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ORIGINAL PAPER

Macroporous polymeric hydrogels formed from acrylate modified polyvinyl alcohol macromers

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Abstract Macroporous polymeric hydrogels for the last several years have found broad application in areas connected with medicine, especially in such new disciplines as cell and tissue engineering. In present work a novel combine approach is proposed for preparation of polyvinyl alcohol macroporous hydrogels by cross-linking of polyvinyl alcohol acrylic derivatives in the presence of heterophase of frozen aqueous media. Hydrogels prepared using this method does not need additional structure fixing and are characterized by high thermal stability in swollen state sustaining even heating to more than 100 °C. The influence of different factors and reaction conditions on the cross-linked hydrogel formation process was studied. The high yield of products (80÷95%) was observed when reaction was conducted at temperature range −12÷−18 °C, concentration of macromer 4–12 weight %, and amount of initiator 0.8÷1.6 mg/ml. Moreover, the equilibrium swelling of synthesized macroporous hydrogels was investigated and it was shown that synthesized cross-linked hydrogels are characterized by high water absorption which is weakly depended on solution pH and ionic force values.

Keywords Hydrogel · Cryogel · Macroporous · Cross-linking · Polyvinyl alcohol · Swelling

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Introduction

Hydrogels are swelling in water three-dimensional polymeric networks, synthesized on the basis of hydrophilic monomers. The nature of cross-linking in hydrogels can vary from simple covalent to ionic or hydrogen bonding or even to Van der Waals interactions [1]. Due to several unique properties of polymeric hydrogels such as similar to living tissues water content or their mechanical properties, for the last several decades hydrogels have found broad application in medicine and biotechnology [2–6]. Another type of gels which represent hydrogels prepared at cryo-conditions are defined as cryogels and also attracts intent attention due to simplicity of the network formation process, when frozen zones of the reaction system act as an inert diluent during gelation, which can easily be removed from the gel by thawing, leading to a porous structure [7].

Finally, for the last several years, the major attention among hydrogels is paid to cross-linked macroporous polymeric hydrogels which are gel networks formed by cross-linking of appropriate monomers or polymeric precursors at subzero temperature. The uniqueness of cross-linked macroporous hydrogels lies in the fact that the methodology to produce them leads to formation of an interconnected open macroporous network of pores, which can be used for multiple applications depending upon the type of polymers used. The beneficial feature of these

systems comparing to existing physical hydrogels and cryogels is a unique combination of high porosity with adequate mechanical strength and osmotic stability, due to which they are being envisaged as promising material for various biomedical applications [8–11]. One of the important aspect of cross-linked hydrogels is simple approach by which they can be synthesized and use of aqueous solvent for their synthesis which make them suitable for different biological applications [12–14].

For the present time, the most widely used and studied macroporous systems are physical cryogels of polyvinyl alcohol (PVA) [15]. At the same time such systems have several essential disadvantages. They are thermally reversible and transfer to solution under heating [16, 17] or demand complicated methods of structure stabilization using bifunctional linking agents [18–21] or hard radiation [22–25].

In present study, for the purpose of physical cryogels deficiency remediation we propose to use polyvinyl alcohol acrylic derivatives for creation of cross-linked polymeric macroporous systems. In this case introduced in polymer structure unsaturated groups are able to form covalent cross-links in the presence of radical polymerization initiators. Hydrogels prepared using this method does not need additional structure fixing and are characterized by high thermal stability sustaining even heating to more than 100 °C.

The influence of process conditions on yield of polymeric hydrogels was also studied. The high yield of products (80÷95%) was observed when reaction was conducted at temperature range −12÷−18 °C, concentration of macromer 4–12 weight %, and amount of initiator 0.8÷1.6 mg/ml.

The microscopic examination of prepared macroporous hydrogels surface confirmed the presence of linked pores system in them with pore size of dozens of micrometers. It was shown that such structure provide obtained systems with higher values of equilibrium swelling and higher rates of water absorption, regardless of hydrogel particles size, which is typical for macro- and superporous hydrogels [8, 9, 26, 27].

Experimental

Materials

Polyvinyl alcohol, BF-03 sort with molecular weight M=12000 (CCP, Taiwan) was additionally purified by methanol extraction. Glycidylmethacrylate, N,N,N′,N′-tetramethylethylenediamine, potassium persulphate (Sigma, USA) were used as received. All other chemicals used were reagent grade and used as purchased without further purification. All solvents and components of buffer solutions were analytical grade preparations. Distilled-deionized water was prepared with a Milli-Q Plus System (Millipore, USA).

Macromer synthesis

An acrylate modified PVA (Acr-PVA) was synthesized by esterification of the pendant alcohol groups on the PVA with glycidylmethacrylate in dimethyl formamide. Polyvinyl alcohol was solved in dimethylformamide (DMFA) (10 wt. %) at 120 °C. The glycidylmethacrylate was added to the system after full dissolution of the polymer (the amount of introduced glycidylmethacrylate was determined by needed substitution degree of the resulting polymer). Obtained mixture was stirred at 120 °C for 6 h. After the end of reaction the mixture was cooled, polymer was precipitated in acetone, filtered and dried in vacuum until constant weight. The percent acrylation of the PVA macromers was determined using ^{1}H–NMR (Varian VXR-300S), and all spectra were collected in D_2O. The –CH=CH_2 protons (two doublets and one split doublet at 5.6–6.4 ppm) were rationed by the PVA backbone protons (–CH_2–) and (–CH–) at 1.4–1.8 ppm and 3.8–4.1 ppm to calculate the percent acrylation.

For all the studies in this work 3,5% acrylation was attained.

Gel preparation

The cross-linking of modified macromers with formation of macroporous polymeric hydrogel was carried out in thin-walled forms with the size of 100×50×3 mm. General scheme of this process is presented on Fig. 1.

The accurate amounts of macromer with substitution degree about 3.5 mol % were diluted in distilled water while heating (70 °C). For elimination of dissolved oxygen the obtained solution was vacuumed, argon blown and cooled to 0÷5 °C temperature and then initiating system (potassium persulphate and N,N,N′,N′-tetramethylethylenediamine) was added and reaction mixture was flooded in forms. After that prepared forms were placed in cryothermostat (Julabo F-32, USA) where the adjusted temperature (−5÷−20 °C) was maintained during necessary time. After finishing of the cross-linked macroporous hydrogel formation process, reaction system was quickly thawed and obtained hydrogels were rinsed in boiling water until complete removal of unreacted components in rinsing water. The quality of washing was controlled by measuring absorption intensity for wave-length range 200÷300 nm using spectrophotometer (Beckman DU-65, Germany). Rinsed hydrogels were frozen in liquid nitrogen and lyophilized by Alpha I-4LD freeze dryer system (Martin Christ GmbH, Germany).

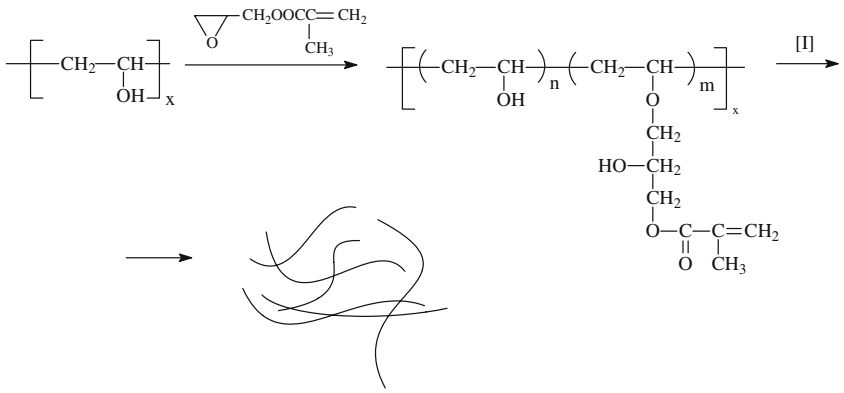

Fig. 1 Scheme of the acrylate modified polyvinyl alcohol synthesis and resulting hydrogel formation

Hydrogel equilibrium swelling degree measurements

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

The value of equilibrium swelling was determined using following equation:

$$S = \frac{m_w - m_d}{m_d} \cdot \frac{1}{\rho},$$

Where

m_w weight of swollen gel
m_d weight of dry gel
ρ density of water or buffer solution.

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

Hydrogel texture determination

Scanning electron microscopy studies were carried out at magnifications of 100×, 300× and 1,000× on scanning microscope equipped with WinEDS system at accelerating voltage 15 kV and electron ray current 1×10^{-10}A (JSM U3, Japan).

Results and discussion

Preparation of polymeric hydrogels

In this work, we report on a novel cross-linked macroporous polymeric hydrogels that can be used for various biomedical applications. First of all we investigated the process of synthesis of polymeric hydrogels on the basis of acrylate modified polyvinyl alcohol macromers and influence of gel formation conditions on yield, structure and properties of end-products.

On the preliminary investigation stage the acrylated polyvinyl alcohol solutions with concentration from 1.0 to 25.0 wt. % containing no initiating system were frozen, incubated for 24 h at −25 °C with following defrosting at room temperature. The formation of physical cryogels after defrosting process was observed only for solutions with polymer concentration higher than 18.0 wt. %. It was determined that such hydrogels are thermal reversible as it was predicted.

After addition of initiating system to polymer solutions before freezing the formation of hydrogels took place even for solution with 1.0 wt. % concentration. At the same time hydrogels prepared with initiator introducing kept stability even after several hours of boiling in water due to presence of covalent intermolecular linkages in them.

The influence of initiating system amount on gel yield is show on Fig. 2. Curves of yield changes during the time of reaction realized at different initiator concentrations are presented on Fig. 3. As one can see, in all cases reaction is practically completed with high yield after 3–4 h, and increase of initiator amount can reduce reaction time to 1 h only.

Process realization at relatively low initiator concentrations (under 0.4 mg/ml) leads to formation of product with low yield. Probably it is caused by low content of free radicals in the system. Increase of initiator concentration provides considerable amount of free radicals which initiate kinetic chains in many active centers simultaneously,

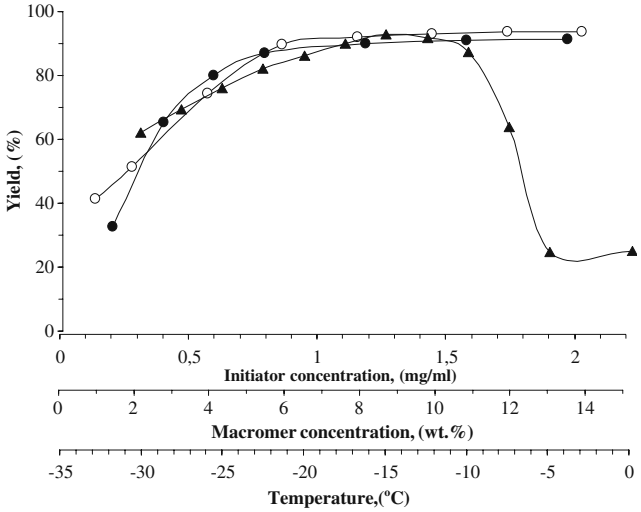

Fig. 2 Influence of initiator concentration in reaction system (●), macromer concentration (○) and temperature (▲) on polymeric hydrogel yield (reaction time 6 h.)

ensuring significant increase of reaction rate and product yield (Figs. 2 and 3). But at further increase of initiator concentration to more than 0.8 mg/ml the change of reaction rate is much less intensive, probably due to chain breaks with participation of both macro-radicals and initiator radicals as a result of large quantity of reactive centers in the system. Moreover, when initiator concentration exceeds 1.2 mg/ml the yield of product rise steadily, but prepared gel was isotropic gel, which can be explained by presence of large number of reactive centers forming part of gel-fraction before the final formation of ice crystals during the cross-linked hydrogel formation reaction.

As formation of cross-linked polymer in water-frozen systems takes place in solution which is in the intercrystalline space it was necessary to discover influence of macromer concentration on the yield of obtained product. Dependence between hydrogel yield and macromer concentration is presented on Fig. 2 and the curves character-

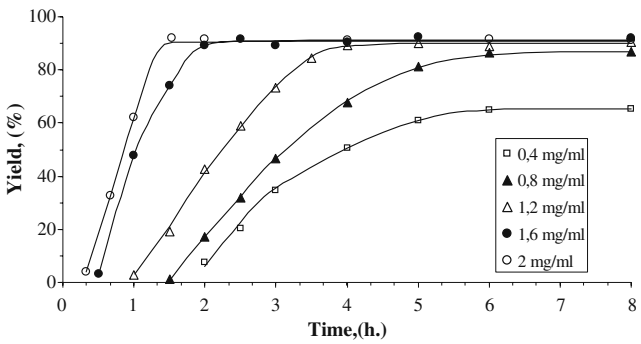

Fig. 3 Influence of reaction time on hydrogel yield at different initiator concentration. (Reaction temperature – −15 °C, macromer concentration 6 weight %)

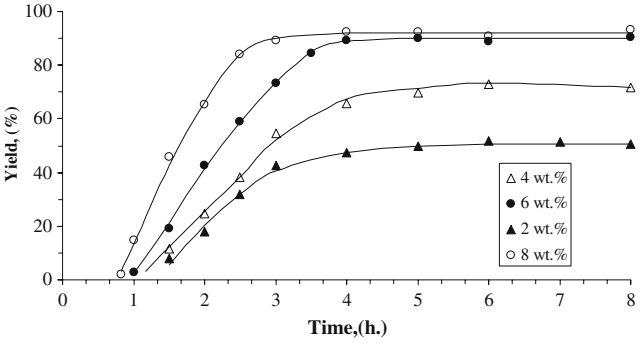

Fig. 4 Influence of reaction time on hydrogel yield at different macromer concentration. (Reaction temperature – −15 °C, initiator concentration 1.2 mg/ml)

izing relation between product yield and time of reactions realized at different macromer concentrations are shown on Fig. 4. As we can see from presented plots, product yield predictably increases with increase of reaction system concentration. It should be mentioned that in sub-zero conditions formation of cross-linked gel with significant yield occurs at 1 weight % macromer concentration already, whereas at positive temperatures noticeable formation of hydrogel was observed only at concentrations over 3÷4 weight % (Table 1). Such situation can be probably explained by increase of active components concentration in liquid inclusions existed in solid frozen solvent. By in spite of the fact that formation of hydrogel took place already at macromer concentrations equal approximately 1 weight %, the practical interest was paid to concentration range 4÷10 weight % as hydrogels prepared from solutions with concentration lower 4 weight % possessed low mechanical stability, and increasing of macromer concentration up to 12÷14 weight % leads to formation of physical polyvinyl alcohol cryogels before the cross-linking process beginning.

To determine optimal synthesis temperature we carried out polymerization reactions in different conditions. The influence of synthesis reaction temperature on products yield is presented on Figs. 2 and 5. As we can see from the

Table 1 Polymeric hydrogel yield. (Temperature +20 °C, iniciator concentration 1.2 mg/g, reaction time 6 h.)

Macromer concentration, wt.%	Yield, %
1	–
2	–
3	34
4	48
5	67
6	78

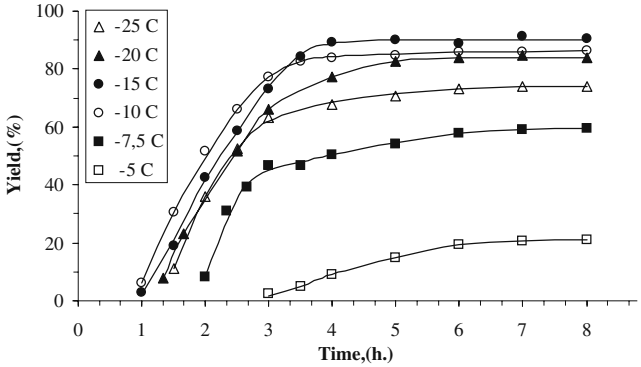

Fig. 5 Influence of reaction time on hydrogel yield at different temperature. (Macromer concentration 6 weight %, initiator concentration 1.2 mg/ml)

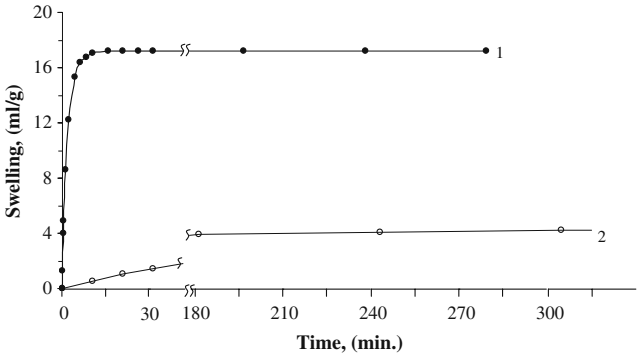

Fig. 7 Influence of time on hydrogel swelling for hydrogels prepared at negative (1) or positive (2) temperatures

plots, temperature decrease from 0÷−5 °C when system stays liquid to−10÷−15 °C when reaction mixture is in solid state leads to the marked increase of product yield. The further temperature lowering decreases hydrogel yield. Such dependences can be described by complex nature of contribution of different physico-chemical process, determining reaction passing.

The low yield of the product at temperatures near 0 °C when reactive system was still in liquid phase, first of all can be explained by low concentration of groups with double bond in reactive system. As the degree of macromer substitution was only 3.5%, the concentration of methacrylate links in reactive system was only about 3.0–9.0 mmol/l. That is why in spite of relatively high amount of free radicals generated by initiating system the initiating efficiency was rather low. Moreover, the low concentrations of reactive groups evidently lead to increase of relative share of termination processes and their prevailing over growth processes.

Freezing of the system leads to noticeable increase of reagents effective concentrations [28, 29]. As a result, in intercrystalline space, interacted components are spatially approached and in this case one of the important reaction realization conditions is met.

During the further temperature decreasing, probably the kinetic factors gain dominant influence, resulting in lowering reaction rate according to Arrhenius equation. Moreover, during the temperature decrease, the considerable increase of non-frozen water viscosity takes place [30], which complicates diffusion of reagents and influence reaction rate. It is also necessary to take into account that at temperature decreasing generation of free radicals (potassium persulphate decay) is also slowed and relative share of chain-break processes is rather high.

Hydrogel equilibrium swelling study

The interior morphology of prepared polymeric hydrogel samples was studied by using scanning electron micrographs (SEM). Figure 6 shows typical SEM of several samples of cross-linked hydrogels in their dried states. The hydrogel exhibit a discontinuous morphology, consisting of

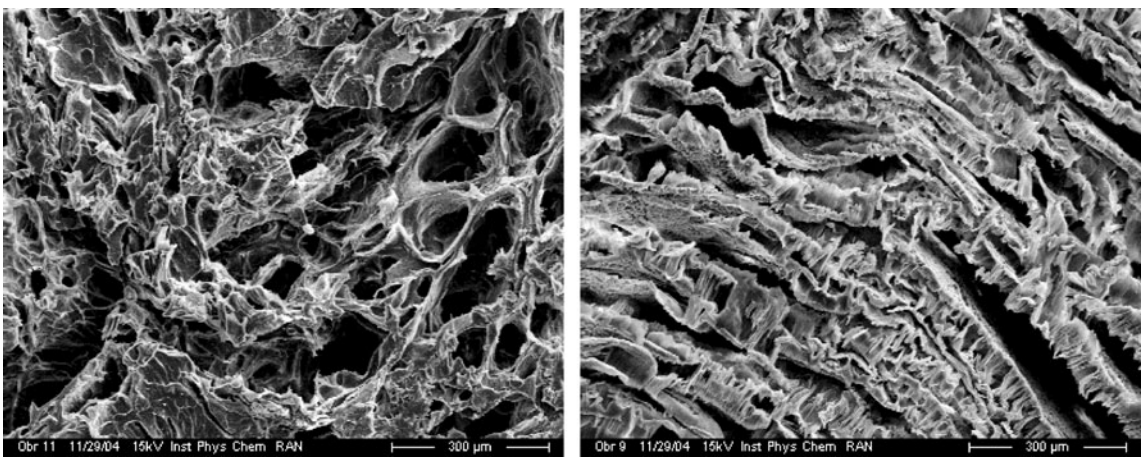

Fig. 6 Typical SEM of prepared macroporous cryo-formed hydrogels

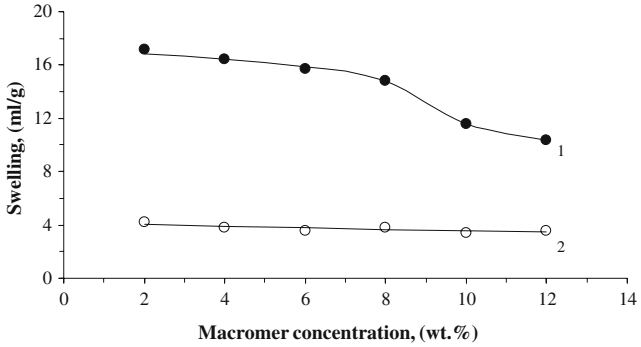

Fig. 8 Influence of macromer concentration in reaction mixture on equilibrium swelling of macroporous hydrogel (1) and hydrogel swelling after removing free water from macropores (2). (Reaction temperature – −15 °C, initiator concentration 1.2 mg/ml)

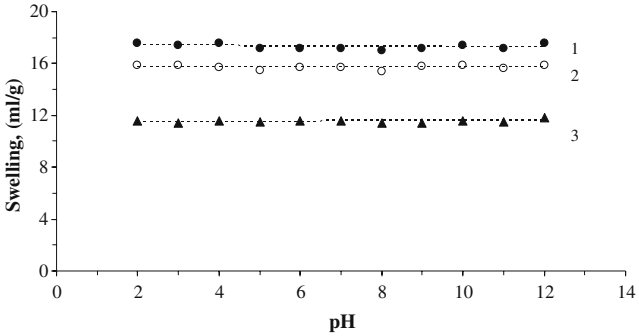

Fig. 9 pH-dependence of macroporous hydrogels swelling. (Reaction temperature – −15 °C, initiator concentration 1.2 mg/ml; 1—macromer concentration 2 weight %, 2—macromer concentration 6 weight %, 3—macromer concentration 10 weight %)

polymer phase separated by irregular shaped voids (pores). The porous structure of hydrogels is due to the action of ice crystals as a pore forming agent during macromer cross-linking. Thus, during freezing of the macromer solution at subzero temperatures, an unfrozen phase containing a high concentration of dissolved macromer and initiating system is formed as water is separated from the solution in the form of ice crystals [28, 29]. After thawing, the voids left from the ice crystals constitute the pore structure of hydrogels.

Table 2 Equilibrium swelling of prepared macroporous hydrogels in water. (Reaction time 6 h.)

Macromer concentration, wt. %	Temperature, °C	Initiator concentration, mg/ml	Equilibrium swelling, ml/g
2	−15	1,2	17,2
4	−15	1,2	16,4
6	−15	1,2	15,7
8	−15	1,2	14,3
10	−15	1,2	11,6
12	−15	1,2	9,8
6	*−10*	1,2	19
6	*−12,5*	1,2	17
6	*−15*	1,2	15,7
6	*−17,5*	1,2	14,2
6	*−20*	1,2	13,8
6	*−22,5*	1,2	13,6
6	*−25*	1,2	13,5
6	−15	*0,4*	16,4
6	−15	*0,6*	16,2
6	−15	*0,8*	16
6	−15	*1,2*	15,7
6	−15	*1,6*	10,2

From the SEM images, one can identify the pores in the cross-linked polymeric gel networks, bounded by sections of cross-linked polymer and the connectivity of these pores. The connectivity of the pores plays a crucial role in fast swelling of the prepared hydrogels (Fig. 7); water and other solvents can enter or leave the cross-linked gels through the interconnected pores by convection [8, 26, 27].

In contrast to the prepared in cryo-conditions cross-linked macroporous hydrogels, the swelling and deswelling of the usual isotropic hydrogels obtained at positive temperatures are controlled by the diffusion of solvent molecules through the gel network, which is a slow process. Thus, equilibrium swelling of cryo-formed macroporous hydrogels was achieved after 20÷30 min, as isotropic gels needed 4 h or more to achieve maximum swelling.

As we can see from Fig. 8, the major portion of liquid absorbed by polymeric hydrogel is placed in pores free space which occupies the bulk volume of prepared samples. The share of the polymeric cross-linked part of the hydrogel material is relatively low.

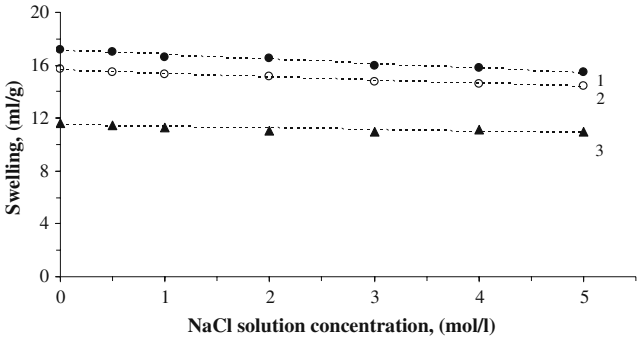

Fig. 10 Influence of solution ionic force on equilibrium swelling of hydrogels. (Reaction temperature – −15 °C, initiator concentration 1.2 mg/ml; 1—macromer concentration 2 weight %, 2—macromer concentration 6 weight %, 3—macromer concentration 10 weight %)

The investigation of swelling process for prepared macroporous gels showed that to a considerable extent it depends on macromers concentration and reaction temperature. The experimental data on cross-linked polymeric hydrogels equilibrium swelling in water are given in Table 2. As in each particular case of practical application the solutions with different ionic force and pH value are used, it was found interesting to investigate synthesized gels behavior in solutions with different ionic force and pH.

As it is shown on Fig. 9, the change of pH value did not exert considerable influence on gels equilibrium swelling, even some small decrease of swelling took place both in acidic and in alkaline media.

While changing molar concentration of NaCl solution from 0 to 5 M some decrease of gels equilibrium swelling was observed (Fig. 10), and relative change of equilibrium swelling was several times higher for samples synthesized at low macromer concentration in reaction system. It should be also mentioned that observed increase in swelling was not considerable and in all cases did not exceed 5%.

Conclusison

In the present work we have investigated possibility of preparation in subzero conditions of cross-linked macroporous polymeric hydrogels on the basis of polyvinyl alcohol acrylic derivatives. It was determined that the rate of cross-linking of modified polyvinyl alcohol in water-frozen systems in the presence of initiating systems consisting of potassium persulphate and N,N,N′,N′-tetramethylethylenediamine and the yield of prepared polymeric cross-linked hydrogel achieve their maximum in the range of temperatures −12÷−18 °C. It was shown that synthesized cross-linked macroporous polymeric hydrogels are characterized by high value of equilibrium swelling, weakly depending on ionic force and pH of the solution.

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