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pH-responsive poly(*N*,*N*-dimethylaminoethyl methacrylate-co-2acrylamido-2-methyl-propanosulfonic acid) cryogels: swelling, elasticity and diffusive properties

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Tough and fast responsive ionic P(DMAEMA-co-AMPS) cryogels were prepared below the bulk freezing temperature of water.



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Abstract

A series of ionic poly(N,N-dimethylaminoethyl methacrylate-co-2-acrylamido-2-methylpropanosulfonic acid) (P(DMAEMA-co-AMPS)) cryogels were prepared by free-radical crosslinking copolymerization of N,N-dimethylaminoethyl methacrylate (DMAEMA) and 2acrylamido-2-methyl-propanosulfonic acid (AMPS) in aqueous solution. The swelling properties and the elastic behavior of P(DMAEMA-co-AMPS) cryogels drastically change at a gel preparation temperature, -18 °C, below the bulk freezing temperature of the polymerization solvent, water. The equilibrium and dynamic swelling/deswelling properties of the prepared cryogels responding to pH as well as in aqueous solutions of NaC1, KCl, $MgC1_2$, Na_2S0_4 , K_2S0_4 , and $MgS0_4$ were investigated. The influence of the relative content of ionic comonomer AMPS on the swelling properties was examined in water and the obtained results were compared with P(DMAEMA-co-AMPS) hydrogels synthesized at usual polymerization temperature. The ionic gels prepared with higher DMAEMA contents exhibited a highly pH- dependent equilibrium swelling behavior. The extent of transition from the swollen state to the shrunken state was strongly related to DMAEMA content of the network. The results indicated that P(DMAEMA-co-AMPS) cryogels not only had considerable swelling ratio as well as the rapid swelling/deswelling kinetics, but also exhibited improved mechanical properties.

Introduction

Stimuli-responsive smart materials that undergo phase transition in response to external stimuli have been widely investigated in nanomedicine, gene delivery, shape-memory materials, artificial muscles, and microfluidic devices.¹⁻⁵ In particularly, numerous researches are focused on pH-responsive materials that are able to respond in a physiological environment.⁶⁻¹⁰ Specifically, poly(*N*,*N*-dimethylaminoethyl methacrylate) (PDMAEMA)-based gels are the most frequently studied class of pH-responsive smart materials in drug delivery systems, biological-therapeutic demands, and sensing applications.¹¹⁻¹⁷ PDMAEMA is a typical cationic polymer containing tertiary amino groups which are positively charged in the acidic pH region, also showing sensitivity to temperature. pH and temperature critical

points of PDMAEMA-based gels are different for various systems, since they are affected by several external factors.¹⁸⁻²⁰

Since the distinguishing characteristics of stimuli-responsive hydrogels is their ability to respond to rapid changes in their external environment, their response rate is usually expected to be as fast as possible in some specific fields, such as chemical sensors and artificial organs, where a high response rate is required. Besides the responsiveness, improving the mechanical strength of hydrogels is also important since conventional covalently-crosslinked hydrogels generally exhibit weak and brittle mechanical performance under stress. The lack of the mechanical strength in the hydrogels is usually due to the random nature of cross-linking reactions, the low friction between chains and the spatial inhomogeneity formed in the gelation process.²¹⁻²⁴ Recently, some novel types of hydrogels, including hybrid structures, nanocomposites, double-network gels, and porous gels have been developed to overcome the poor mechanical properties of conventional hydrogels.²⁵⁻²⁹ Wang et al. prepared poly(DMAEMA)/graphene oxide hybrid hydrogels to investigate pH- and temperaturesensitivity as well as Cr(VI) adsorption. All their results showed that hybrid hydrogels exhibited obvious deswelling when pH of swelling medium increased from 5 to 10 gradually.³⁰ In most of the studies on DMAEMA-based ionic gels, the polymerization reactions were performed usually at relatively high temperatures which limits the use of resulting gels in the physical immobilization of biologically active agents due to their low swelling ratio for water based applications and weak mechanical properties.³¹ Zang et al. reported the synthesis of degradable intelligent hydrogels of poly(N-isopropylacrylamide) (PNIPA) and PDMAEMA by the combination of raft polymerization and click chemistry at 30 °C.32 Chen and coworkers prepared temperature and pH double-responsive hybrid hydrogels with co-crosslinked networks via in situ free-radical polymerization of NIPA and DMAEMA in the presence of crosslinker N,N-methylenebis(acrylamide) (BAAm) and inorganic crosslinker octavinyl polyhedral oligomeric silsesquioxane at 80 °C.³³ Moreover, the swelling behavior of poly(DMAEMA-co-AMPS) hydrogels prepared by chain polymerization in water at 60 °C by tetraethyleneglycol diacrylate as crosslinking agent has been shown to vary depending on the temperature and comonomer AMPS content with the least amount of swelling for an equimolar composition.³⁴

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Numerous investigations were performed in the last 10 years to prepare macroporous hydrogels with high toughness and superfast responsivity by cryogelation technique. The reaction solution containing the monomers and the initiator is concentrated in the unfrozen reaction zones of the apparently frozen system and the copolymerization reaction is carried out below the freezing point of the polymerization solvent. The increased monomer concentration in the unfrozen reaction zones is the essential characteristic of the cryogelation reaction. A macroporous structure in the final material appears due to the existence of ice crystals acting as a template for the formation of pores.³⁵⁻³⁹ Burova et al. synthesized thermoresponsive cryogel of NIPA and N-(3-N,N-dimethylamino propyl)acrylamide in a frozen (-10 °C) aqueous medium in presence of the drug substance ibuprofen and studied a reversible volume phase transition upon heating of the swollen cryogel samples.⁴⁰ Tuncel and Cicek prepared lightly crosslinked hydroxyethylmethacrylate and DMAEMA copolymer gels at a very mild temperature of 4 °C initiated by potassium persulfate in an aqueous medium. The effective diffusion coefficient of water was determined by applying unsteady state diffusion model on the dynamic swelling of the produced gels.⁴¹ Yun et al. prepared polyacrylamide (PAAm)-based cryogel beads and grafted with DMAEMA to provide anionexchange beads with tertiary amine functional groups suitable for binding proteins.⁴² An effective method for grafting functional polymer of DMAEMA onto superporous PAAm cryogels was also reported by Savina et al. as supermacroporous monolithic matrices.⁴³

Most of the articles investigated so far deal with the formation and morphological properties of PAAm and PNIPA cryogels as well as some novel cryogels based on DNA, silk fibroin and cellulose derivatives. However, the literature survey so far conducted reveals very few reports on PDMAEMA-based cryogels due to a number of preparation difficulties such as controlling the transition from conventional gelation to the cryogelation regime and scaling-up the preparation process. The present work introduces, for the first time, the preparation protocol of polymeric cryogels based on PDMAEMA as well as the relationship between the structural parameters and the preparation process. A relatively soft procedure was proposed for the tertiary amine containing monomer imparts pH-sensitivity and the ionic comonomer AMPS introduces the ionic moiety. The equilibrium swelling behavior as well as the elasticity of ionic P(DMAEMA-co-AMPS) cryogels were investigated and the effective diffusion coefficient of water was estimated. An innovative strategy has been employed in order to

evaluate the influence of pH and salt on controlled release characteristics of P(DMAEMA-co-AMPS) cryogels. In order to make comparison, the conventional P(DMAEMA-co-AMPS) hydrogels were also prepared. It was shown that, in addition to the polymerization temperature, the ionic comonomer concentration is an important parameter in the design of pH- sensitive P(DMAEMA-co-AMPS) cryogels with superfast responsive properties. The salt sensitive swelling behavior of the resulting cryogels was investigated as a function of the valence of the counterion, co-ion as well as the charge density.

Experimental

Materials

N,*N*-dimethylaminoethyl methacrylate (DMAEMA, Fluka) as main monomer, 2-acrylamido-2-methyl-propanosulfonic acid (AMPS, Merck) as ionic comonomer, diethylene glycol dimethacrylate (DEGDMA, Fluka) as crosslinking agent were used as received. Ammonium persulfate (APS, Merck) and *N*,*N*,*N'*,*N'*-tetramethylethylenediamine (TEMED, Merck) were used as redox-initiator system. Hydrochloric acid (Merck), potassium dihydrogen phosphate (Riedel-de Haen), potassium phosphate (J.T. Baker), disodium hydrogen phosphate (Merck), Sodium chloride (NaCl, Merck), Potassium chloride (KCl, Merck), Magnessium chloride (MgCl₂, J.T. Baker), Sodium sulphate (Na₂S0₄, Merck), Potassium sulphate (K₂S0₄, J.T.Baker), and Magnesium sulphate (MgS0₄, Riedel-de Haën) were used for the swelling experiments. All of the reagents and the solvents were of the highest available purity and were used as received. Distilled water was used for the preparation of gels.

Synthesis of P(DMAEMA-co-AMPS) cryogels and hydrogels

P(DMAEMA-co-AMPS) gels were prepared by free-radical crosslinking copolymerization of DMAEMA and AMPS in the presence of DEGDMA as crosslinking agent in aqueous solution. The gels were both prepared below (-18 °C) and above (21 °C) the bulk freezing temperature of water, producing cryogels and hydrogels, respectively. The polymerization reactions were initiated using 2.63 mM APS and 24.9 mM (0.375 v/v %) TEMED. The mole fraction of AMPS in the monomer mixture (x_i) was varied between 0 and 0.50, while the cross-linker ratio X (mole ratio of the crosslinker DEGDMA to the monomers DMAEMA + AMPS) was fixed at 1/50. P(DMAEMA-co-AMPS) gels with different feed compositions were prepared by keeping the total monomer concentration constant at 1.0 M. The concentration of DEGDMA in the feed was adjusted so that it made for 2.0 mol % of the total

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monomer content. The formation of P(DMAEMA-co-AMPS) gels by free-radical crosslinking copolymerization of DMAEMA and AMPS was schematically illustrated in Figure 1. To illustrate the synthetic procedure, the details for the preparation of the cryogel sample with $x_i = 0.20$ in the comonomer feed can be described as follows: DMAEMA (1.37 mL), AMPS (0.29 g), DEGDMA (0.045 mL) and TEMED stock solution (1.0 mL) were mixed in a graduated flask. After bubbling nitrogen for 20 min to eliminate oxygen, APS stock solution (1.0 mL) was added, after shaking the flask, the pre-gel solution was poured into several syringes and the polymerization reaction was conducted for 48 h at -18 °C and 21 °C for cryogels and hydrogels, respectively. The prepared gel matrix was taken out of the syringe and washed in distilled water and subjected to the swelling and mechanical tests.

Equilibrium swelling measurements

The equilibrium swelling degree of P(DMAEMA-co-AMPS) gels was measured in deionized water, in various pH medium ranging from 2.1 to 11.2 as well as in aqueous salt solutions. The gel samples were placed in vials filled with 25 mL of the solvent (deionized water, different buffer solutions or salt solutions) and were periodically removed from the vials, wiped with filter paper to remove the superficial water or buffer /salt solution. The swelling equilibrium was tested by measuring the diameter as well as the mass of the gel samples. The swelling capacity is expressed in terms of the equilibrium volume swelling ratio of gels (V_{eq}) which is defined as the volume of equilibrium swollen hydrogel to the volume of the hydrogel just after preparation as follows:

$$V_{eq} = (D/D_0)^3 \tag{1}$$

where D_0 and D are the diameters of gel samples after preparation and after equilibrium swelling, respectively. Then, the volume fraction of the crosslinked polymer in the equilibrium swollen gel (v_2) was calculated using:

$$v_2 = v_2^0 / V_{eq}$$
(2)

where v_2^0 is the volume fraction of the crosslinked polymer after preparation. In order to determine v_2^0 , P(DMAEMA-co-AMPS) gels after preparation were first swollen in water to extract non-polymerizable or soluble components and then carefully dried at room temperature to constant mass. v_2^0 was experimentally calculated using the equation:

5

$$v_{2,\exp}^{0} = \left[1 + \frac{(q_{F} - 1)\rho}{d_{1}}\right]^{-1}$$
(3)

where ρ and d₁ are the densities of P(DMAEMA-co-AMPS) and water, respectively. The values of ρ and d₁ used in this study were 1.2 and 1.0 g / mL, respectively. q_F is the dilution degree after preparation (mass of gel after preparation / mass of dried gel). The experimental values of v_2^0 of P(DMAEMA-co-AMPS) cryogels were given in Table 1. By assuming that the monomer conversion is complete after the crosslinking, the theoretical values of v_2^0 can be calculated from the initial molar concentration of the monomers C_0 using the equation, $v_2^0 = 10^{-3} C_0 \overline{V}_r$ where, \overline{V}_r is the average molar volume of the polymer repeat units (in mL / mol) and for P(DMAEMA-co-AMPS) cryogels, it can be given as:

$$\overline{V_r} = 131.02 + 41.70x_i \tag{4}$$

The gel fraction after the crosslinking copolymerization (Wg) was also determined by the extraction of P(DMAEMA-co-AMPS) gels in water. The crosslinked gels, 4.5-5.0 mm in diameter, were freed from the syringes and cut into samples of about 6 mm length. Then, each sample was placed in an excess of water and, after extraction, they were carefully washed several times with acetone and dried to constant weight. The gel fraction was calculated as $W_g = m_{gel} / m_{mon}$, where m_{gel} and m_{mon} are the weights of extracted dry gel and of the initial monomer (DMAEMA+AMPS+DEGDMA), respectively and the results were given in Table 1. It was observed that the gel fraction of both P(DMAEMA-co-AMPS) cryogels and hydrogels increases from 0.84 to 0.93 with increasing ionic comonomer concentration, while the conversion of hydrogels was higher than that of the corresponding cryogels. The obtained gel fractions Wg higher than 84% for all the samples indicate the high efficiency of the crosslinking reactions as well as the conversion of the monomers to the crosslinked polymer. The swelling behavior of P(DMAEMA-co-AMPS) gels was also performed in aqueous solutions at room temperature. The concentration of the salt solutions ranged from 10^{-5} to 1.0 M. The diameter of P(DMAEMA-co-AMPS) gel samples after equilibrium swelling in water was first measured and then transferred to the vials containing the least concentrated aqueous salt solution. The samples were allowed to swell in the solution at least 10 days, during which the salt solution was refreshed once to keep the concentration as feeded. When the swelling equilibrium was established, the diameter of the samples was measured again and then transferred into the next dilute salt solution. The sample diameters were monitored until the changes were within 1% of the previous measurement. Each swelling data reported in this study is an average of at least four separate measurements.



Figure 1. Formation of P(DMAEMA-co-AMPS) cryogels by free-radical crosslinking polymerization of DMAEMA and AMPS in the presence of APS - TEMED.

Measurements of swelling and deswelling kinetics

The dynamic swelling experiment was performed by immersing the dried gels in a buffer solution of pH 2.1 to reach the equilibrium at 21 $^{\circ}$ C. The weight of the cryogel sample was measured after wiping off the excess solution on the surface with filter paper. The measurements were continued until a constant weight was reached for each sample. The amount of water absorbed was gravimetrically monitored and the water uptake, WU(t), at time *t* was defined as:

$$WU(t) = \frac{W_t - W_d}{W_d}$$
(5)

where w_t is the weight of the wet cryogel sample at time t and w_d is the weight of dry cryogel. The deswelling kinetics of the cryogel was investigated by immersing the swollen gels in a buffer solution of pH 8.0. The water retention, WR(t), corresponding to deswelling ratio was calculated from the following equation:

$$WR(t) = \frac{W_t - W_d}{W_s - W_d}$$
(6)

where w_s is the weight of the swollen cryogel at equilibrium and the other symbol is the same as defined above. The equilibrium swelling ratio, SR(eq), was defined as follows:

$$SR(eq) = \frac{W_s - W_d}{W_d}$$
(7)

The water transport and mechanism of diffusion in polymeric networks, swelling-time curves is described by the equation given by Ritger and Peppas:⁴⁴⁻⁴⁸

$$F = \frac{M_t}{M_{\infty}} = \frac{WU(t)}{SR(eq)} = kt^n$$
(8)

where *F* denotes the water fraction at time t, M_t represents the amount of water absorbed at time t, M_{∞} is the water uptake at equilibrium, *k* is a constant related to the structure of the network, and the exponent *n* is a number to determine the type of diffusion which is dependent on the geometry of the sample as well as the physical mechanism of water uptake or release. Eq. (8) is strictly dependent on the geometry of the gel and applicable to the initial stages of the swelling curves only and yields straight lines up to nearly 60% of the maximum amount of water which a gel is capable of absorbing. Eq. (8) couples both Fickian and non-Fickian mechanisms for a thin slab or cylindrical sample since the water transport mechanism in gels processes fall between these limiting cases. For Fickian kinetics, in which the rate of diffusion of the solvent is rate-limiting, *n* equals 0.5, whereas the values between 0.5 and 1 indicate contributions from anomalous (non-Fickian) processes such as polymer relaxation. When the water penetration rate is much below the polymer chain relaxation rate, *n* values are below 0.5. This situation, which is regarded as Fickian diffusion, is named as "Less Fickian" behavior.⁴⁹

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Uniaxial compression measurements

The elastic modulus of ionic P(DMAEMA-co-AMPS) gels was evaluated by uniaxial compression measurements performed on equilibrium swollen gel samples in water. All the mechanical measurements were conducted in a thermostated room at 21 °C. A cylindrical P(DMAEMA-co-AMPS) gel sample, 4 mm in diameter and 7 mm in height, was placed on a digital electronic balance. Then, a load was transmitted vertically to the gel through a rod fitted with a PTFE (Teflon) end-plate. Each sample was subjected to a compressive force and measurement was made immediately following the application of the force, approximately ten records were taken during the elasticity tests, which is required 180 s. The force acting on the gel (F) was calculated from the reading of the balance m as F = mg, where g is gravitational acceleration. The resulting deformation $\Delta l = l - l_o$ where l_o and l are the initial un-deformed and deformed lengths, respectively, was measured using a digital comparator (IDC type Digimatic Indicator 543-262, Mitutoyo). The deformation ratio α (deformed length / initial length) was calculated using the equation, $\alpha = 1 - \Delta l/l_o$. The force and the resulting deformation were recorded after 20 sec of relaxation and all the measurements were conducted up to a maximum of 20% of its original length. Strain rates for compressive testing were chosen in order to adhere to previously developed laboratory protocols. The time elapsed between the application of the force and the measurement was in the order of seconds. The deformation ratio of the cryogel samples under the applied force remained constant after about 10 s, indicating that 20 s of relaxation time for the network chains was sufficient for the present gels. This time was sufficiently long for the equilibrium state measurements. The stress f was calculated as f = F/A, where A is the cross-sectional area of the sample, i.e., $A = \pi (D_0 / 2)^2$. For uniaxial deformation, the statistical theories of rubber elasticity yield an equation of the form:^{50,51}

$$f = G\left(\alpha - \alpha^{-2}\right) \tag{9}$$

where G is the elastic modulus of the gel sample which can be determined from the slope of this linear equation.^{52,53} The typical stress-strain curves of both P(DMAEMA-co-AMPS) cryogels as well as hydrogels after equilibrium swelling in water were collected in Figure 2(A) and (B), respectively. As can be seen from the figure, the slope of the stress-strain isotherms varies depending on the mole fraction of the ionic comonomer AMPS used in the

preparation and the magnitude of % strain at deformation decreases with increasing ionic group content.



Figure 2. Typical stress - strain isotherms of P(DMAEMA-co-AMPS) cryogels after equilibrium swelling in water (A) and that of corresponding P(DMAEMA-co-AMPS) hydrogels (B). The mole fraction of ionic comonomer AMPS (x_i) is indicated in the figure.

Results and Discussion

Herein, a series of copolymerized gels based on pH-sensitive monomer DMAEMA and strong ionic comonomer AMPS were synthesized both at -18 °C and 21 °C, which were designated as P(DMAEMA-co-AMPS) cryogels and hydrogels, respectively. The effect of the composition as well as the charge density on the mechanical properties and dynamic swelling-deswelling behavior of both P(DMAEMA-co-AMPS) hydrogels and cryogels have been evaluated systematically.

Effect of ionic comonomer on the equilibrium swelling of P(DMAEMA-co-AMPS) gels

Both P(DMAEMA-co-AMPS) cryogels and hydrogels were subjected to the swelling measurements in distilled water. The equilibrium swelling of P(DMAEMA-co-AMPS) gels were volumetrically determined and the equilibrium volume swelling ratio of gels (V_{eq}) was calculated using Eq. (1). Figure 3 shows V_{eq} (A) and the modulus of elasticity G of swollen

P(DMAEMA-co-AMPS) cryogels and hydrogels (B) as a function of the mole fraction of ionic comonomer AMPS content. As seen in Figure 3(A), V_{eq} values of P(DMAEMA-co-AMPS) gels increase with increasing amount of ionic comonomer AMPS in the feed. This is a consequence of the osmotic pressure exerted by the counterions of AMPS units in the network chains. The increase in concentration difference of the counterions between the inside and outside of the gel phase also increases the osmotic pressure. The equilibrium swelling capacity of P(DMAEMA-co-AMPS) cryogels is much smaller than those of the corresponding P(DMAEMA-co-AMPS) hydrogels. Due to the formation of P(DMAEMA-co-AMPS) cryogels below the freezing point of water, the polymerization and crosslinking reactions at -18 °C take place only in the unfrozen microzones of ice which contains concentrated dissolved monomer and the crosslinker. Thus, P(DMAEMA-co-AMPS) cryogels swell in water much less than the corresponding hydrogels. Although the initial monomer concentration in the gel preparation is set to 1.0 M, the actual monomer concentration in the reaction zones of cryogels is much higher and this results in the entanglement of the polymer chains, so that the network formed in a concentrated solution swells less than that of formed in a dilute solution.



Figure 3. The equilibrium swelling ratio V_{eq} (A) and the modulus of elasticity G of swollen P(DMAEMA-co-AMPS) cryogels (open symbols) and hydrogels (solid symbols) (B) as a function of the mole fraction of the ionic comonomer AMPS, x_i .

Similar results were reported for ionic PAAm hydrogels and cryogels prepared by free-radical crosslinking copolymerization of AAm and AMPS in the presence of BAAm in aqueous solution. The ionic PAAm cryogels exhibited lower swelling capacity and released a much smaller amount of water when compared to the hydrogels.⁵⁴ Fig. 3(B) showed that *G* decreases as the comonomer content AMPS in the feed augments which means that *G* values of both hydrogels and cryogels drop as the swelling capacity given in Fig.2 increases, i.e., as the water content increases. As compared with hydrogels in Fig. 3, P(DMAEMA-co-AMPS) cryogels exhibited larger elastic moduli than the corresponding hydrogels. For non-ionic PDMAEMA cryogels, the elastic modulus was found as 15 kPa where it was 1400 Pa for non-ionic PDMAEMA hydrogels.



Figure 4. Typical stress-strain data of P(DMAEMA-co-AMPS) cryogels as the dependence of f on 1 - α . The mole fraction of ionic comonomer AMPS (x_i) is indicated in the figure.

The moduli of elasticity G of P(DMAEMA-co-AMPS) cryogels were in the range of 10^3 - 10^5 Pa, while it was 10^2 - 10^3 Pa for corresponding P(DMAEMA-co-AMPS) hydrogels. An important point was that P(DMAEMA-co-AMPS) cryogels prepared at -18 °C were very

tough and can be compressed up to about 95% strain without any crack development. This behavior was illustrated in Fig. 4 where the force *f* acting on the cryogel samples formed at various ionic comonomer AMPS content is plotted against the fractional deformation $(1 - \alpha)$. The mechanical stability of P(DMAEMA-co-AMPS) cryogels increased with decreasing ionic comonomer concentration and the cryogels formed at -18 °C were more stable than hydrogels formed at usual polymerization temperature. The cryogels containing 50 mol% of AMPS broke at a stress of 5 kPa and a strain of about 45%. However, non-ionic PDMAEMA cryogels did not break even at a strain of about 95%.



Figure 5. Photographs of P(DMAEMA-co-AMPS) cryogels (A,B,C) and hydrogels (D,E) taken during compression test. The mole fraction of ionic comonomer AMPS in the swollen sample x_i is 0.20 (left panel) and that of 0.40 (right panel).

Photographs in Figure 5 demonstrate how P(DMAEMA-co-AMPS) cryogels sustain a high compression. During the compression test, as the cryogel sample is squeezed under the piston, it releases its water, so that it can be compressed to high strains. As seen from the images denoted by (D) and (E) in Figure 5, the swollen hydrogel samples prepared at 21 °C fractured under low deformation suggesting that cracks develop easily in the hydrogel. However, those obtained at -18 °C (A, B, C) remain mechanically stable up to complete compression.

Table 1. The composition and characteristic data of P(DMAEMA-co-AMPS) cryogels. \overline{V}_r = average molar volume of the polymer repeat units (mL / mol), $v_{2,exp}^0$ = experimental values of volume fraction of the crosslinked polymer after the preparation, G = elastic modulus of cryogels at swelling equilibrium, v_2 = volume fraction of crosslinked polymer after equilibrium swelling calculated using Eq.(2), W_{g,c} and W_{g,h} = gel fractions of cryogels and hydrogels, respectively. The numbers in parenthesis are the standard deviations of the separate measurements.

AMPS	\overline{V}	ν_{2}^{0}	G/Pa	$\nu x 10^{-2}$	Waa	Wah
x_i	v r	2,exp	0/10	v 2 MIO	vv g,c	vv g,n
0	131.0	0.1171	14484 (576)	7.41	0.8472	0.8608
0.05	133.1	0.1241	13492 (743)	4.56	0.8671	0.8899
0.10	135.1	0.1260	12603 (901)	3.45	0.8687	0.8912
0.15	137.2	0.1402	11119 (862)	2.95	0.8721	0.8887
0.20	139.3	0.1258	9134 (614)	2.65	0.8737	0.8904
0.25	141.4	0.1394	5179 (563)	2.02	0.8823	0.9047
0.30	143.5	0.1300	4214 (639)	1.56	0.8947	0.9212
0.40	147.7	0.1333	3394 (239)	0.74	0.8997	0.9303
0.50	151.8	0.1428	918 (66)	0.46	0.9158	0.9355

The pH-sensitive swelling of both P(DMAEMA-co-AMPS) hydrogels and cryogels was investigated in buffer solutions ranging from pH 2.1 to 11.2 at room temperature. Figure 6 shows the effect of pH on the water content of gels prepared at different ionic comonomer concentrations. P(DMAEMA-co-AMPS) hydrogels remain in the swollen-state in acidic conditions and in the collapsed-state in alkaline conditions, demonstrating the same characteristics of P(DMAEMA-co-AMPS) cryogels. In a narrow range of pH between 7.7 and 8.0, both P(DMAEMA-co-AMPS) hydrogels and cryogels exhibit pH-sensitive phase transition. Since the tertiary amine side groups of DMAEMA chains become protonated in acidic conditions, the charge density on the network increases by increasing degree of the protonation. The internal osmotic pressure also increases the attractive interactions between

the network chains and the water molecules, which in turn, increase the equilibrium swelling capacity of P(DMAEMA-co-AMPS) gels in the acidic pH region. Since the amino nitrogens are deprotonated in basic pH region between 8.0 and 11.2, the excess swelling is reduced and both P(DMAEMA-co-AMPS) hydrogels and cryogels shrank in basic condition owing to the coiled conformation due to ionic affinity.

Another point shown from the Figure 6 is that the swelling degree of P(DMAEMA-co-AMPS) hydrogels in acidic pH region is higher than that of corresponding P(DMAEMA-co-AMPS) cryogels which is in accord with the swelling capacity of both gels in water. The extent of the transition is also affected by the preparation temperature of P(DMAEMA-co-AMPS) gels. As can be seen from inner figure given in Fig.6 (A), for PDMAEMA hydrogels (with 0 mol% of AMPS), a sharp pH-dependent phase transition was observed at a pH of 7.7. However, the phase transition of P(DMAEMA-co-AMPS) hydrogels completely disappears with increasing ionic comonomer content in the copolymer feed up to 40 mol% of AMPS. The inner figure in Fig.6 (B) also indicates that pH-dependent phase transition in P(DMAEMA-co-AMPS) cryogels was not as sharp as those obtained with P(DMAEMA-co-AMPS) hydrogels. Increasing AMPS content in the copolymer structure decreased the extent of the pH-sensitive phase transition. The effect of increasing AMPS content can be explained by the role of hydrophilicity/hydrophobicity in the phase transition behavior of P(DMAEMAco-AMPS) hydrogels and cryogels. At a constant pH, incorporation of the hydrophilic comonomer increases the gel hydrophilicity which in turn lowers the amount of amine side groups of DMEAMA chains in the network structure and the degree of protonation which also causes a gradual decrease in the swelling ratio of the hydrogels. pH-dependent swelling behavior of P(DMAEMA-co-AMPS) hydrogels and cryogels and drastic change in the gel volume can also be seen in the photographs shown in Fig. 7 taken at pH = 2.1 and 8.0 by a digital camera (Sony, Cyber-shot, 8.1 Mega pixel).



Figure 6. The equilibrium volume swelling ratio V_{eq} of P(DMAEMA-co-AMPS) hydrogels (A) and cryogels (B) shown as a function of the pH value. The inner figures show the variation of V_{eq} of both hydrogels and cryogels containing 10 mol% of AMPS (solid symbols) and that of 40 mol% of AMPS (open symbols). The mole fraction of ionic comonomer AMPS, $x_i = 0$ (\bullet), 0.05 (\bigcirc), 0.10 (\blacktriangle), 0.15 (\triangle), 0.20 (\triangledown), 0.25 (\bigtriangledown), 0.30 (\blacksquare), 0.40 (\square).

Dynamic swelling-shrinking of P(DMAEMA-co-AMPS) cryogels

The kinetics of the swelling process is affected by several factors, since the swelling is a diffusion phenomenon driven by the affinity of the molecules of swelling media for the polymer matrix. Most of the applications proposed for responsive gels depend on the kinetics of observed property. The swelling rate of P(DMAEMA-co-AMPS) cryogels with different ionic comonomer AMPS contents was determined in pH = 2.1 buffer solution and was collected in Figure 8(A). The data of this figure showed that both the swelling ratio and the swelling rate increased with increasing amount of ionic comonomer. P(DMAEMA-co-AMPS) cryogel containing 10 mol% of AMPS absorbed about 69% water within 80 min, whereas the cryogel sample containing 40 mol% of AMPS absorbed about 83% within the same duration.



Figure 7: Photographs of P(DMAEMA-co-AMPS) hydrogels (upper) and cryogels (lower) taken after equilibrium swelling in buffer solutions of pH = 2.1 and 8.0. The mole % of ionic comonomer AMPS is already indicated in the figure.

lable	e 2. Ini	itial diffusio	on coeffic	cient of w	ater <i>D</i> , kn	netic e	exponer	it <i>n</i> and charac	teristi	c c	onstant
k of v	water	penetrated	through	cryogels	obtained	from	fitting	experimental	data 1	to	Ritger-
Peppa	s mod	lel.									

$\operatorname{AMPS}_{x_i}$	п	lnk	k	r^2	$D (10^{-7} \text{ cm}^2/\text{s})$
0	0.307	-1.594	0.2030	0.9976	1.72
0.10	0.246	-0.883	0.4131	0.9958	2.15
0.20	0.179	-0.235	0.7902	0.9869	2.78
0.30	0.194	-0.364	0.6947	0.9766	3.28
0.40	0.138	-1.024	0.3591	0.9884	4.03

To find out the transport mechanism, the initial swelling data fulfilling the condition $WU(t)/SR(eq) \le 0.6$ were fitted to Eq. (8) and the constants k and n were determined from the intercept (lnk) and slope (n) of lnF vs. lnt plot of P(DMAEMA-co-AMPS) cryogels given in Figure 8(B).⁴⁸ For water uptake of P(DMAEMA-co-AMPS) cryogels in the solution of pH 2.1, the values of n and k were summarized in Table 2 together with the regressian coefficient r^2 . As shown, the values of n for P(DMAEMA-co-AMPS) cryogels in buffer solutions of pH 2.1 are in the range of 0.14 - 0.30 depending on AMPS content. This indicates that the water diffusion in the cryogels follows Less-Fickian type which is attributed to polymer relaxation; the water penetration rate is much below the polymer chain relaxation. The diffusion coefficient values of water moving through the P(DMAEMA-co-AMPS) cryogels were determined from the following equation, and the results were also collected in Table 2:

$$D = \pi l^2 \left(\frac{k}{4}\right)^{1/n} \tag{10}$$

where *D* is the diffusion coefficient (cm²/s) and *l* is the dry diameter of the cryogel sample. The diffusion coefficient of P(DMAEMA-co-AMPS) cryogels were found in the range of $1.72 - 4.03 \times 10^{-7} \text{ cm}^2/\text{s}$, all falling well within which is typical for solute diffusion coefficients $(10^{-6}-10^{-7} \text{ cm}^2/\text{s})$ in rubbery polymers.⁵⁵ The diffusion coefficient *D* increased with an increase of ionic comonomer AMPS content in the feed. These relatively low values are attributed to the hydrophilic nature of the gel matrix and the expansion of the network structure resulting from the increase of the ionic comonomer. As these gels contain ionizable the tertiary amino groups in DMAEMA bacbone and strongly acidic -SO₃H groups in AMPS, more complications arise, because both the water and ions must be transported into the gel to

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reach equilibrium. Therefore, under such conditions, in addition to water diffusion and polymer relaxation, other factors that contribute to swelling are believed to be ion diffusion and fixed charge group ionization.^{56,57}



Figure 8. Dynamic swelling curves of P(DMAEMA-co-AMPS) cryogels with different ionic comonomer AMPS content in pH = 2.1 buffer solution.

The dynamic shrinking kinetics of P(DMAEMA-co-AMPS) cryogels from the swollen state (in pH = 2.1 buffer solution) to the collapsed state (in pH = 8.0 buffer solution) was measured, and the results were collected in Fig. 9. The swelling ratio of the cryogels decreases, however, the data illustrates that due to the increase of diffusion path with increasing AMPS content, lower water loss rates was observed for cryogels with higher AMPS content in pH = 8.0 solution. P(DMAEMA-co-AMPS) cryogel containing 40 mol% of AMPS had its water retention reduced from 100% to close 29% within 130 min, whereas the sample containing 10 mol% of AMPS was reduced to about 81%. However, the non-ionic PDMAEMA cryogel was reduced to about 89% within the same time frame. The incorporation of more ionic comonomer AMPS into PDMAEMA backbone would reduce the shrinking rate mainly due to the the dominant effect of strongly acidic -SO₃H groups in AMPS on swelling in comparison with weakly basic tertiary amino groups in DMAEMA and formation of ionic complex between pendant groups of DMAEMA and AMPS.



Figure 9. Deswelling kinetics of P(DMAEMA-co-AMPS) cryogels with different AMPS content in buffer solution at pH 8.0 as measured from an equilibrium swelling condition in pH 2.1.

Swelling of P(DMAEMA-co-AMPS) gels in aqueous salt solutions

The swelling behavior of P(DMAEMA-co-AMPS) gels in aqueous salt solutions of NaC1, KC1, MgC1₂, Na₂S0₄, K₂S0₄, and MgS0₄ was investigated as a function of the salt concentration, the valence of the counterion and co-ion as well as the charge density. The gel samples were allowed to swell in the most concentrated salt solution and then transferred into the next dilute one when the equilibrium was reached. Figure 10 shows the equilibrium swelling ratio of P(DMAEMA-co-AMPS) hydrogels (left panel; A, B, C) and cryogels (right panel; D, E, F) as a function of the concentration of aqueous salt solutions of KCl, NaCl, and MgCl₂, respectively. The water absorbency of P(DMAEMA-co-AMPS) cryogels in the solutions of Na₂S0₄ (A), K₂S0₄ (B), and MgS0₄ (C) was also given in Figure 11. The swelling measurements in all types of salt solutions showed that P(DMAEMA-co-AMPS) gels exhibit strong salt-sensitive swelling behavior over the entire range of the ionic comonomer AMPS concentrations.

As can be seen from these figures that, in the medium range of salt concentration, the swelling ratio of both hydrogels and cryogels increases with the increasing mole fraction of the ionic comonomer AMPS in the feed, x_i , following the sequence from 0 to 0.40 and decreases with increasing salt concentration. Finally, the swelling curves converge together in concentrated salt solutions (1.0 M). The results showed the tendency that the water absorbency by the gel and the swelling ratio decrease as the salt concentration increases due to the ionic strength of the salt solution. This phenomena can be attributed to the electrostatic repulsion between charged groups on the network chains and to the concentration difference of mobile ions inside the gel and the external solution governed by the Donnan potential. At low ionic strength, the repulsions are long-range interactions, and the gel expands to minimize the repulsion free energy.

As the salt concentration rises, the electrostatic force is shielded to a lower strength and the mobile ion concentration in the external solution approaches that inside the gel, and the gel deswells. The osmotic pressure attributable to the polymer network is the driving force for the water absorption and, consequently, the swelling. At thermodynamic swelling equilibrium, the chemical potential of water in the gel network equals that of water surrounding the network. Addition of a salt to the solution leads to network contraction, and the decreasing chemical potential of water in the external solution. Therefore, the gel cannot imbibe as much salt water as pure water. Another point shown from Figs. 10 and 11 is that the swelling of P(DMAEMA-co-AMPS) cryogels in saline solutions was distinctly decreased when compared to the values measured for P(DMAEMA-co-AMPS) hydrogels. The salt effect is clearly evidenced as a result of the osmotic pressure difference between the internal solution in the gel and the external solution due to the different ion concentrations.⁵⁸



Figure 10. The equilibrium swelling ratio of P(DMAEMA-co-AMPS) hydrogels (left panel; A, B, C) and cryogels (right panel; D, E, F) as a function of the concentration of aqueous salt solutions of KCl, NaCl, and MgCl₂. The mole fraction of AMPS in the comonomer feed, $x_i = 0$ (\bullet), 0.05 (\bigcirc), 0.10 (\blacktriangle), 0.15 (\triangle), 0.20 (\triangledown), 0.25 (\bigtriangledown), 0.30 (\blacklozenge), 0.40 (\diamondsuit).

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Figure 11. The equilibrium swelling ratio of P(DMAEMA-co-AMPS) cryogels as a function of the concentration of aqueous salt solutions of K_2SO_4 (A), Na_2SO_4 (B), and $MgSO_4$ (C). The AMPS contents of the hydrogels are already indicated in the figure.

It was also observed that the swelling degree of both P(DMAEMA-co-AMPS) hydrogels and cryogels decreases even more with increasing MgCl₂ and MgSO₄ concentrations. This is mainly due to the divalent Mg²⁺ ions create additional crosslink points in the gel by salt formation on adjacent chains or chain segments of the copolymer. Consequently, the crosslink density of the network increases and hence the swelling capacity of gels decreases. In addition, the equilibrium osmotic pressure is reached earlier in the presence of the divalent ion

as a result of the higher ionic strength of $MgCl_2$ and $MgSO_4$ solutions compared with that of other salt solutions with the same concentration.⁵⁸⁻⁶⁰



Figure 12. The equilibrium swelling ratio of P(DMAEMA-co-AMPS) cryogels containing 30 mol% of AMPS in the feed showing the dependence on the counterion species in the six types of salt solutions.

The equilibrium swelling ratio of P(DMAEMA-co-AMPS) cryogels containing 30 mol% of AMPS in the salt solutions was collected in Figure 12 as a function of the ionic strength. The co-ions in the salts are identical in each plot; i.e., C1⁻ is in Fig. 12(A) and SO₄²⁻ in Fig.12(B), and three counterions are also concerned in each plot. The equilibrium swelling ratio of P(DMAEMA-co-AMPS) cryogels can not be fit with a single curve, especially for those in MgCl₂ and MgSO₄ solutions which show the dependence of swelling capacity of ionic cryogels on the chemical nature of counterions even at low and moderate salt concentrations. In order to observe the counterion effect, the swelling capacity of P(DMAEMA-co-AMPS) cryogels containing 30 (open symbols) and 40 mol% of AMPS (solid symbols) in salt solutions with the same cation K^+ , Na^+ , Mg^{2+} and different co-ions was collected in Figure 13. It was clearly observed that the extent of swelling ratio at the same ionic strength decreases with the change in counterion species as the sequence of K^+ , Na^+ , and Mg^{2+} . This experimental finding indicates the osmotically passive counterions inside the swollen gel and the binding of counterions on the network chains will reduce the repulsion force between the charged groups leading to the decrease in swelling capacity of the resulting gels. Thus, the counterion species is very important for the swelling equilibrium in the presence of the same type of cation and, for P(DMAEMA-co-AMPS) cryogels, the effect of the counterion on swelling capacity seems to be independent of the type of anion at the same ionic strength.



Figure 13. Swelling curves of P(DMAEMA-co-AMPS) cryogels containing 30 (open symbols) and 40 mol% of AMPS (solid symbols) in salt solutions with the same cation K^+ , Na⁺, Mg²⁺ and different co-ions.

Conclusions

P(DMAEMA-co-AMPS) gels were prepared both below (-18 °C) and above (21 °C) the bulk freezing temperature of the polymerization solvent, water, which are called the cryogels and the hydrogels, respectively. The mole fraction of the ionic comonomer AMPS used in the gel preparation was varied over a wide range. The swelling capacity of P(DMAEMA-co-AMPS) gels in water rapidly increases and the modulus of elasticity in the swollen state rapidly decreases as the mole fraction of the ionic comonomer AMPS is decreased. The uniaxial

compression testing showed that P(DMAEMA-co-AMPS) cryogels exhibit larger elastic moduli than that of the corresponding P(DMAEMA-co-AMPS) hydrogels. The swelling measurements of both P(DMAEMA-co-AMPS) hydrogels and cryogels in the solutions with different pH value indicated that P(DMAEMA-co-AMPS) gels are very sensitive to the pH change, especially in the range of 2.1-7.7 and over 8.0. With increasing pH value, the equilibrium swelling ratio of both hydrogels and cryogels decreased and pH-dependent phase transition was obtained. The dependence of the swelling capacity on the counterion species showed that the extent of equilibrated volume decreases with the counterion in the following sequence of K⁺, Na⁺, Mg²⁺ at the same ionic strength. The impact of salts on water absorbency of both P(DMAEMA-co-AMPS) hydrogels and cryogels is relative to the concentration of salt solution as well as the valence of cations.

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