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Polyvinyl alcohol hydrogels I. Microscopic structure by freeze-etching and critical point drying techniques

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Abstract: Freeze-etching (FE) and critical point drying (CPD) techniques were employed to prepare samples for investigating surface and bulk structures of polyvinyl alcohol (PVA) hydrogels by scanning electron microscopy. The hydrogels were obtained by freezing homogeneous solutions containing PVA polymer in either water or an aqueous solution of dimethyl sulfoxide (DMSO). An oriented porous structure was observed in the PVA hydrogel prepared without DMSO. The structure on the surface was found to be more porous than in the bulk for PVA hydrogels prepared from aqueous DMSO solutions. For given compositions of the hydrogels, samples prepared by FE technique showed a highly porous fibrillar structure on the surface, while those prepared by CPD technique showed a collapsed fibrillar structure with much less porosity. This marked difference indicates a collapse of the surface structure caused by the CPD technique. The CPD technique also led to significant reduction in porosity and loss of fibrillar structure in the bulk. Volume shrinkage of hydrogels caused by dehydration in ethanol may be responsible for the surface collapse as well as alteration of bulk structure. The FE technique reveals a more native structure of hydrogels than the commonly used CPD technique. However, it suffers from disadvantages such as charging and structural damage at high magnifications.

Key words: Hydrogels – polyvinyl alcohol – structures – SEM – freeze-etching – critical point drying

Introduction

Investigations of hydrogel structure by scanning electron microscopy (SEM) are complicated by the presence of water in the native state of hydrogels. Any attempt to remove water prior to SEM examination will inevitably influence the morphology. Various techniques have been employed to investigate the hydrogel structure [1–3]. Each technique has its own advantages and disadvantages. However, freeze-etching (FE) is reported to be the most preferred method for studying hydrogel surface structure [1, 2]. Vacuum drying subsequent to dehydration in alcohols has been applied to prepare xerogels of polyvinyl alcohol (PVA) for SEM studies [4, 5]. This simple drying method is expected to result in significant alteration of the gel structure. Critical point drying (CPD) technique, a common preparation method for biological samples, has also been used to prepare PVA xerogels for SEM examination [6, 7]. CPD is generally thought to be a better method than vacuum drying due to the absence of phase transition above the critical point of the transitional fluid.

The structure of PVA hydrogels has been investigated in previous studies [5–8]. However, different structures were reported for PVA hydrogels even for similar processing methods such as repeated freezing-thawing technique [4, 5, 7]. Reported PVA hydrogel structures include fibrillar network [5, 6], irregular porous network [8] and

honeyecomb-like structure [4, 7]. This discrepancy has prompted further investigation of PVA hydrogel structure. In applications such as biomedical implants, the hydrogel surface plays an important role due to its direct contact with host tissues. Thus, a comparison between surface and bulk structures of PVA hydrogels is of practical interest. However, this has not been reported by previous investigators. In the present work, both FE and CPD techniques are applied to examine the structure of PVA hydrogels at the as-cast surface as well as within the bulk or freeze-fractured surface. The results are compared to evaluate the difference between the two techniques. The PVA hydrogels used in this study are prepared from homogeneous solutions of PVA polymer in either water or an aqueous solution of dimethyl sulfoxide (DMSO) [8].

Materials and methods

Materials

The polymer used in this study is atactic polyvinyl alcohol from Air Products and Chemicals, Inc., Allentown, Pennsylvania, USA. AIRVOL 165 was selected because this grade of PVA has the highest commercially available weight-average molecular weight (160 000) and degree of hydrolysis (99.3 + %). The polymer was kept in a dry environment to prevent it from absorbing moisture prior to use. Reagent grade dimethyl sulfoxide was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin, USA.

Preparation of polyvinyl alcohol hydrogels

PVA solutions were prepared by heating a mixture of PVA polymer in either distilled water or an aqueous solution of DMSO for 1 h at 100 °C in nitrogen atmosphere. Water and DMSO vapors were recovered with a condenser during heating. Initial PVA concentrations and weight ratios of DMSO to water were varied as listed in Table 1. The PVA solutions were placed in an ultrasonic bath at 50 °C for 30 min to facilitate the release of air bubbles. When the temperature reached 50 °C and air bubbles were removed, the solutions were cast between glass plates and quenched for 15 h in a freezer at either − 20 ° or − 60 °C. Following the gelation period, the PVA hydrogel sheets were removed from the glass plates and submerged in copious distilled water for at least 1 month to extract DMSO. The water content of PVA hydrogels was measured using a COMPUTRAC LX10 Solid Analyzer.

Freeze-etching (FE)

After extraction of DMSO, the hydrogel sheets (approximately 1 mm thickness) were cut into small samples with surface area between 1 to 2 cm^2. The samples were always maintained wet but with minimal excess water. The samples were then placed flat on a square aluminum holder (2.5 in × 2.5 in × .5 in). The aluminum holder and the samples were submerged in liquid nitrogen for approximately 3 to 4 min until bubbling stopped. For examination of bulk structure, the PVA samples were fractured in liquid nitrogen with the fractured surface facing upward. To minimize condensation of moisture on the sample surface, the aluminum holder and the frozen samples were immediately transferred to the chamber of an AMRAY 1810 scanning electron microscope. The samples were left under vacuum in the SEM chamber for about 20 min in order to remove any ice on the surface that came from excess water or

Table 1. Processing conditions and equilibrium swelling of PVA hydrogels

Sample ID	DMSO/water weight ratio	Initial PVA concentration (weight % of PVA)	Quench temperature (celsius)	Swelling in water (weight % of water)	Swelling in ethanol (weight % of ethanol)
A	0/100	8	− 20	95.2	70.9
B	50/50	4	− 60	94.8	73.9
C	25/75	12	− 20	96.3	71.0
D	75/25	12	− 20	81.9	47.1

condensed moisture. The electron beam was then turned on at an accelerating voltage of 10 KV. As the samples were heated by the electron beam, residual ice on the sample surface underwent sublimation, thereby revealing the PVA polymer structure.

Critical point drying (CPD)

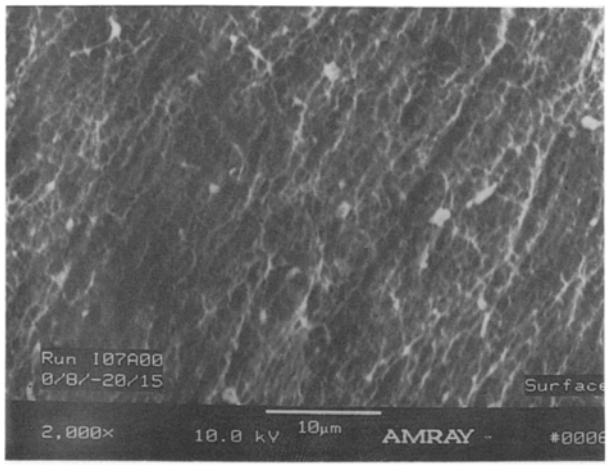
PVA xerogels were prepared for SEM examination using a PELCO CPD2 critical point dryer. PVA hydrogel sheets were cut into small samples of about 1 cm^2 in area. The hydrogel samples were then dehydrated in a series of graded ethanol/water solutions (30 wt%, 60 wt %, 90 wt%) and twice in 100% ethanol. During each dehydration step the samples were allowed to remain in ethanol/water or pure ethanol for a minimum of 2 h while the fluid was slightly agitated by a Burrell shaker. After the final dehydration step, the PVA samples were placed in a specimen basket and immediately transferred to the CPD chamber. The CPD chamber was promptly flooded with liquid CO_2 in order to minimize artifacts which may be caused by premature drying of the PVA samples. The samples were dried above the critical point after five soak-drain cycles of 15 min each. Some PVA xerogels removed from the CPD chamber were freeze-fractured in liquid nitrogen for examination of bulk structure. The samples were then sputter-coated with gold-palladium alloy.

Results and discussion

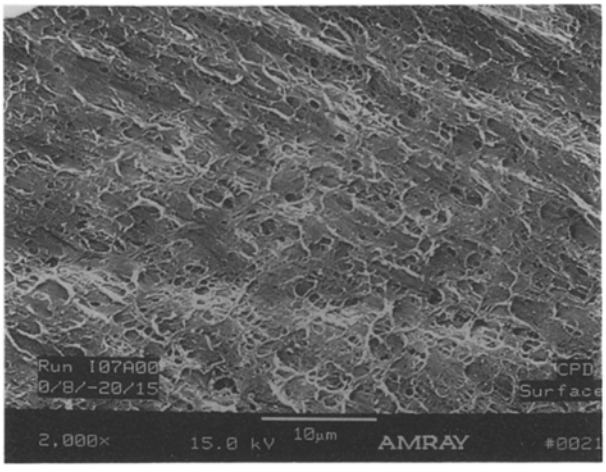
Several preliminary trials were carried out to examine the freeze-etched surface of PVA hydrogels in order to determine the optimum operating parameters for SEM observations. Both the sample and the aluminum holder were completely submerged in liquid nitrogen to quickly freeze the gel structure and to avoid moisture condensation on the sample surface during the freezing period. The aluminum sample holder with its relatively large mass helped to keep the hydrogel sample frozen for at least 30 to 45 min. This time interval allowed the operator to observe and photograph the sample at various locations. An accelerating voltage of 10 KV provided good resolution at relatively high magnifications (2000 × and sometimes 4000 ×), yet did not significantly damage the sample. However, charging was frequently observed at such a high accelerating voltage because the sample had no conductive coating. As ice on the sample surface underwent sublimation, more PVA polymer was exposed and it became more susceptible to charging due to poor charge neutralization. Minor adjustments in the procedure were frequently required to handle different hydrogel samples due to variations in water content, porosity, etc. For PVA xerogels prepared by CPD, an accelerating voltage of 10 KV was also selected to minimize possible damage to the porous structure.

Figures 1a through e illustrate the surface and bulk structures of PVA hydrogel sample A prepared via the FE and CPD techniques. The samples were prepared from a stock solution of 8 wt% PVA in pure distilled water. The equilibrium water content of this PVA hydrogel sample is 95.2 wt%. Figures 1a and b reveal that the surface structure obtained via either FE or CPD is oriented. At 2000 × magnification, the freeze-etched surface exhibits a porous fibrillar structure while the PVA xerogel surface shows a relatively dense structure with numerous cavities and deposited fibrils. This difference indicates a collapse of the surface structure caused by the CPD technique. Such collapse of surface structure has not been reported in previous studies of PVA hydrogels. The pores of the freeze-etched surface in Fig. 1a are non-uniform in size (up to about 4 microns) and shape, and the polymer shows some orientation.The orientation is much more obvious in the bulk of sample A as shown in Figs. 1c and d. This structural orientation is local and manifests macroscopically as opaque domains. The domains are randomly distributed within the gels and result from phase separation of PVA during freezing.

Yokoyama et al. [4] reported that pores in PVA hydrogel are oriented nearly normal to the freezer plate due to the growth of ice crystal in this direction. This phenomenon was observed more frequently at PVA concentrations lower than 5 wt%. In contrast to the observation by Yokoyama et al., the orientation on the surface as well as within the bulk of sample A is parallel to the freezer plate. Phase separation was visually observed along the glass plates during freezing of the solution. This visual observation is consistent with the SEM results shown in Figs 1a and d. Ice



(a)



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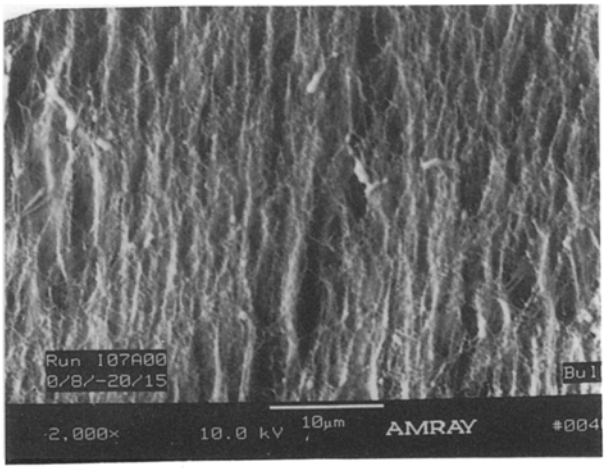

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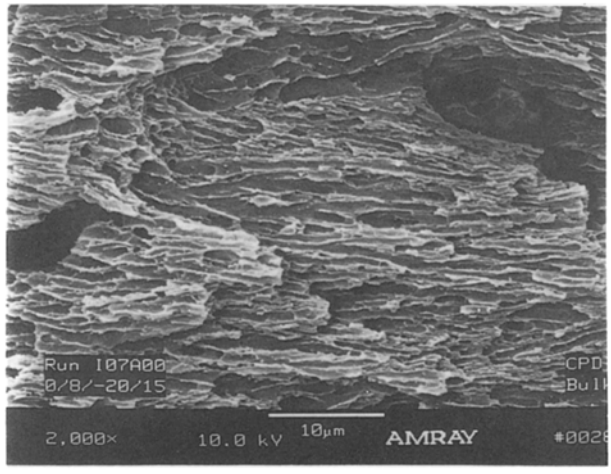
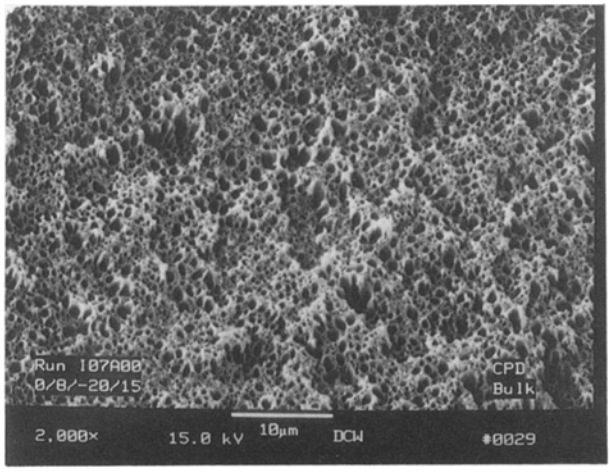


(d)



(e)

Fig. 1. SEM photographs of PVA hydrogel sample A obtained from 8 wt % PVA in pure distilled water. a) Surface structure via FE; b) surface structure via CPD; c) bulk structure via FE; d) bulk structure via CPD with fracture surface parallel to the direction of pore orientation; e) bulk structure via CPD with fracture surface normal to the direction of pore orientation. All photographs were taken at 2000 × magnification

crystals are expected to grow more rapidly along the isothermal planes parallel to the freezer plate where the freezing temperature is simultaneously reached rather than along the direction of temperature gradient normal to the freezer plate. Similar orientation was reported by Tong et al. [9] for ice crystal formation in agar-ice composites. The agar and ice domains developed in the direction

parallel to the surface of the cooling annulus due to the rapid growth of ice in that direction.

The large cavities in the bulk structure of xerogel shown in Fig. 1d are artifacts caused by freeze-fracture. When Figs. 1c and d are compared, one observes that the fibrillar structure of sample A is lost during the CPD preparation. The pores in the bulk of xerogel sample appear to be more discrete as the fine fibrillar structure is absent. Figures 1a and c show some differences in the size and shape of the pores at the surface as compared to those in the bulk of freeze-etched samples. Comparing Figs. 1b and d, a significant variation in porosity is also found between the surface and bulk structures of the sample prepared by CPD. This is partially due to the collapse of surface structure. Figure 1e shows that the bulk structure of xerogel sample A is quite similar to the porous honeycomb-like structure reported by Yokoyama et al. [4] and Watase et al. [7] when the xerogel was fractured perpendicular to the pore orientation. On the other hand, the surface structure of xerogel sample A shows fibrillar characteristics similar to those reported by Urushizaki et al. [5] and Lozinski et al. [6].

Surface and bulk structures of hydrogel sample B containing an initial concentration of 4 wt% PVA in aqueous solution of DMSO are illustrated in Figs. 2a through d. The equilibrium water content of this sample is 94.8 wt%. As shown in Fig. 2a, the freeze-etched surface of sample B has a fibrillar network structure which is very porous. The mesh size is relatively non-uniform and varies from submicron to about 5 microns. In contrast to sample A, there is no orientation in the structure. The presence of DMSO in the solution prevented phase separation which caused the oriented structure observed in sample A. In Fig. 2b, the surface of xerogel prepared by CPD has a much denser fibrillar structure with mesh size smaller than 1 micron. Comparison of Figs. 2a and b suggests a dramatic collapse of surface structure for the xerogel. A three-dimensional network of fibrillar structure is observed in the bulk of sample B as shown in Fig. 2c. The bulk mesh size is fairly uniform, approximately 2 microns, and smaller than that on the surface. Figure 2d does not show a fibrillar structure in the bulk of the xerogel prepared by CPD. Rather the morphology appears to be rough due to freeze-fracture and the pores are very fine compared to the freeze-etched sample. The difference in xerogel morphology and porosity demonstrate a significant alteration by CPD of bulk structure in addition to surface structure.

The structures of hydrogel sample C which had an initial concentration of 12 wt% PVA are depicted in Figs. 3a through d. The equilibrium swelling of this sample is 96.3 wt% in water. It is not surprising that both surface and bulk structures of this sample are very porous at such a high level of water content. Figures 3a and c show a continuous fibrillar network structure for both surface and bulk. The meshes on the surface are in the range from submicron to about 8 microns, while in the bulk they are generally smaller than 3 microns. Comparison of Fig. 3a with 3b suggests that a dramatic collapse of surface structure has occurred during the CPD process. Figure 3d shows the freeze-fractured surface of the sample prepared by CPD. Besides the surface being rough, the bulk structure in the CPD sample as observed at high magnifications has pores in the submicron range, significantly smaller than in the freeze-etched sample. The absence of fibrillar structure in the bulk is again a striking difference between the samples prepared by the two techniques.

Figures 4a through d reveal the structures of sample D which was prepared from 12 wt% PVA solution with a DMSO/water ratio higher than in sample C (Table 1). The equilibrium water content of sample D is 81.9 wt%, the lowest value. The PVA content in this hydrogen is 18.1 wt%, about five times higher than in sample C. Hence, less porosity would be expected in this sample. Figure 4a shows that the surface structure obtained by freeze-etching has a non-uniform fibrillar structure with a mesh size smaller than 3 microns. The bulk structure obtained by FE reveals no porosity at 2000 × magnification (Fig. 4c). No micrograph was taken above this magnification due to severe charging and damage to the sample. As expected, Fig. 4b shows the collapse of surface structure for the sample prepared by CPD. The fractured surface in Fig. 4d shows negligible porosity.

No porosity gradient was observed across the sample thickness for all PVA hydrogel samples. The bulk structure immediately under the surface is similar to that in the middle of the sample.

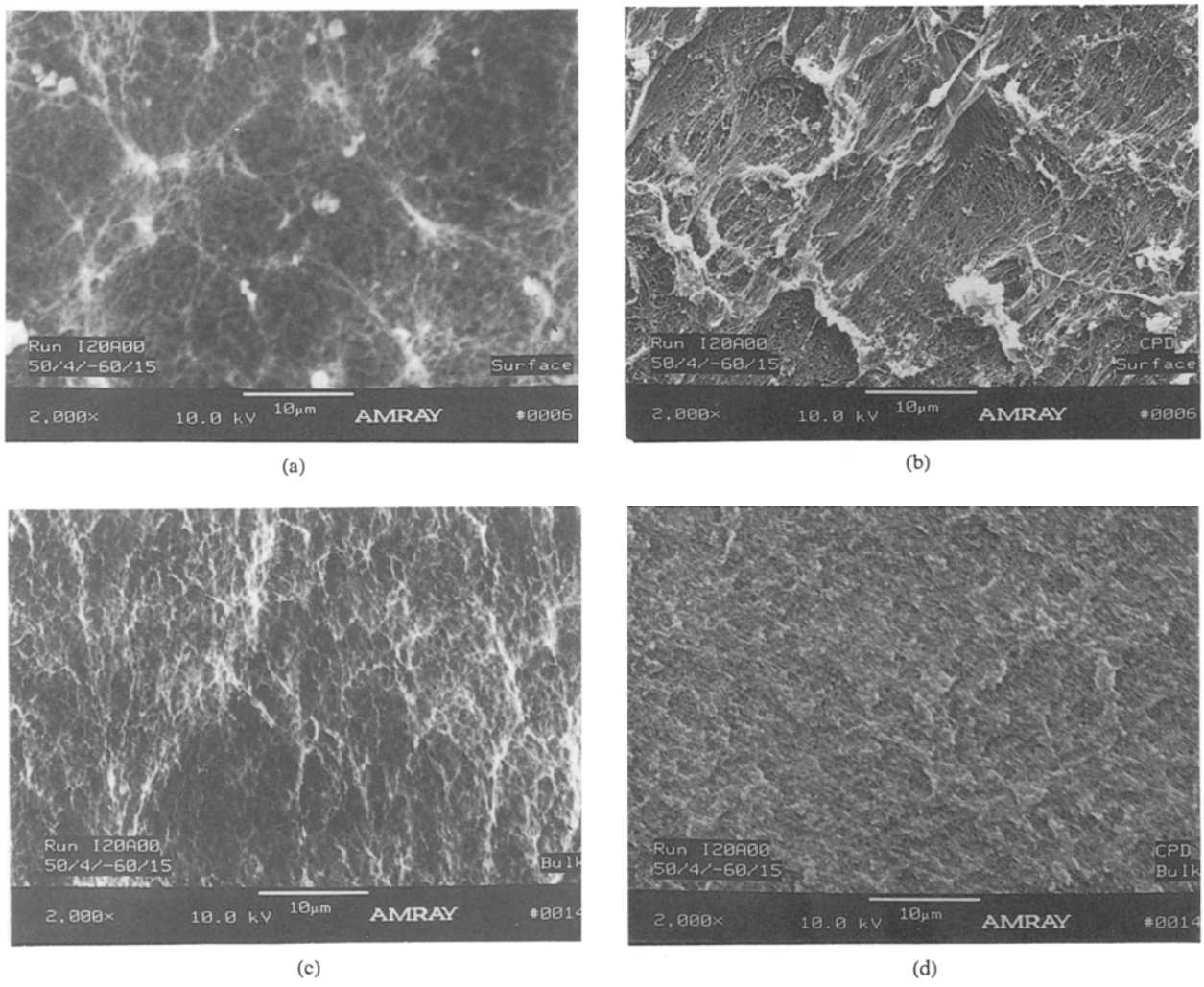


Fig. 2. SEM photographs of PVA hydrogel sample B obtained from 4 wt % PVA in DMSO/water mixed solvent with a weight ratio of 50/50. a) Surface structure via FD, b) surface structure via CPD; c) bulk structure via FE; d) bulk structure via CPD. All photographs were taken at 2000 × magnification

Immediately beneath the porous surface layer, freeze-etched samples showed a denser structure with lower porosity except for the sample prepared without DMSO. Thus, large pores or meshes are apparently confined to a thin surface layer of about 10 microns or less.

The collapse of surface layer by CPD may be attributed to more than one factor. The most probable cause is the significant swelling reduction or volume shrinkage during dehydration prior to drying. As is evident from Table 1, the hydrogel samples shrink significantly in volume when fully dehydrated with ethanol. The collapse of surface structure could also be attributed to its insufficient mechanical strength to withstand the stress caused by CPD. A highly porous surface structure is expected to collapse easily because of weak support. For freeze-etched samples, ice on the sample surface was gradually removed, but the bulk of the sample was still frozen, which kept the surface from shrinking. The highly porous surface did not collapse upon removal of ice in the FE process. This suggests that the surface layer is sufficiently strong to withstand CPD. Premature

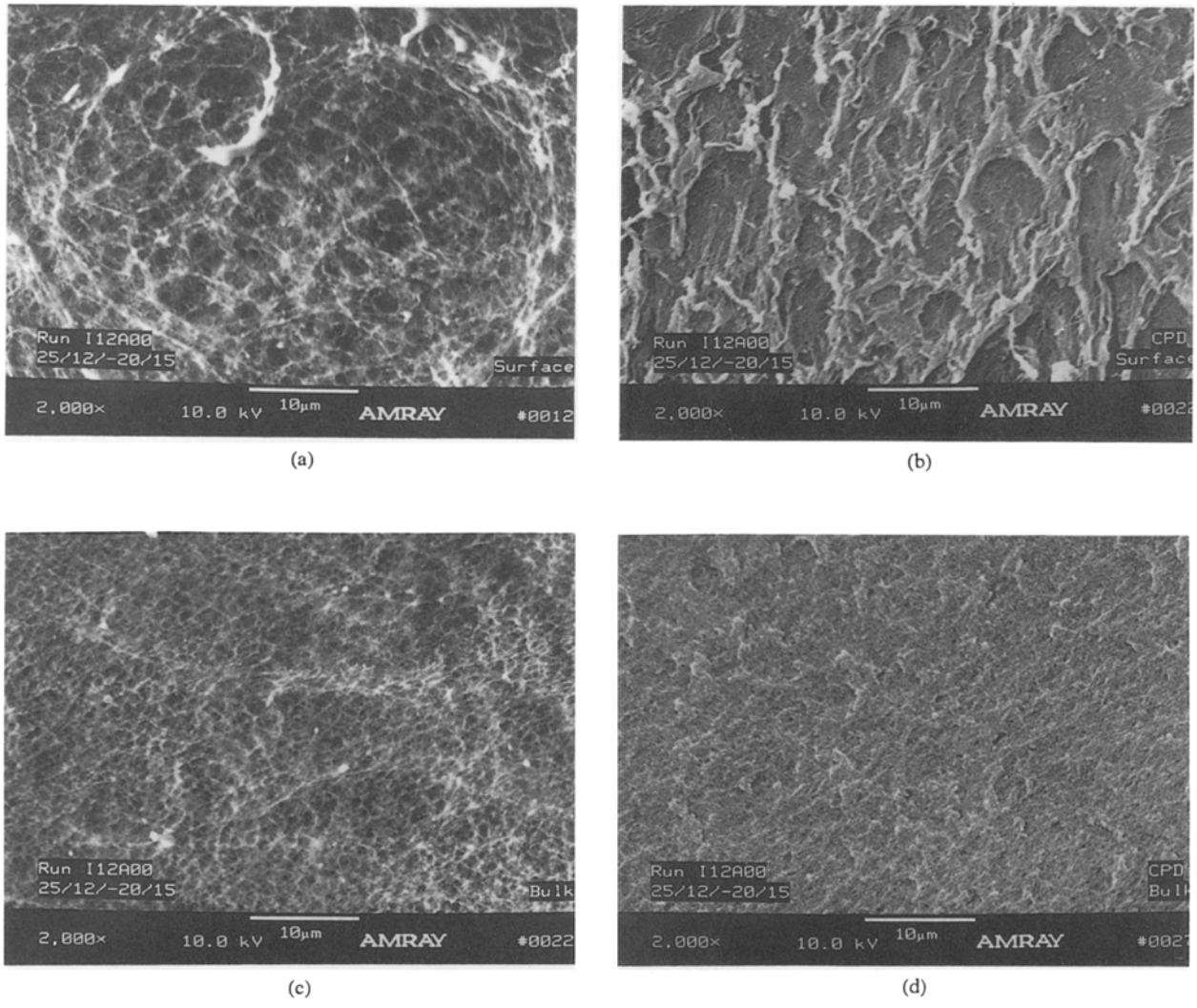


Fig. 3. SEM photographs of PVA hydrogel sample C obtained from 12 wt % PVA in DMSO/water mixed solvent with a weight ratio of 25/75. a) Surface structure via FE; b) surface structure via CPD; c) bulk structure via FE; d) bulk structure via CPD. All photographs were taken at 2000 × magnificaton

drying of the top thin layer of the sample surface prior to submersion in liquid CO_2 can be ruled out because the samples were immediately transferred to the CPD chamber and promptly flooded in liquid CO_2. Therefore, the collapse of surface structure is most likely caused by the significant volume shrinkage in ethanol. Such shrinkage of the hydrogel could also be the main cause for alteration of the bulk structure.

The marked differences in hydrogel structure of samples A through D are essentially attributed to the changes of gelation mechanism with processing parameters. The phase diagram for PVA/water system has been reported by Komatsu et al. [10]. In the case of sample A prepared without DMSO, liquid-liquid phase separation or spinodal decomposition occurs as PVA solution temperature drops below the spinodal curve in the temperature versus concentration phase diagram. Upon further cooling, gelation takes place as the temperature drops below the sol-gel transition curve. As temperature drops below 0 °C freezing starts to occur which leads to directional growth of ice crystals. The gelation

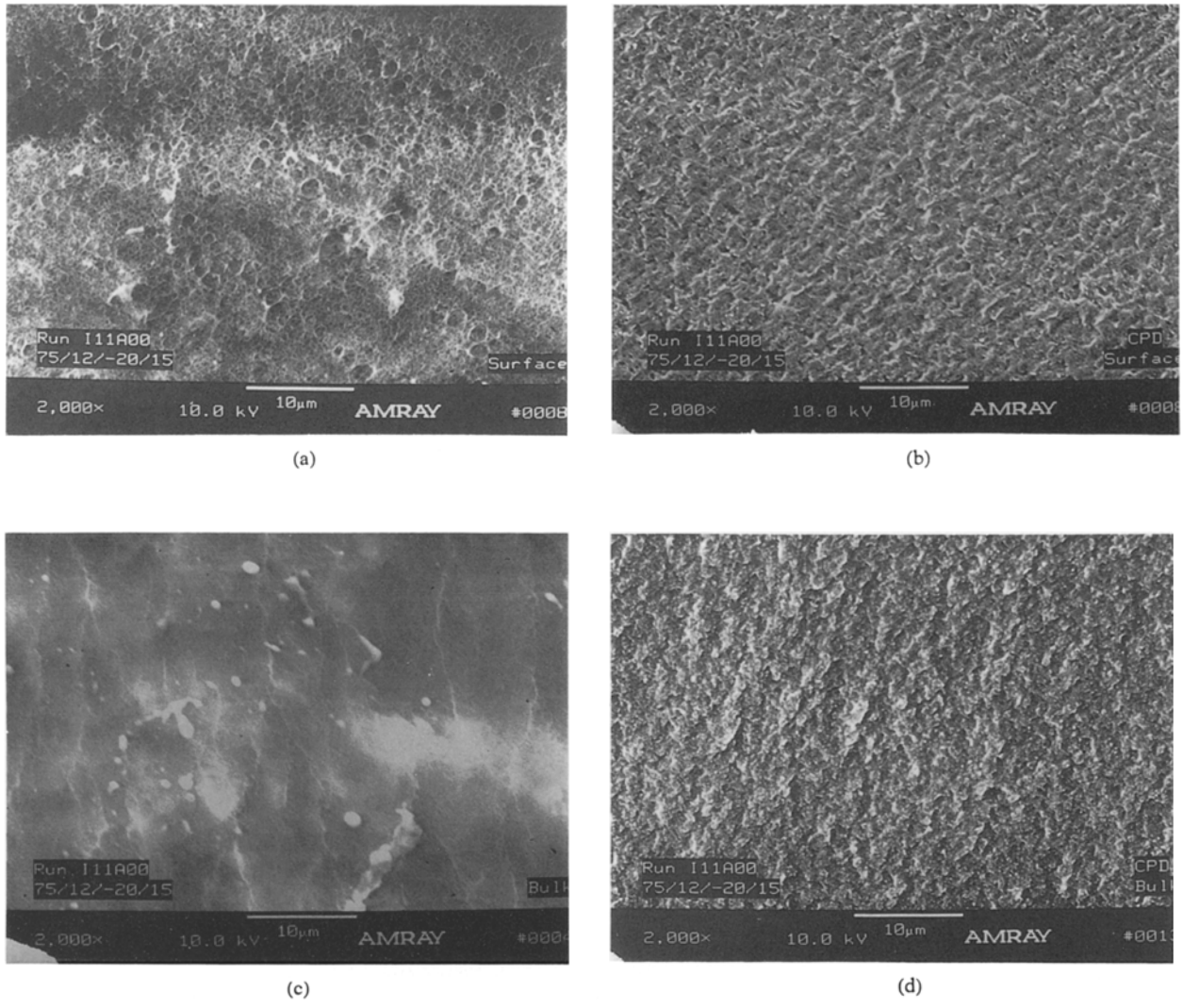


Fig. 4. SEM photographs of PVA hydrogel sample D obtained from 12 wt % PVA in DMSO/water mixed solvent with a weight ratio of 75/25. a) Surface structure via FE; b) surface structure via CPD; c) bulk structure via FE; d) bulk structure via CPD. All photographs were taken at 2000 × magnification

behavior of samples B through D is very different from that of sample A due to the presence of DMSO. DMSO and water form complexes which cause remarkable freezing depression [11]. This enables gelation to take place below 0 °C with delayed freezing or even no freezing at all. Gelation occurs very fast at low quench temperature, thereby obstructing phase separation [12]. An increase in PVA concentration changes the phase diagram and results in more homogeneous hydrogels. Processing parameters like DMSO concentration, initial PVA concentration and quench temperature influence the gelation mechanism and kinetics, thereby changing the structure and properties of PVA hydrogels. A detailed analysis of the role of the processing parameters will be reported in the second paper [13].

Summary

PVA hydrogels prepared without DMSO show an oriented structure on the surface as well as within the bulk. Such local orientation is not

present in PVA hydrogels prepared from aqueous DMSO solutions due to the absence of directional growth of ice crystals. The pore size on the surface does not differ significantly from that in the bulk for PVA hydrogels processed without DMSO. For hydrogels prepared with DMSO as a cosolvent, the three-dimensional fibrillar structure has a significantly higher porosity at the surface than in the bulk. However, large mesh size was confined to only a thin surface layer. The surface layer is significantly different from the bulk irrespective of the solvent composition.

FE and CPD techniques produce very different surface and bulk morphology of PVA hydrogels. The hydrogel surfaces prepared by FE show highly porous fibrillar structures while those prepared by CDP reveal collapsed, dense fibrillar structures. For PVA hydrogels with high water content, only FE technique shows fibrillar structure in the bulk.

FE technique is also vulnerable to several problems. One has to compromise between poor resolution at low accelerating voltage and severe charging and structure damage at high accelerating voltage. FE technique is satisfactory for highly porous hydrogels where very high magnification in not required.

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