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Biocompatible zwitterionic copolymer networks with controllable swelling and mechanical characteristics of their hydrogels

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Abstract The equilibrium swelling ratio in both water and physiological solution of the biocompatible copolymer networks of 3-dimethyl(methacryloyloxyethyl)ammonium propane sulfonate (DMAPS) and *N*-vinyl-2-pyrrolidone (NVP) is determined as a function of copolymer composition. It is established that equilibrium swelling ratio of the polymer networks in physiological solution increase with raise of zwitterionic monomer unit fraction. A sharp decrease of this ratio in water with increase of zwitterionic monomer unit fraction is related to the formation of thermolabile physical junctions produced by dipole–dipole interactions between the zwitterionic side groups. The same fact affects considerably the storage and loss moduli of the copolymer hydrogels as well as the morphology of the dried networks. Scanning electron microscopy images provided evidence of the occurrence of a lamellar structure forming the morphology of the polymers. This was corroborated by differential scanning calorimetry experiments. In this way a possibility for effective control on swelling ratio in different solutions and the mechanical properties of these novel biocompatible networks are established.

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1 Introduction

The steadily increasing interest to polymer hydrogels is a result of both their characteristic physico-mechanical behaviour as soft materials and of their expanding biomedical and industrial applications [1–7]. Poly(*N*-vinyl-2-pyrrolidone) (PNVP) is a hydrophilic, non-charged and biocompatible polymer with a widespread application in pharmaceutical, cosmetic, food and beverage industries [8–10]. Polyzwitterions (PZI), mainly polysulfo- and polyphospho-betaines, are polyampholytes in a permanent isoelectric state having the best biocompatibility found yet among the other synthetic polymers [11–15]. This fact is related to the specific intra- and inter-molecular interactions between the PZI side groups [16–19] and to the structure similarity between PZI and phospholipids, the main biomembrane components. Dipole–dipole clusters (DDC) of oppositely oriented dipoles (Fig. 1) [20–24], formed as a result of the above mentioned interactions, play the role of junction points producing physical network. The concentration, the size and the strength of these junctions define PZIs' solubility, their transport and optical properties in solutions, their ability to interact with low-molecular-mass salts (LMS), surfactants and other polymers, as well as the physico-mechanical properties of their hydrogels [25–28]. Though the investigation of these properties gives new information continuously, PZI have already found applications as the best materials for vascular prosthesis, angiosurgery stents, different kind of catheters, hemodialysis membranes, ion exchangers, materials for controlled drug release, for purification of waters and soils from heavy metals, for production of soft contact lenses and high quality cosmetic compositions [29–35]. PZI are cross-linked in most of these applications. Therefore the synthesis and the characterization of ZI

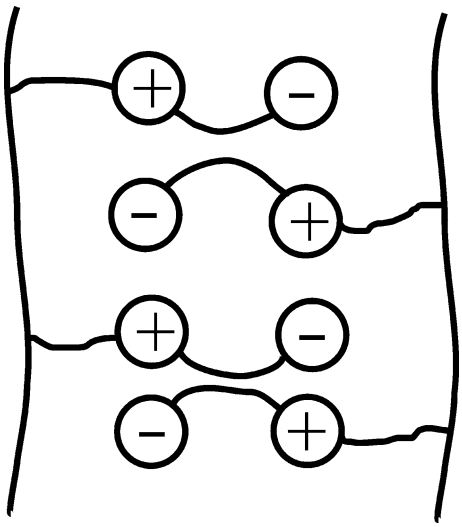

Fig. 1 Scheme of a dipole–dipole cluster from oppositely oriented dipoles, belonging to the PZI macromolecule side groups

copolymer networks are of great interest, both from scientific and practical point of view. The zwitterions' cross-linking and copolymerization reveals an opportunity to control the size, the stability and the DDC concentration (Fig. 1). In this way controlling of the ratio between chemical and physical cross-linking could be changed the physico-mechanical characteristics of the synthesised ZI copolymer networks. The nature of a non-zwitterionic comonomer is an additional factor influencing of these properties. There are some reasons to choose *N*-vinyl-2-pyrrolidone (NVP) as a second comonomer: (i) The above mentioned PNVP non-toxicity and biocompatibility creates expectation that poly(3-dimethyl(methacryloloxyethyl) ammonium propane sulfonate-co-*N*-vinyl-2-pyrrolidone) (DMAPS-co-NVP) should save the unique PZI hemo- and biocompatibility; (ii) The high PNVP hydrophilicity should contributed the possibilities for alteration of copolymer DMAPS-co-NVP hydrophilicity depending on the copolymer composition; (iii) According to the available literature, the copolymerization of DMAPS with NVP is not investigated up to now, which makes these copolymer networks very interesting from scientific point of view.

In this paper, original results on the synthesis of copolymer (DMAPS-co-NVP) networks, their hydrogel equilibrium swelling ratios in water and physiological solution, their morphology and thermo-mechanical behaviour are presented. The variation of the latter parameters with the temperature is explained with alteration of the ratio between strong covalent junctions and weak thermolabile physical nodes (presented in Fig. 1). The morphology of the solid PNVP and the copolymer networks are very different too.

2 Experimental

2.1 Materials and methods

N-vinyl-2-pyrrolidone (NVP, Fluka Chemical Co., Buchs, Switzerland) was purified by vacuum distillation. 3-dimethyl(methacryloyloxyethyl) ammonium propane sulfonate was provided by (DMAPS, Merck Darmstadt, Germany). The initiator potassium persulfate (KPS) was purchased by (Merck Chemical Co., Darmstadt, Germany). The cross-linking agent *N*,*N*′-methylenebisacrylamide (MBA, Sigma-Aldrich Chemical Co., Munich, Germany) and *N*,*N*,*N*′,*N*′-tetramethylene diamine (TEMED, Fluka Chemical Co., Buchs, Switzerland) were used as received. Sodium chloride was provided by (Merck Chemical Co., Darmstadt, Germany).

2.2 Preparation of homo- and copolymer hydrogels

The homopolymers of DMAPS (PDMAPS) and NVP (PNVP), as well as the copolymers of DMAPS with NVP were synthesized by free radical copolymerization (FRC) in deionised water into glass ampoules. Three copolymer samples were synthesized from NVP and DMAPS (Fig. 2),

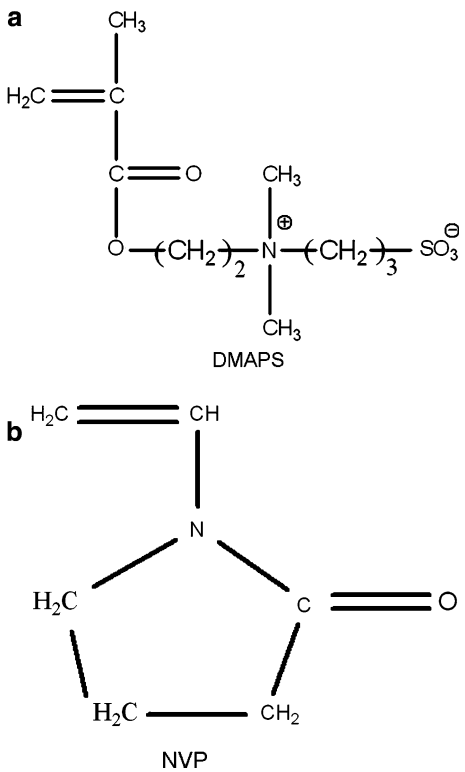

Fig. 2 Chemical structures of the monomers used for preparation of hydrogels: (**a**) 3-dimethyl-(methacryloyloxyethyl)ammonium propane sulfonate, DMAPS and (**b**) *N*-vinyl-2-pyrrolidone, NVP

using three different component DMAPS/NVP weight ratios in the initial monomer mixture: 1/3 (Co 1/3), 1/1 (Co 1/1) and 3/1 (Co 3/1). To the 40% aqueous solution of the monomer mixture 1 wt.% MBA as a cross-linking agent, 0.1 wt.% KPS and 0.05 wt.% TEMED as an accelerator were added. The FRC was carried out at room temperature (25 °C) for 24 h. The obtained hydrogel rods were immersed in excess amount of deionised water for 168 h in order to remove any low molecular weight compounds, incorporated into the networks.

2.3 Determination of equilibrium swelling ratio

2.3.1 Determination of equilibrium swelling ratio of PNVP, PDMAPS and copolymer hydrogels (Co1/3, Co1/1 and Co3/1) in water

For the water content determination from each swollen hydrogel rod were cut cylinders with approximately thickness 5 mm and diameter 13 mm. They were immersed in deionised water at room temperature until swelling equilibrium was established. The weight of the wet sample (m_{wet}) was determined after removing the surface water by blotting with filter paper. After that the samples were dried to constant weight in a vacuum oven at 50 °C. The dried plates were weighted (m_{dry}) and the equilibrium swelling ratio (Q) was calculated by the following equation:

$$Q = (m_{wet} - m_{dry})/m_{dry}$$

The final Q value is averaged for three different specimens of each gel and is given as mean Q with its standard deviation, SD.

2.3.2 Determination of Q *of PNVP, PDMAPS and copolymer hydrogels (Co1/3, Co1/1 and Co3/1) in physiological solution*

Determination of Q of PNVP, PDMAPS and copolymer hydrogels (Co1/3, Co1/1 and Co3/1) was carried out in 0.9 wt.% NaCl aqueous solution [36]. The Q values were measured by the above mentioned method. During the sample drying process the weight of precipitated salt was removal from the total weight of the dry samples.

2.4 Dynamic mechanical analysis

The dynamic mechanical analysis (DMA) was carried out by a dynamic mechanical thermal analyzer (Rheometric Scientific) DMTA MK III, operating in the "compression" mode. The test performed was a temperature ramp from 25 to 90 °C with heating rate of 3 °C/min at constant compression strain and 1 Hz frequency. A compressive contact force was applied to the specimen. The preliminary experiments were performed and the value of 0.1 cN was chosen to ensure that the collected data were reproducible. This also ensured that the upper compression plate would not lost contact with the hydrogel sample during the experiment. Upon completion of the acquisition of data, the DMTA software calculates the moduli for the specimen and exports the data for plotting as a function of temperature. The samples' dehydration was recorded by weighting the sample immediately after the DMA experiment and calculating the weight loss as the difference between the weight of the sample before and after the experiment in percents.

2.5 Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) thermograms of the samples were recorded with a Mettler DSC 30 apparatus interfaced to a model TC11 processor. The samples were dried at room temperature for 168 h before the experiment. Specimens of 10 ± 3 mg, taken from the dried networks, were sealed in standard aluminium pans. The following heating–cooling sequences were systematically repeated until reproducible scans were obtained: heating from −50 to 280 °C at a heating rate of 10 °C/min and cooling back to −50 °C with 40 °C/min. The second scans are presented in these investigations. These heating–cooling cycles provide also for a more thorough drying of the samples.

2.6 Scanning electron microscopy

Scanning electron microscopy (SEM; JOEL-JSM 5510, JAPAN) images were obtained from the gold-coated (Jeol Fine Coater 1200, Japan) fracture surfaces of the air dried hydrogel samples, using 0.1 keV electron energy beam. Several different peaces of each polymer network were examined in order to determine the influence of the composition on the specimens' morphology.

3 Results and discussion

3.1 Equilibrium swelling ratio as a function of copolymer network composition in water and physiological solution

The dependences of Q versus copolymer network compositions (Co1/3, Co1/1, Co3/1 and PNVP, PDMAPS) in

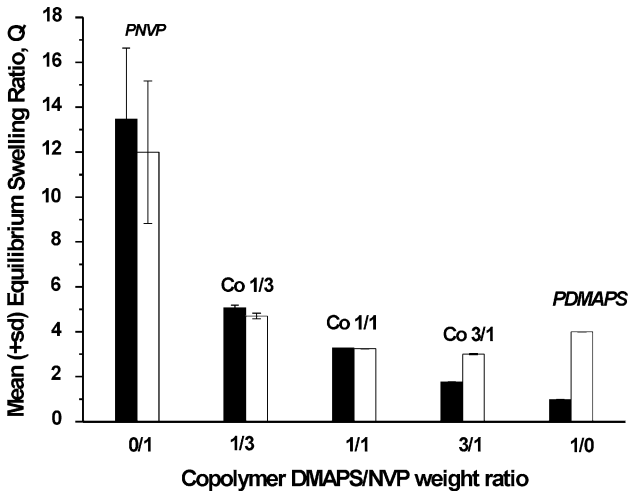

Fig. 3 Influence of copolymer composition on the equilibrium swelling ratio (Q) of the PNVP, PDMAPS and copolymer hydrogels in water (black columns) and in physiological solution (white columns) at 25 °C

water (black columns) and in physiological solution (white columns) at 25 °C are presented in Fig. 3. The goal of this study was to investigate the influence of varying the swelling media (water and physiological solution) on the specific structural organization of the swelling polymer networks. As it could be observed, the DMAPS/NVP weight ratio considerably affects the Q values of the copolymer networks in both media.

In water, Q value decreases with increase of the DMAPS/NVP ratio. The PDMAPS network has the lowest Q value, while the PNVP network exhibits the highest one. Incorporation of zwitterionic monomer units into the copolymer chains decreases significantly the equilibrium water content of the gel compared with the pure PNVP sample. Swelling change is registered for ZI copolymer networks with 2-hydroxyethyl methacrylate (HEMA) [37, 38], sodium acrylate (SA) [39] and *N*-isopropylacrylamide (NIPAAm) [40]. This common tendency for ZI copolymer networks could be explained with the increase of the concentration of the DDC (Fig. 1), playing the role of physical junctions in the networks. The greater the DMAPS/NVP weight ratio, the larger the probability for the formation of such junctions, and as a result—Q value decreases. The NVP monomer units dilute DMAPS units and when their concentration increases, the content of physical junctions decreases (DDC, Fig. 1). By this reason the swelling ratio of the copolymer networks increases too. The DDC are also responsible for the specific antipolyelectrolyte effect (APE) in PZI aqueous solutions with LMS [41, 42], as well as for the PZI unique hemo- and biocompatibility [24, 43–45]. As it could be seen in Fig. 3, Q values of PDMAPS and copolymer hydrogels (Co3/1) are higher in physiological solution than these in water. These results are expression of the specific APE for PZI. The Q value of PNVP network decreases in physiological solution. This result could be explained with dehydration effect of the LMS (salting effect) in PNVP segments. As well it is shown in Fig. 3 that for the copolymer hydrogels (Co1/1 and Co1/3) did not observe significant effect of added LMS. This could be related with the existing of both opposite effects: APE and above mentioned diluting effect of NVP units addition. The latter process leads to considerably decrease of both DMAPS units in copolymer chains and the possibility of formation DDC. In previous our paper it has been proposed hypothesis explaining the PZI swelling behavior in physiological solution [45]. It is shown that at these conditions PZI possess amphiphilic properties. By this way it could be explained their unique hemo- and biocompatibility. It is interesting to test this explanation by dynamic mechanical analysis of the synthesized networks in their equilibrium swollen state.

3.2 Dynamic mechanical analysis

Mechanical damping is often the most sensitive indicator of all kinds of molecular motions, which take place in a material, and these movements, apart from their scientific interest, are of great importance for the determination of the mechanical behaviour of polymer materials [46]. The temperature dependence of the storage (E′) and the loss (E″) moduli for all hydrogel samples are determined by means of dynamic mechanical analysis (Fig. 4a–e). The gels with higher Q value (PNVP and Co1/3 (Fig. 4a and b) have a low storage modulus which varied little over the temperature range of the experiment. This result is consistent with the gels being in the rubbery region [47]. It is explained both with the water plastification effect and with the lack (PNVP) or the too small (Co1/3) DDC concentration—the physical network junction points. The latter, as it was mentioned in the previous section, is the main reason also for the high Q values of these two hydrogels. The practical independence of E′ and E″ of the temperature is another, particularly strong consequence of the same reason. In the first case (PNVP), the junction points are covalent only, which are not influenced by the temperature in the range 20–75 °C, while in the second case (Co1/3), the added by DMAPS units physical junctions are so small, that their destruction does not act on the E′ and E″ values markedly. However, it is observed two differences between the discussed dependences of the above mentioned hydrogels. For the copolymer hydrogel (Co1/3; Fig. 4b) the E′ and E″ values are higher than those for PNVP (Fig. 4a). Therefore, the appearance of physical junctions in Co1/3 hydrogel makes it harder and with higher viscoelasticity under mechanical exposure in a comparison with PNVP

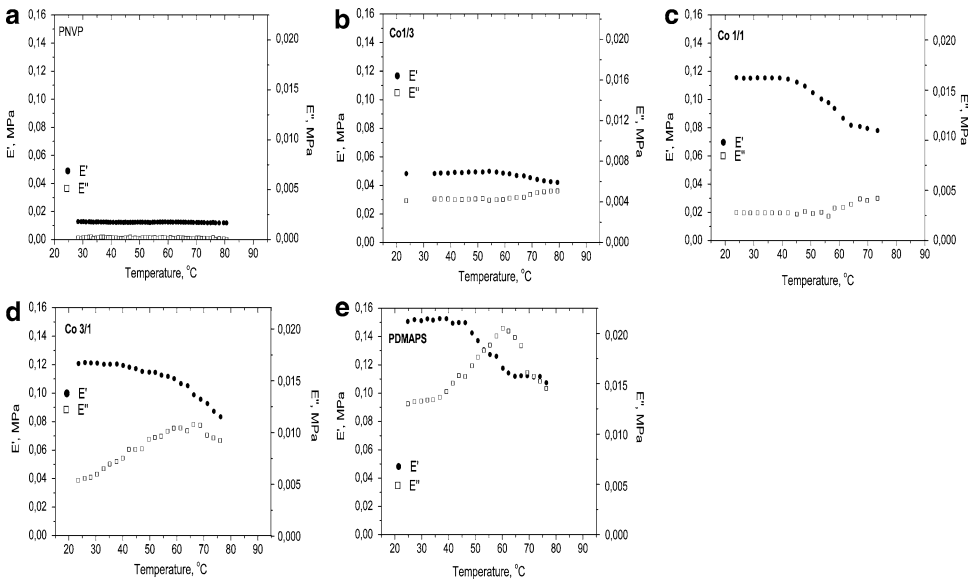

Fig. 4 Temperature dependences of the storage (E′) and loss (E″) modulus (1 Hz) of (**a**) PNVP; (**b**) Co1/3; (**c**) Co1/1; (**d**) Co3/1; (**e**) PDMAPS hydrogels

hydrogel without such junctions. The second difference, which should be noted, is that at temperatures over 55 °C, the copolymer hydrogel becomes more elastic and the E′ values begins to decrease as a result of the DDC destruction (Fig. 1). These two trends are investigated for other (with higher percent of ZI units) hydrogels (Fig. 4c–e) too. As could be expected, this temperature effect is more significant for hydrogels with a higher concentration of the DDC, i.e. with higher wt.% of DMAPS [23]. The E′ decreases with temperature from 20 to 70 °C as follows: PNVP—0%, Co1/3—20%, Co1/1—27%, Co3/1—25%, PDMAPS—33%. The deviation from this tendency for Co3/1 is in the range of experimental error. The above mentioned trend of E′ values decrease with temperature as a result of destruction of the physical junctions (Fig. 1). In the temperature interval (45 °C ≤ T ≤ 70 °C) E′ value decreases, the E″ values are changed too but lower than E′ values in the whole temperature interval used. This behavior is typical for the gel state [48]. For the Co3/1 and PDMAPS hydrogels, the E″/T dependences present thermostimulated molecular structure transformations—E″/T maximum [23]. As could be expected, this maximum is more intensive for the PDMAPS hydrogel because of both the higher DDC concentration and their destruction degree in this case. The higher relative part of ZI monomer units in the hydrogel, the greater hydrogel hardness and E′ values (Table 1). The hydrogel viscoelasticity (registered by the E″ values) increases also with a ZI monomer unit part. The presented E′/T and E″/T dependences for Co3/1 (Fig. 4d) and PDMAPS (Fig. 4e) possess one more significant difference from others. They have stepped character in contrary to the smooth E′/T and E″/T dependences for the remained samples. This difference could be related to the distinct DDC distribution on size and stability (physical junctions) as a function of the ZI monomer unit wt.% in copolymers [24]. The smaller, unordered and unstable DDC are destroyed at lower temperatures and depict the lower temperature steps of the curves discussed. The higher temperature steps are result of the destruction of the greater and more stable DDC. In addition, these original experimental results prove the high sensibility of the dynamic mechanical analysis to the molecular and structural transformations of the investigated soft material samples. It is interesting to attempt the registration of these transformations in polymer networks by another widely used method as DSC.

Table 1 Storage (E′) and loss (E″) modulus of hydrogels at equilibrium swelling stage, 30 °C and frequency 1 Hz

Samples	E′ × 10^{-3}, MPa	E″ × 10^{-3}, MPa
PNVP	12.84	~0.5
Co1/3	48.31	4.0
Co1/1	108.64	3.0
Co3/1	121.09	6.5
PDMAPS	141.19	11.0

3.3 DSC of the dried networks

The DSC traces of the samples are shown in Fig. 5. It could be seen that in the temperature interval from −50 to 280 °C there are no registered thermal transitions for all of the samples, except for PDMAPS. Two small endothermal peaks are observed at 200 and 250 °C for PDMAPS. The glass-transition and decomposition temperatures for PDMAPS reported in the literature were 261 and 295 °C, respectively [49]. Hence, at least one of the observed

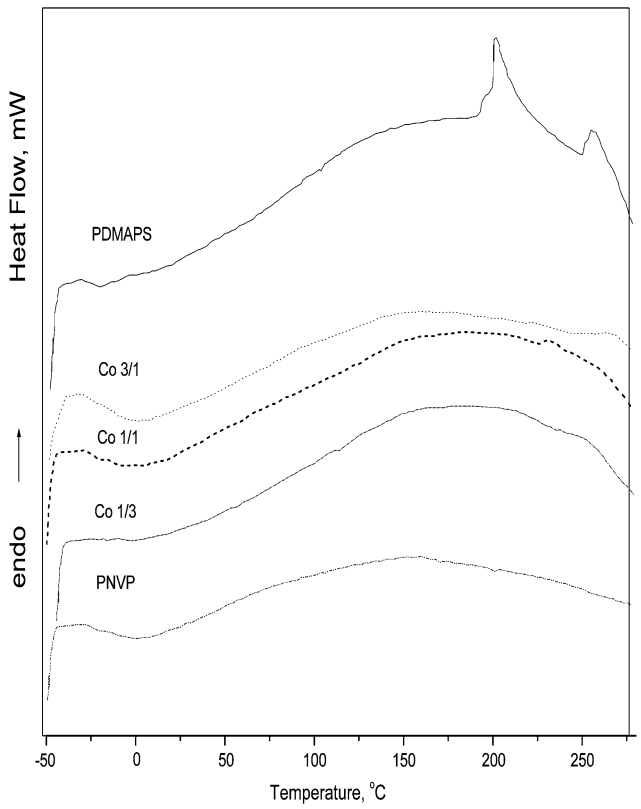

Fig. 5 DSC thermograms of the dried PDMAPS, PNVP, Co1/3; Co1/1 and Co3/1 polymer networks. Curves are shifted vertically

thermal processes is most likely due to the destruction of the above mentioned DDC. DSC experiments with other zwitterionic polymers show similar thermal transitions which are related to the melting of the ion-rich ordered domains formed from the charged dipoles [49–52]. The lack of endothermal peaks at high temperatures for NVP containing samples is in correspondence with all the above reported results, regarding the retardation of the DDC formation in the presence of the NVP monomer units. While the physical nodes destruction in the copolymer and PDMAPS hydrogels is in the temperature range 40–70 °C, in the dried networks it begins at 200 °C, about 50 °C before the PDMAPS glass transition temperature.

3.4 Scanning electron microscopy

The results from the SEM morphology analysis of the dried homo- and copolymer networks are presented in Fig. 6a–f. All samples show behaviour of a rigid and fragile material after drying in contrast to elastomeric consistence after swelling in water. The change of the morphology with alteration of the monomer ratio for the different samples is clearly visible from these images (Fig. 6a–f). Whereas PNVP has smooth surface (Fig. 6a), the copolymers and particularly PDMAPS samples, have rough fracture surface where could be seen different, randomly located, ribbed parts. The roughness of the surface could be more clearly observed for PDMAPS than those of the other polymers. Higher magnifications of the ribbed parts, of the PDMAPS revealed some lamellar structures in this polymer sample (Fig. 6f). According to the previous studies [49, 50, 53] and the above discussed results, PZI show presence of ionic aggregates in the solid state. The morphology of the synthesized polymer networks is related to their ionic content and the above mentioned DDC formation (Fig. 1), which are densely packed and form lamellas in the PZI solid state similar to these point to the above mentioned papers.

4 Conclusion

For the first time, the copolymer 3-dimethyl(methacryloyloxyethyl)ammonium propane sulfonate with *N*-vinyl-2-pyrrolidone networks and hydrogels are synthesized and characterized by means of swelling behavior, dynamic mechanical analysis, scanning electron microscopy and

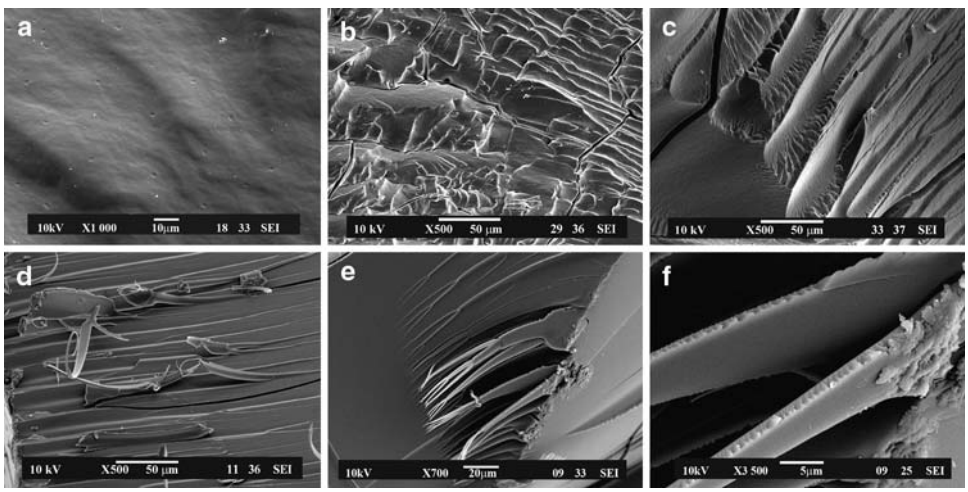

Fig. 6 SEM images of the dried networks: (**a**) PNVP; (**b**) Co1/3; (**c**) Co1/1; (**d**) Co3/1 and (**e**, **f**) PDMAPS: Magnification: 1000 (**a**), 500 (**b**), 500 (**c**), 500 (**d**), 700 (**e**) and 3500 (**f**)

differential scanning calorimetry. A considerable effect of the copolymer composition on the self-organization of PZI hydrogels and solid-state networks is observed. These effects are related to the formation and thermostimulated destruction of the DDC which act as physical network junctions. The dipole–dipole interaction between the zwitterionic side chains reduces with the increase of the NVP monomer unit content in copolymer networks and hydrogels. This leads to increase the swelling and the decrease of the gel stiffness and viscoelasticity. These relationships provide the strategy to optimize the experimental conditions to control the physico-mechanical properties of these new biocompatible materials.

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