Pore Structure of Macroporous Monolithic Cryogels Prepared from Poly(vinyl alcohol)

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ABSTRACT: Macroporous monolithic cryogels made from poly(vinyl alcohol) (PVA) with degree of saponification 87.7% have been prepared using a crosslinking reaction with glutaraldehyde under acidic conditions at subzero temperatures. The porous structure of the monolithic cryogels from PVA (cryoPVA), analyzed using optical microscopy, scanning electron microscopy, and environmental scanning electron microscopy, revealed interconnected macropores up to 150 μm in size with a noticeable microporosity of the gel walls. Differential scanning calorimetry measurement showed that more than 90% of the water in the cryoPVA monoliths was freezable water, while the amount of polymer bound water increased with increase in the polymer concentration in the cryoPVA sample. The swelling degree of cryoPVA depended on concentration of polymer in the initial reaction mixture and degree of crosslinking. The cryoPVA monoliths were elastic and spongy-like materials that can be dried, stored in dried state, and re-swelled when required. Derivatization of hydroxyl-groups of the cryoPVA monolith allowed incorporation of required functionality.
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Key words: monolith; poly(vinyl alcohol); degree of saponification; cryogel; crosslinking; pore structure; freezable water

INTRODUCTION

There is a considerable need for the preparation of macroporous materials from hydrophilic polymers for chromatography. The majority of the macroporous materials prepared and used so far as chromatography media are of hydrophobic origin based on poly(styrene) or poly(methacrylate) with traditional beaded shape or as a single piece of a highly porous material (so called monolith). The monolithic columns are considered as a new chromatography medium that allows carrying out high-speed separation of proteins without losing the column efficiency.[1–3] The low hydraulic resistance is the most advantageous feature of the monolithic columns as compared with traditional columns packed with beaded adsorbents. Among the monolithic chromatography media, the rigid and hydrophobic poly(glycidyl methacrylate) monoliths are the most well-known.[1,4,5]

The hydrophilic monolithic cryogels based on crosslinked poly(acrylamide) (pAAm-monoliths) have been recently introduced as a very promising medium for chromatography of biological nano- and microparticles.[6–10] These monoliths are prepared through polymerization reactions at subzero temperatures when most of the solvent (water) is frozen while the dissolved reagents are concentrated in small nonfrozen regions, so called "liquid microphase." The gel formation occurs in this liquid microphase and the crystals of frozen solvents perform like porogen. After melting the ice crystals, a system of large interconnected pores is formed. The large interconnected pores endow the cryogel monoliths with unique elastic and spongy morphology and very low back pres-

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sure.[9,11,12] The elasticity and spongy-like morphology make these monoliths fundamentally different from rigid poly(glycidyl methacrylate) monoliths[1,4,5] or macroporous monolithic agarose.[13,14] The prepared pAAm-monoliths were obtained through the polymerization reaction using different low molecular weight monomers as starting material. The aim of this work is to prepare macroporous cryogel monolith with unique properties, as elasticity and spongy-like structure, from a hydrophilic polymer as starting material.

Poly(vinyl alcohol) (PVA) is one of the well studied hydrophilic polymers with a good status in biomedical industry. PVA is nontoxic and readily available polymer that can be used in biomedical and drug delivery applications[15,16] and many biotechnological applications.[17] PVA with degree of saponification (DS) greater than 97% is a hydrophilic polymer whose concentrated aqueous solution can form a weak gel during prolonged storage at room temperature because of the formation of hydrogen bonds. Cryogenic treatment (freezing–thawing) facilitates the gelation process as a result of cryoconcentration effects in unfrozen liquid microphase.[18] Freezing–thawing cycle results in the preparation of the three dimensional network of polymer chains held together by crystallites, acting as physical crosslinks,[16,18] that dissolve in water at temperatures above 50°C.[16,18] The combination of freezing–thawing with crosslinking through γ-radiation induced both physical and chemical crosslinking of filled PVA hydrogels.[19,20] Normally, PVA with DS greater than 97% are required for the preparation of physically crosslinked elastic cryogels with high mechanical strength.[15,18] When the DS of PVA is less than 90% (so called "partly saponified or hydrolyzed" PVA), physically crosslinked cryogel can not be formed because the high concentration of residual acetyl groups prevents hydrogen bond formation.[18,21] This property (impossibility for PVA with DS less than 90% to form gel through hydrogen bond formation) was used during the preparation of macroporous chemically crosslinked cryogel monoliths from PVA (cryoPVA monoliths). PVA with molecular mass 67,000 and DS 87.7% was used for the preparation of the monolithic cryogel through the crosslinking reaction with glutaraldehyde (GA) under acidic conditions.

EXPERIMENTAL

Materials

Poly(vinyl) alcohol (PVA) of the trademark "Mowiol" with molecular weight 67,000 and DS 87.7% was obtained from Clariant (Frankfurt at Main, Germany). Glutaraldehyde (GA, 50% w/v aqueous solution), Cibacron Blue 3GA (C. Blue), and epichlorhydrin (ECH, 99%) were from Sigma (St. Louis, USA). *N*,*N*-dimethyltrimethylenediamine (DMTMDA), albumin fraction V from bovine serum (BSA), sodium chloride, and acetone were from Merck (Darmstadt, Germany).

Production of the cryoPVA monoliths

PVA was dissolved in water (5%, w/v) by stirring at elevated temperature (90°C). After cooling the PVA solution to room temperature, the pH of the solution was adjusted to 1.0–1.2 with 5*M* HCl, and the solution was cooled in an ice bath for 30 min. The crosslinker GA (final concentration, 1.0% w/v) was added, and the reaction mixture was stirred for 1 min. The solution was poured into glass tubes of 13 mm in diameter and was frozen at −18°C. After kept frozen at −18°C overnight, the frozen monoliths were defrosted and washed with water until the solution is neutralized, and kept in 0.3*M* ethanolamine, pH 8.2, for 2 h to block possible free aldehyde groups. The cryoPVA monoliths were washed until neutral, dried in oven at 60°C overnight, and stored at room temperature.

Characterization of the cryoPVA monoliths

Water vapor adsorption experiments were performed according to Plieva et al.[9,11] Briefly, PVA-monoliths, previously dried till constant weight, were placed in a water vapor saturated chamber with no direct contact of the sample with water. The increase in sample weight with time due to absorbed water vapor was checked for 14 days. This value gave the weight of the gel matrix with polymer bound water ($m_{\text{dry polymer + bound water}}$). The content of dry polymer and polymer with bound water in the swollen gel was determined as percent of swollen gel weight ($m_{\text{swollen gel}}$). The total volume of macropores in the swollen cryogel was roughly estimated as follows: the weight of the sample ($m_{\text{squeezed gel}}$) was determined after squeezing the free water from the swollen gel matrix, the porosity was calculated as follows:

$$(m_{\text{swollen gel}} - m_{\text{dry polymer + boundwater}})/m_{\text{swollen gel}} \times 100\%$$

DSC experiments were performed on a DSC 6200 from Seiko Instruments (Shizuoka, Japan). The instrument was calibrated using indium and double-distilled water, and an empty aluminum pan was used as reference. To prepare cryoPVA samples for DSC, discs of the swollen cryoPVA monolith of appropriate size (10-mg sample) were placed in a weighed aluminum sample pan (TA Instruments, New Castle, USA). The pan with wet gel was immediately sealed and weighed. Samples were frozen within the instrument to −40°C and then heated to 60°C, with a scanning rate of 2.5°C/min. Transition enthalpy (Δ*H* expressed as Joules per gram total water) for the melting of

frozen water was determined by EXSTAR6000 Thermal Analysis System connected to a Hewlett-Packard computer. After the scan, the pans were punctured and dried in an oven at 105°C to determine the dry weight. The total water content of each sample was determined gravimetrically by weighting the wet samples before and after encapsulation and after the drying. The amount of freezable water was calculated from the ΔH values, assuming melting of ice has ΔH = 334.45 J/g. Non freezable water content was then determined by subtracting freezable water from the total water content. All experiments were repeated at least in triplicate.

The sorption capacity of the cryoPVA monoliths was measured by immersing the dried cryoPVA monoliths in the water or the buffer medium of different ionic strength until the hydrated weight reached a constant value. Then, the swollen gel was accurately taken out from the buffer solution using a thin needle. After gentle shaking (to remove free liquid droplets from the surface of the swollen gel), the hydrated weight of the swollen cryoPVA sample was measured. The swelling ratio of each sample was calculated as follows: swelling ratio = $(W_s - W_d)/W_d$, where W_s and W_d are the fully swollen and dry weight of cryoPVA sample, respectively.

Microscopy of cryoPVA monoliths

Dried cryoPVA monolith (5 mL) was inserted into the glass column (i.d. 10 mm) and re-swollen in deionized water. The column was equipped with adaptors and connected to a pump. The monolithic cryoPVA column was washed with water (50 mL) at a flow rate of 1 mL/min. A solution of dye C. Blue (50 mg of the dye in 15 mL of deionized water with 5 mL of 4*M* NaCl and 0.5 mL of 2*M* NaOH) was applied to the cryoPVA monolith at a flow rate of 1 mL/min in recycle mode for 48 h. The C. Blue-cryoPVA monolith was washed first with deionized water until the solution was colorless, then with 0.5*M* NaCl (50 mL) and finally with water. The dyed cryo-PVA monolith was removed from the glass column and cut to disks with ~4–5 mm thickness. Thin slices of C. Blue-cryoPVA (10 μm) were prepared as follows: a piece of the colored cryogel (4–5 mm height) was fixed on the special metallic mold and frozen using liquid nitrogen. The frozen samples were cut to thin slices (10 μm) using a microtome. The slices were transferred to a glass slide and thawed. A drop of mounting liquid (85% w/v of glycerol with 15% v/v of Na-phosphate buffer) was added and covered with a cover slip. Samples were studied using a Nikon Labophot-2 light microscope.

Samples for scanning electron microscopy (SEM) were fixed in 2.5% GA in 0.1*M* HCl for 1 h and postfixed in 1% osmium tetroxide for 1 h. Samples were dehydrated in increasing concentrations of ethanol ranging from 0 up to 99.5% and critical point dried. The sample was coated with gold/palladium (40/60) and examined using a JEOL JSM-5600LV scanning electron microscope.

CryoPVA monoliths were also visualized by environmental scanning electron microscopy (ESEM) using a Philips XL30 ESEM with tungsten filament, equipped with a Peltier cooling stage. Cryogels were soaked in water and placed into the ESEM chamber. Temperature was fixed at 2°C and pressure inside the microscope was modified from 6.0 Torr to 1.9 Torr to control the dehydration process. The microscope operated at 15.0 kV. Photographs were obtained at 250× magnification.

Coupling of epoxy-groups to the cryoPVA monolith

A dried cryoPVA monolith prepared from 5% w/v PVA solution (3 mL) was inserted into a glass column (i.d., 10 mm) and re-swollen in deionized water. The column was equipped with adaptors and connected to a pump. The swollen cryoPVA monolith was washed by passing degassed deionized water (50 mL) at a flow rate of 1 mL/min. Functional epoxy-groups were coupled to the surface of cryoPVA by passing of epichlorhydrin (ECH) (10%, v/v, 20 mL) in NaOH (0.5*M*)/dioxane solution (5:1) for 24 h, at a flow rate of 1.2 mL/min in recycling mode. Finally the epoxy-containing cryoPVA (epoxy-cryoPVA) monolith was washed with deionized water until pH became neutral. Then, the epoxy-cryoPVA monolith column was washed with 50 mL of deionized water, followed by 50 mL of 0.1*M* Na-carbonate buffer, pH 9.5, at a flow rate of 1 mL/min. The solution of *N*,*N*-dimethyltrimethylenediamine (DMTMDA) (0.3*M* in 0.1*M* Na-carbonate buffer, pH 9.5, 30 mL) was passed through the column in a recycling mode for 24 h. Finally the anion-exchange cryoPVA monolith was washed with water until neutral.

Chromatographic experiments

All chromatography experiments were performed using Biologic DuoFlow Chromatography System (Bio-Rad, Hercules, USA). The cryoPVA monolith (3 mL) in the glass column (i.d. 10 mm) was equipped with upper and lower commercial adaptors and connected to the Biologic DuoFlow Chromatography System. The marker (acetone, MW 0.058 kDa) was applied in deionized water. Chromatographic peaks were recorded at 280 nm after injecting 50 μL marker solution (2%, v/v) at a flow rate of 1 mL/min. Pressure drop experiments through different columns were performed in water as equilibration medium, and at flow rates from 1 to 10 mL/min corresponding to linear flow rates from 76 to 764 cm/h. The water was passed

TABLE I
Preparation of CryoPVA Monoliths from PVA of Different DS

PVA grade	Degree of saponification (mol %)[a]	Molecular weight (g/mol)[a]	Chemical crosslinking	Ability to form a cryogel	Water flow through the monolithic columns (cm/h)[b]
Mowiol 20–98	98.4 ± 0.4	125,000	None	Elastic cryogel	Negligible flow
Mowiol 4–98	98.4 ± 0.4	27,000	None	Elastic cryogel	Negligible flow
Mowiol 8–88	87.7 ± 1.0	67,000	None	No gel formed	—
			Crosslinking with GA	Elastic and spongy-like cryogel	510
Mowiol 4–88	87.7 ± 1.0	31,000	None	No gel formed	—
			Crosslinking with GA	Elastic and spongy-like cryogel	670

PVA solution (5%, w/v) in plastic syringe (5 mL) was frozen at −18°C for 16 h. After defrosting, the monolithic cryogel formed was washed extensively with water.

[a] Data according to the manufacturer (Clariant, Frankfurt at Main, Germany).

[b] The water flow through the monolithic columns was measured at the constant hydrostatic pressure equal to 1 m of water column (pressure about 0.01 MPa).

through the column for 1 min at each flow rate. BSA chromatography on ion-exchange cryoPVA monolith column was performed in 20 m*M* Tris–HCl buffer, pH 7.0, at a flow rate of 2 mL/min. Elution was performed with 1.5*M* NaCl in 20 m*M* Tris–HCl buffer, pH 7.0.

RESULTS AND DISCUSSION

Preparation of cryoPVA monoliths

PVA is manufactured by saponification (or hydrolysis) of poly (vinyl acetate). PVA with DS less than 90% may be considered as copolymers of vinyl alcohol (88–90%) and vinyl acetate (10–12%). The content of the remaining acetyl groups (presented as DS) in PVA has an overall effect on the chemical properties, solubility, and the potency of the polymer to crystallize. On a molecular level, the crystallites of PVA can be described as layered structures. The randomly oriented polymer chains run parallel to one another in certain areas, forming crystalline regions.[15]

The ability of PVA with different DS to form cryogel and the porosity of the cryogels estimated as water flow through cryoPVA monoliths are summarized in Table I. The cryoPVA monoliths prepared from PVA with DS values higher than 97% (Mowiol 4–98 and 20–98) were elastic but not spongy. These physically crosslinked cryogels have pores in the range of 0.1–1 μm.[18,22] The absence of the so-called "flow-through" pores (with the dimension of at least 1.5–2 μm^4) resulted in a negligible flow through these monoliths (Table I). No physical gel was formed for PVA with DS values less than 90% (Mowiol 4–88 and 8–88) because of the high content of residual acetyl groups that prevented the hydrogen bond formation.

Thus, the physically crosslinked cryoPVA monoliths prepared from PVA with DS values higher than 97% (Mowiol 4–98 and 20–98) could hardly present any interest as potential chromatographic materials due to their high flow resistance. On the contrary, the macroporous monoliths that were obtained using chemically crosslinking of PVA with DS values less than 90% (Mowiol 4–88 and 8–88) had low flow resistance, indicating the presence of flow-through pores. Such materials are of a clear interest as new chromatographic adsorbents.

Further on, the PVA with DS 87.7% and molecular weight 67,000 (Mowiol 8–88) was used in this study. It was found that the crosslinked cryogel monoliths were both spongy and elastic. Chemical crosslinking of PVA chains is used for the preparation of mechanically stable gels. In the late 1960s, PVA crosslinked with formaldehyde to form a highly porous sponge (Ivalon®) was used for medical application.[23] GA as a bifunctional agent was widely used for the PVA crosslinking, as the reaction is simple to carry out and the resultant bonds are stable.[24–29] The crosslinking of the PVA chains restricted the mobility of polymer segments and increased the rigidity of monolithic cryoPVA. The mechanical strength of the prepared cryoPVA monoliths increased when the polymer concentration and the content of the crosslinker in the initial reaction mixture were raised. Highly crosslinked cryoPVA monoliths had higher porosity and hence higher water flow through the monoliths (Table II) as compared with those prepared at the same PVA concentration but at lower crosslinker concentration in the reaction mixture. This effect was previously observed for polyacrylamide based cryogel monoliths.[7] Highly crosslinked PVA monoliths were re-swollen after drying within 1–2 min (Table II).

Pore structure of the cryoPVA monoliths

Pore volume in the cryoPVA-monoliths was determined by DSC measurements and by water vapor

TABLE II
Macroporous CryoPVA Monoliths Prepared from 5% w/v PVA

GA concentration in the reaction mixture (% v/v)	Water flow through the monolithic columns (cm/h)[a]	Re-swelling time of cryoPVA-monolith after drying at 60°C
0.05	n/d	40–48 h
0.2	128.0	60–70 min
0.5	318.2	1–2 min
1.0	509.7	1–2 min

[a] The water flow through the monolithic columns was measured at the constant hydrostatic pressure equal to 1 m of water column (pressure about 0.01 MPa).

adsorption experiments, according to Plieva et al.[9,11] DSC was used to determine the amount of free water that is not bound by hydrogen bonding.[30,31] Free water in gels has the same transition temperature, enthalpy, and DSC curves as pure water. Non freezable water in the gels is composed of water molecules that are bound to polymer chains through hydrogen bonds. Non freezable water shows no endothermic peak in the temperature range of −70 to 0°C.[31,32] Typical DSC thermograms for the cryoPVA monoliths prepared from reaction mixtures with different PVA concentrations are presented in Figure 1. The DSC thermogram for cryoPVA monolith prepared from 8% w/v PVA solution showed broader peak than that for cryogels prepared from 5 and 3.5% w/v PVA solutions, respectively, indicating that ice crystals melted over a wider temperature range. This can be attributed to the increased amount of freezable water that interacts weakly with polymer chains and therefore is harder to melt. Both DSC and water vapor absorption experiments (Table III) showed that the total content of free water in cryoPVA monoliths was more than 90% and decreased slightly with increase in the polymer concentration in the reaction mixture. However,

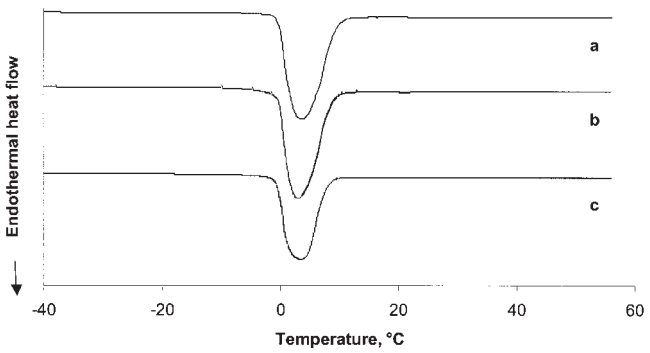


Figure 1 DSC heating thermograms for the cryoPVA monoliths prepared from the reaction mixture with different PVA concentrations: (a) 3.5% w/v, (b) 5% w/v, and (c) 8% w/v.

TABLE III
Different States of Water in CryoPVA Monoliths Prepared from Different PVA Concentration

PVA concentration in reaction mixture (% w/v)	Total water[a] (%)	Free water[b] (%)		Bound water[c] (%)	
		DSC	Vapor ads.	DSC	Vapor ads.
3.5	95.6	91.8	90.7	3.8	4.9
5	94.7	89.6	88.0	5.1	6.7
8	94.4	88.0	84.9	6.4	9.5

[a] Total water content in cryoPVA was determined as difference between the swollen and dried cryogels.
[b] Free water was determined as freezable water using DSC or as difference between total and adsorbed water in water vapor adsorption experiments.
[c] Bound water was determined as the difference between total and free water when using DSC and as the amount of water adsorbed in water vapor adsorption experiments.

the amount of bound water from DSC measurement was lower than that obtained from the water vapor adsorption experiments (Table III). This may be explained by the fact that weakly bound water in fact freezes as free water in the cooling process,[33] alternatively, during water vapor adsorption experiments the capillary condensation of water could result in the accumulation of additional water with respect to bound non-freezable water.

A direct way to study the porosity of the cryoPVA monoliths is microscopy. To facilitate the optical microscopy (OM) studies, the cryoPVA was dyed with C. Blue. OM revealed the porous structure (Fig. 2(a)) (radial cross section was examined) with up to 150–200 μm pores. Microporosity of the gel walls is clearly visible on the microphotographs of cryoPVA monolith prepared from 5% w/v PVA solution (Fig. 2(b)).

SEM provides better resolution of the structure details; however, distortions of the gel structure could occur during the drying process. The SEM micrographs of radial cross section revealed macropores of up till 100-μm size surrounded by dense gel walls of 1–5 μm thickness (Figs. 3(a)–(c)). At the highest magnification, the microporous structure of the pore walls is clearly visible (Fig. 3(c)).

The ESEM allows the examination of the hydrated samples in their natural state.[34–36] One of the important advantages of the ESEM technology is the possibility of monitoring changes in the structure of the material while allowing the sample to dehydrate slowly.[37] The ESEM of microphotographs the cryoPVA monoliths at different stages of the dehydration process are presented in Figures 4(a)–4(e). In the fully hydrated cryoPVA monoliths, it is difficult to visualize the pores as they are filled with water (Figs. 4(a) and 4(f)). As the water evaporates, the surface details became more visible (Figs. 4(c)–4(e), 4(g), and 4(h)). The ESEM micrographs at high degree of the

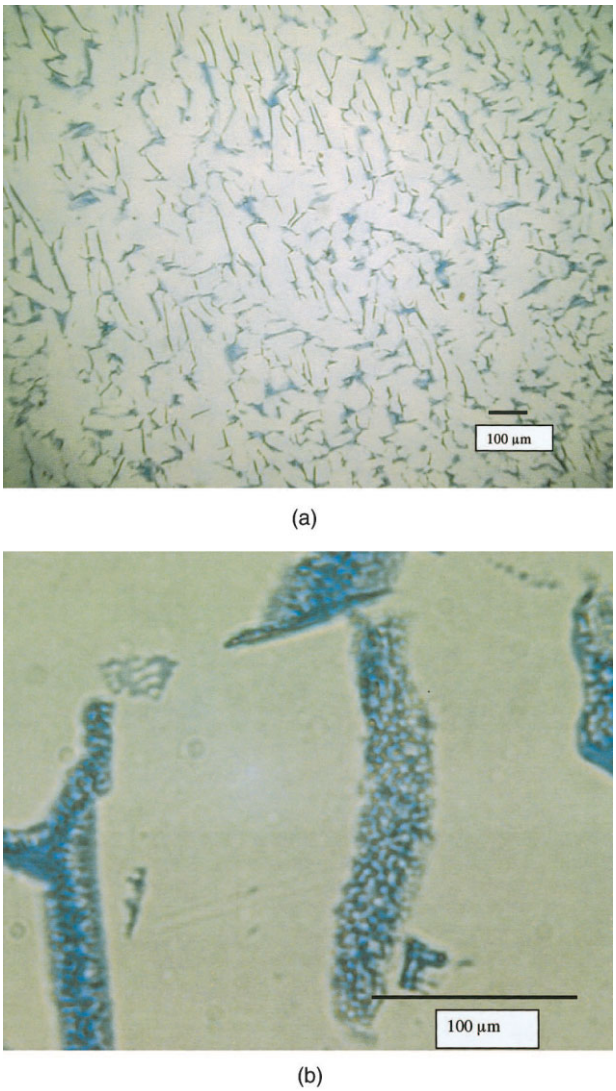


Figure 2 Micrograph of radial cross section of cryoPVA monoliths prepared from 5% w/v PVA solution at different magnifications (a) and (b). For the preparation of cryogel microscopic section for OM see Materials and Methods. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

dehydration showed the macroporous structure of cryoPVA monoliths with pore size of hundreds micrometers (Figs. 4(d) and 4(e)). CryoPVA monolith prepared from 8% w/v PVA solution had thicker pore walls as compared for cryogel prepared from 5% w/v PVA solution (Figs. 4(g) and 4(h)). The microporosity of the walls of the pores was clearly visible for the dehydrated cryoPVA sample (Fig. 4(h)).

Swelling of cryoPVA monoliths

One of the important parameters of the hydrophilic chromatography materials is their swelling ability in buffers with different ionic strength. Increase in swelling is mainly due to the increase in free-water (water

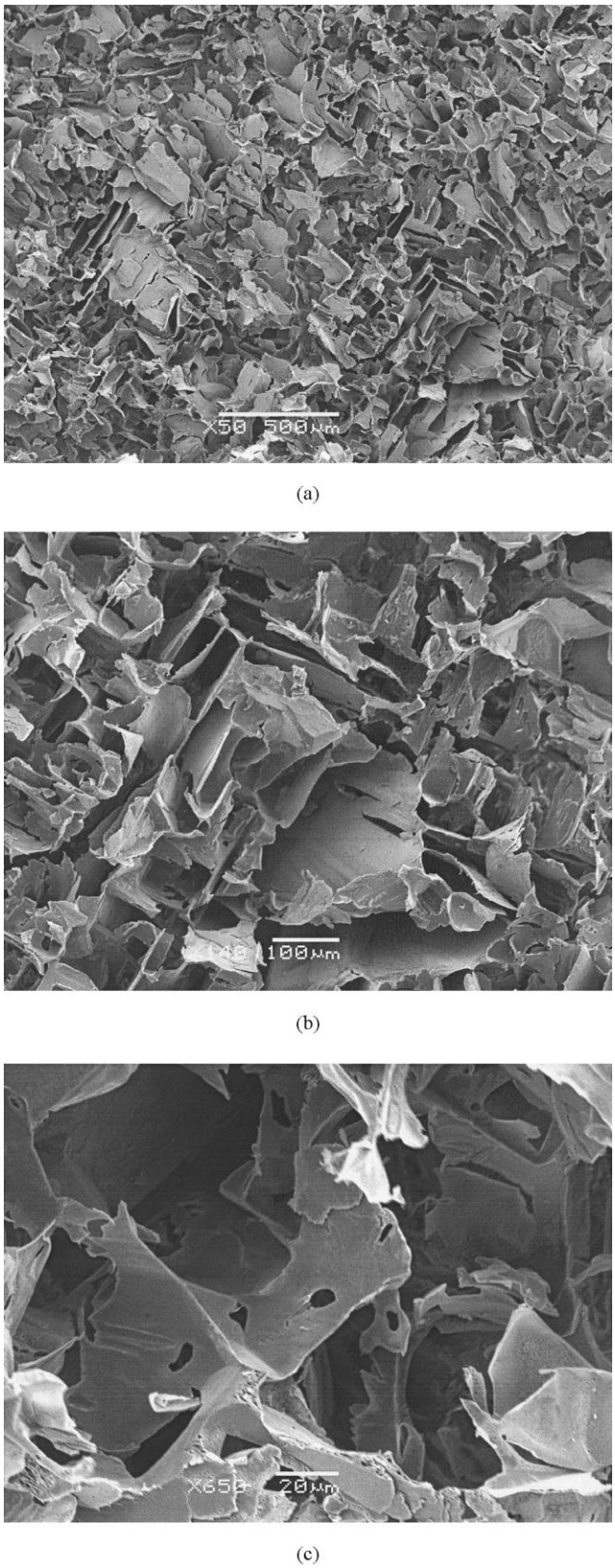


Figure 3 SEM microphotograph of radial cross section of cryoPVA monoliths prepared from 3.5% w/v PVA solution. For the preparation of cryogel samples for SEM see Materials and Methods.

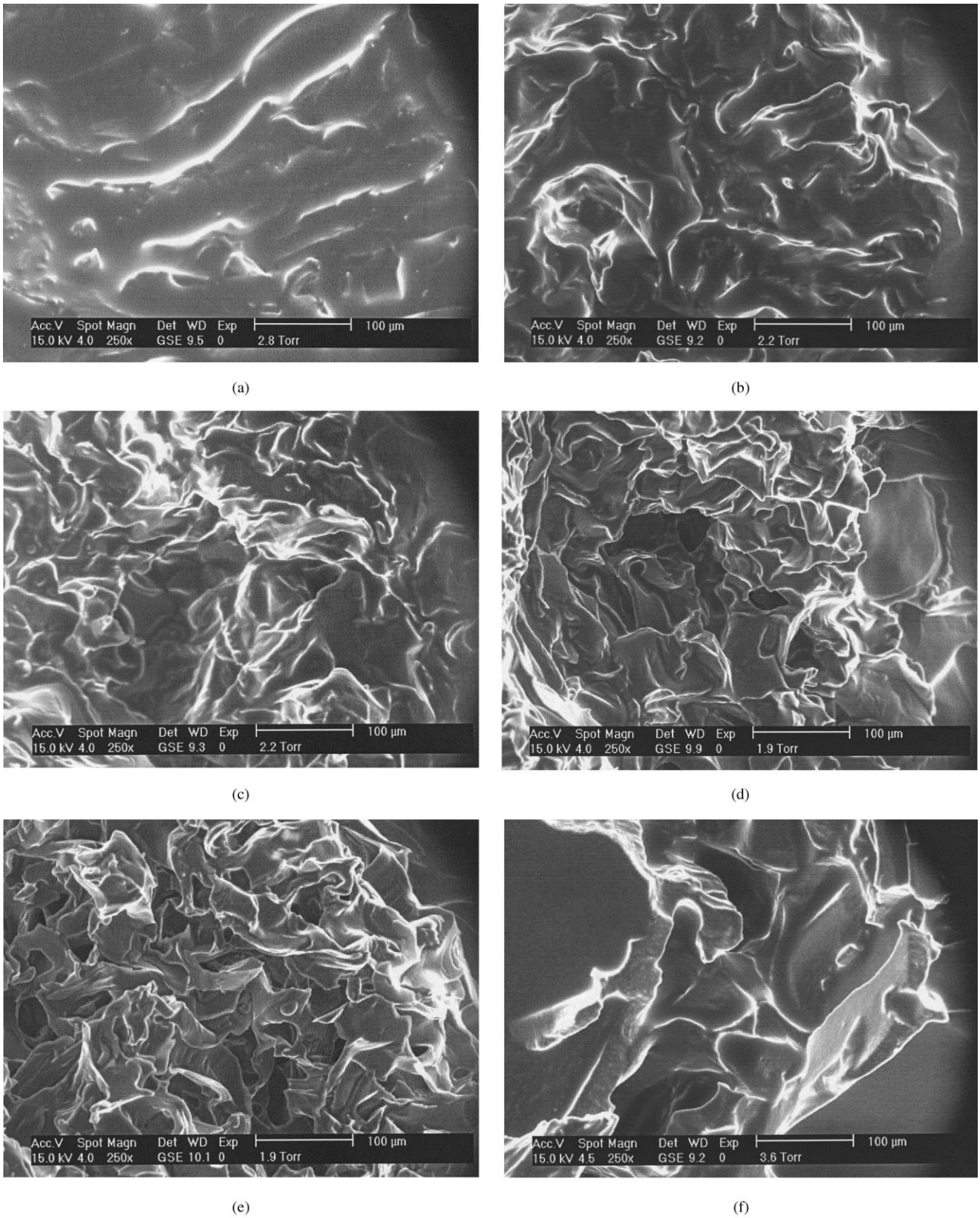
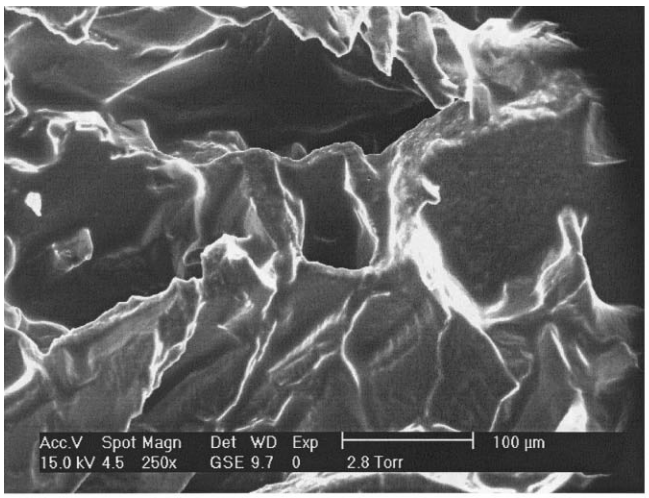
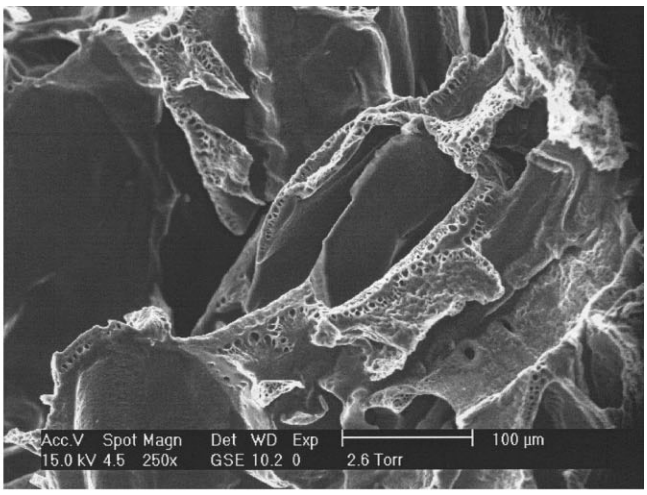


Figure 4 ESEM micrographs of the cryoPVA monoliths prepared from 5% w/v PVA solution (a)–(e) and 8% w/v PVA solution (f)–(h) at different degrees of dehydration. Images (a) and (f) correspond to fully hydrated cryoPVA samples. As the dehydration degree increased the surface topography becomes more visible (from (b) to (e) or from (g) to (h)).



(g)



(h)

Figure 4 (*Continued from the previous page*)

that does not take part in hydrogen bonding with polymer molecules) content in a hydrogel.[38] The water absorbency depends directly on crosslinking density and degree of crystallinity in the hydrogel.[39] For cryoPVA monoliths prepared from the same concentration of PVA (5% w/v), the swelling degree decreased with increase in GA concentration in the reaction mixture (Fig. 5). High crosslinking of the polymeric chains is one of the approaches to prepare rigid chromatographic sorbents, for example, polystyrene networks hypercrosslinked by numerous rigid bridges.[40,41] The independence of swelling degree on the ionic strength for highly crosslinked cryoPVA monoliths makes them attractive as a chromatographic medium.

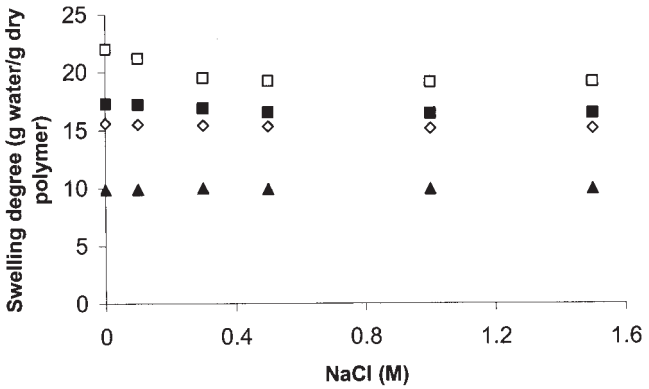


Figure 5 Swelling (water absorbency) of the cryoPVA monoliths at different salt concentrations. The cryoPVA monoliths were prepared from 5% w/v PVA solution and GA concentrations 0.2% v/v (open squares), 0.5% (closed squares), 1.0% (open diamonds) and 2.0% (closed triangles), respectively.

Chromatographic behavior of cryoPVA

The porosity of the chromatographic medium defines the effluent profile of the low molecular weight

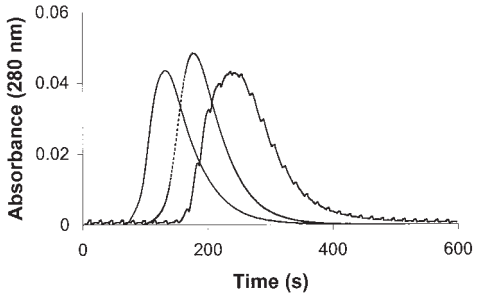


Figure 6 Acetone effluent profile for the cryoPVA monolith prepared from PVA solution with different PVA concentration: 3.5% w/v –solid line, 5% w/v – dash line, and 8% w/v –short and long dash line, respectively. Acetone pulse (2% v/v in deionized water, 50 μL) was passed through the monolith columns at a flow rate of 1 mL/min.

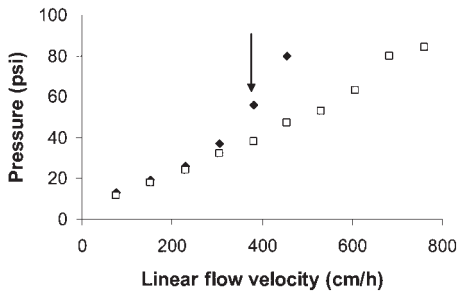


Figure 7 Pressure drop at different flow rates for cryoPVA monoliths prepared from 5% w/v PVA solution and different GA concentration: 0.5% v/v (closed diamonds) and 1.0% v/v (open squares). Arrow indicates at what linear flow rate the cryoPVA monolith (prepared in the presence of 0.5% GA) was compressed up to 75% of its initial height.

marker (acetone). Not surprisingly, the acetone effluent profiles were different for cryoPVA monoliths prepared from PVA solutions with different concentration (Fig. 6). Retention time of the marker increased for the monolithic cryoPVA prepared from PVA solution of high concentration (8% w/v), indicating the formation of the superporous cryoPVA monoliths with smaller size of pores as compared with cryoPVA monoliths prepared from more diluted PVA solutions.

CryoPVA monolith columns had low back pressure indicating the porous structure with large size of the interconnected macropores. The pressure drop through the cryoPVA monolith columns depended on PVA concentration in the initial solution and degree of crosslinking. Less crosslinked cryoPVA monoliths (0.5% v/v and less GA content in the reaction mixture) compressed up to 70–75% of the initial height at high linear velocity, while no compression was observed for highly crosslinked monoliths (GA content 1–2 v/v %) (Fig. 7).

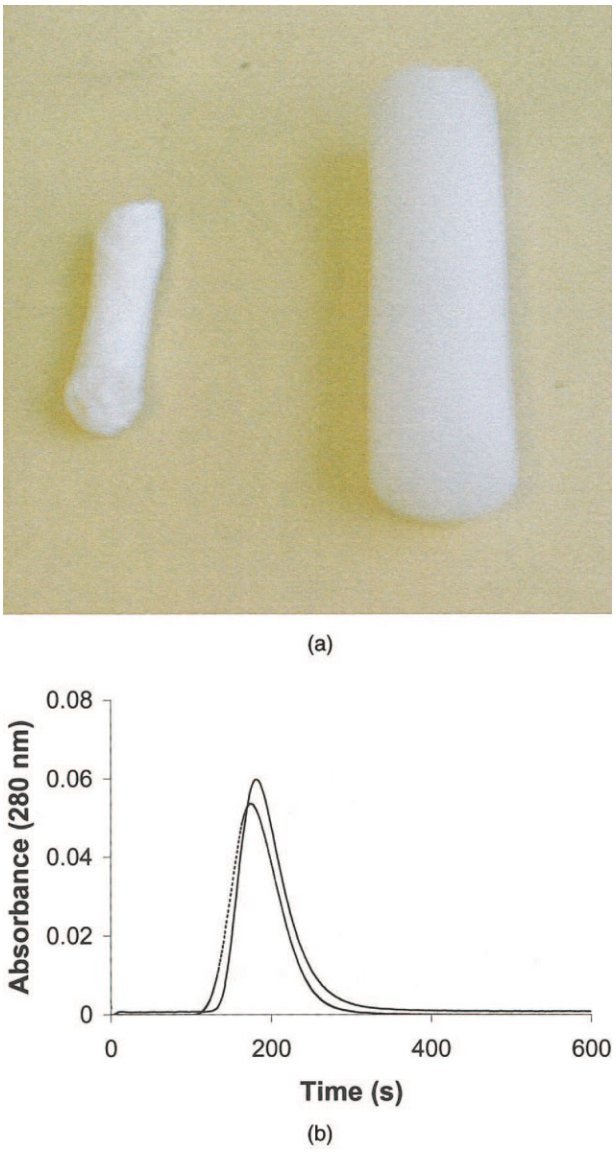


Figure 8 Dried and swollen cryoPVA monolith prepared from 5% w/v PVA solution (a) and acetone effluent profiles for the original cryoPVA monolith (solid line) and for the dried and re-swollen cryoPVA monolith (dash line). Acetone pulse (2%, 50 μL) was passed through the columns in deionized water at a flow rate of 1 mL/min. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

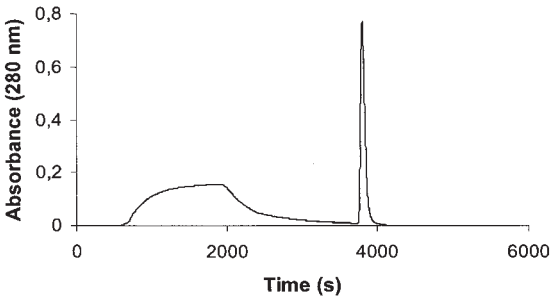


Figure 9 Chromatographic profile for the BSA binding to the anion-exchange cryoPVA column (3 mL) and elution with increased salt concentration. BSA solution (1 mg/mL) was applied to the column in 20 m*M* Tris–HCl buffer, pH 7.0 at a flow rate of 2 mL/min. Elution was performed with 1.5*M* NaCl in the 20 m*M* Tris–HCl buffer, pH 7.0. CryoPVA monolith was prepared from 5% w/v PVA solution and GA concentration 0.5% v/v.

The cryoPVA monoliths can be dried and stored in dried state (Fig. 8(a)). Re-swelling of cryoPVA monoliths after contact with buffer solution took only a few minutes depending on the PVA and GA concentrations in the reaction mixture used for the preparation of cryoPVA monoliths. The drying/re-swelling did not practically affect the chromatographic behavior of cryoPVA monolith (Fig. 8(b)).

Ion-exchange functionality was introduced into cryoPVA monoliths by PVA activation with ECH followed by DMTMDA coupling. The anion exchanger produced was capable of binding a protein, BSA, negatively charged under the experiment conditions. The bound protein was eluted with increasing salt concentration (Fig. 9).

CONCLUSIONS

Elastic and spongy cryogels were prepared from PVA with high residual content of acetyl groups (DS 87.7%), using chemical crosslinking with GA. No cryogel was formed from PVA with this DS without chemical crosslinking. Crosslinked cryoPVA monoliths have a macroporous structure with more than 90% water inside cryogel being free, i.e., nonbound to polymer chains. Pore size visualized by OM, ESEM, and SEM was up to 150 μm. The pore size decreased with increase in the concentration of polymer in the reaction mixture. CryoPVA monoliths present potentially

attractive chromatographic media, as they are chemically and mechanically stable, have low flow resistance, and could be easily modified with required functionality.

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