr>
<tr>
<td>22</td>
<td>−15</td>
<td>1.2</td>
<td>82.3</td>
<td>15.7</td>
</tr>
<tr>
<td>24</td>
<td>−15</td>
<td>1.6</td>
<td>58</td>
<td>10.2</td>
</tr>
</tbody>
</table>

As in each particular case of practical applications of hydrogels, solutions of different ionic strength and pH values are used, it was interesting to study the osmotic stability of synthesized hydrogels. As it is shown on Fig. 10, changing the pH did not affect noticeably the equilibrium swelling of gels, but some slight decrease of swelling took place both in acidic and in alkaline media. Some decrease of hydrogel equilibrium swelling was observed (Fig. 10) while changing the molar concentration of NaCl from 0 to 5.0 mol/l. At the same time the relative variation of equilibrium swelling was slightly larger for samples synthesized from reactive mixtures with a lower concentration of macromer. But it should be also mentioned that the observed variation of swelling was not significant (even for hydrogels synthesized from reactive mixtures with a low concentration of monomers) and that is why we can confidently assert that the synthesized polymeric hydrogels possess high osmotic stability.

Fig. 9. Pore size distribution diagram for hydrogel samples prepared at different initiator concentrations in the reactive mixture. (Macromer substitution degree — 3.5 molar %, reaction temperature — −15 °C, macromer concentration — 6.0 wt.%).

Fig. 10. Influence of pH (●) and solvent ionic force (○, □, ◊) value on hydrogel equilibrium swelling. (Macromer substitution degree — 3.5 molar %, reaction temperature — −15 °C, initiator concentration — 1.2 mg/ml, and macromer concentration: ○ — 2.0 wt.%, □ — 6.0 wt.%, ◊ — 10.0 wt.%, and ● — 6.0 wt.%).
4. Conclusions

In the present work we have investigated the novel macroporous polymeric hydrogels of polyvinyl alcohol prepared using cryostructuring methods. The influence of the conditions of the synthesis process on the structure of the obtained macroporous systems was investigated. The experimentally obtained results allow for the adjustment and control of the properties of the synthesized polymeric hydrogels according to the requirements of their practical application area.

References