Unveiling the self-assembly behavior of copolymers of AAc and DMAPMA in situ to form smart hydrogels displaying nanogels-within-macrogel hierarchical morphology

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\textbf{A B S T R A C T}

Stimulus responsive hydrogels are being considered as one of the most crucial biomaterials of current generation. A new technique has been established for developing hydrogels based on Acrylic acid (AAc) and \textit{N}-[3-(Dimethylamino)propyl]-methacrylamide (DMAPMA), and relevant mechanism has been delineated. Aqueous redox copolymerization of different molar ratios of AAc and DMAPMA at 41°C, leading to the formation of interlocked nanogels of \~300 nm diameter, which acted as the building blocks of a series of superabsorbent hydrogels having robust, honey-comb type three-dimensional architecture. Monomer composition, monomer feed ratio and water content in feed has been found to be important factors in the development of the stable poly(AAc-co-DMAPMA) hydrogel membranes (PADMAs) without any active crosslinking agent. At the cues of pH change from 7.0 to 3.5, pulsatile swelling–deswelling behavior varied, ranging from \~5900\% to \~60\% (mass) respectively, underlining smart hydrogel characteristics needed for specific biomedical applications. Elastic modulus of the gels, equilibrated at pH 7.0, is recorded to be \textgtr15 kPa under uniaxial compression. Underlying mechanism of the formation of such robust three-dimensional structures in poly(AAc-co-DMAPMA) hydrogel membranes, and the origin of hierarchical ‘nano-to-macro’ scale morphological features has been proposed.

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1. Introduction

Stimulus responsive hydrogels are emerging as an important class of tailor-made functional materials due to their innate structural and compositional features, as well as unique physicochemical properties, such as tunable degradability, swelling, mechanics, permeability etc. These properties make such hydrogels appropriate for a wide range of applications, such as Tissue engineering [1], delivery of drugs & soluble factors [2,3], microfluidics [4] and biosensors [5]. Over the past few decades varieties of hydrogel types have been developed from wide range of chemical building blocks and using an array of synthetic techniques [6]. Among these strategies, gelation techniques based on one or more physical phenomena like hydrophobic interaction, hydrogen bonding and coulombic attraction were found to be most promising.

Consequently, constant efforts are being made to explore novel methodologies for inducing physical gelation [7], avoiding need of chemical cross-linkers.

As the landmark of new generation of hydrogels, attempts are being made to fabricate micro/nano-structures at hierarchical levels of hydrogel which would enable us to have a precise control of the mechanical properties including stiffness, toughness, resistance to fracture and degradation rate [8]. Over the years various crosslinking techniques have emerged to introduce sought after features into the hydrogels ranging from nano scale particulates to monolithic matrices [9]. Clear understanding of various physical interactions and the mechanism of hydrogel formation may potentially lead to simple techniques to fabricate a complex architecture like lamellar tubes or spheroids [10].

Supramolecular self-assembly is one of the most important consequences of physical interactions that trigger gelation process, which is frequently observed in amphiphilic block copolymers [11–15]. Amphiphilic random copolymers also found to undergo self-assembly. Ishihara et al. [16] studied the aggregation behavior of poly(2-methacryloyloxyethylphosphorylcholine-co-n-butyl.
methacrylate) in water, and obtained nanospheres with self-assembled core–shell structure. Apart from amphiphilic (co) networks, physical gels of polyelectrolytes and polyampholytes have also been extensively studied, where coulombic attraction in presence/absence of hydrophobic interaction leads to complexation. Depending on the experimental conditions, these complexes furnish a great variety of three-dimensional structures ranging from discrete nano particles to continuous monolithic matrices [17–20]. All of these conventional gels, based on polyelectrolytes or polyampholytes, destabilize and undergo sol–gel transition with the variation of pH due to the pH-dependent formation/cleavage of the intra–inter-polymer ionic crosslinking.

We have recently reported a series of new hydrogel formulations with high hemocompatibility [21]. The hydrogels were prepared by the aqueous copolymerization of acrylic acid (AAC) and N-[3-(Dimethylamino)propyl]-methacrylamide (DMAPMA) without using any chemical crosslinker. Interestingly, the hydrogels (henceforth named as ‘PADMA’) were found to be stable in simulated body fluid (SBF) even after swelling >30 times of its dry weight. The result was apparently unexpected as the pH of SBF (7.4) was much higher than the pKa of PADMA (~4.5). While investigating the unusual stability of PADMA hydrogels in SBF, it was found that a number of interesting and globally important features were hidden in PADMA formulations.

The present study reports the mechanistic aspects of the formation of PADMA membranes. PADMA copolymers have been prepared under various reaction conditions and in five different molar compositions (PADMA50–PADMA90). Evolution of PADMAs as soluble polyampholytes to insoluble gels has been analyzed step by step. The soluble-polymer/insoluble-gels have been characterized by comprehensive spectroscopic, microscopic and physicochemical techniques. Chemical composition and molecular interactions have been studied by infrared (FTIR) and nuclear magnetic resonance (NMR) spectroscopic techniques. Morphological investigations of the PADMA membranes have been carried out by atomic force microscopy (AFM) and scanning electron microscopy (SEM). Crystallinity of the PADMA membranes has been evaluated by wide-angle X-ray diffraction (WAXD), which would infer if the membranes were stable due to physical crosslinking caused by crystal junction zones. These investigations when combined to other measurements, such as viscosity and glass transition temperature (Tg), provided important information regarding the development of PADMA matrices, and led to a new hypothesis of the formation of dimensionally stable hydrogel without crosslinker. To our knowledge, the hierarchical ‘nano-to-macro’ scale morphology of PADMA hydrogels, as displayed in this article, is a rare finding. Attempts are also made to fabricate stable monoliths from homopolymer of AAC (PAAC), homopolymer of DMAPMA (PDMA) and copolymers of Methacrylic acid (MAAc) and DMAPMA, keeping similar reaction conditions to those of PADMA membranes. Morphology and physicochemical behavior of these (co)polymers provided important information regarding the way the PADMA copolymers self assemble in situ and develop stable monoliths with hierarchical morphology.

2. Experimental section

2.1. Materials

Acrylic acid (AAC) (Aldrich, USA), methacrylic acid (MAAc) (Aldrich, USA) and N-[3-(Dimethylamino)propyl]-methacrylamide (DMAPMA) (Aldrich, USA) were distilled under vacuum before use. Ammonium persulphate (APS), 2-butanol (Qualigens Fine Chemicals, India), and N,N,N',N'-tetramethyl ethylene diamine (TEMED; SRL Pvt. Ltd, India) were used without further purification.

2.2. Preparation of monomer mixtures

Five monomer mixtures (MM50-MM90) were prepared in chilled condition using ice bath. Measured volume of DMAPMA was added drop wise to the requisite amount of AAC with continuous stirring over a magnetic stirrer. After thorough mixing, the mixtures were diluted with water. The dilute mixtures were used for preparing copolymers, whereas, the mother MM mixtures were used to investigate their physicochemical properties.

2.3. Preparation of polymers

Five poly(AAc-co-DMAPMA) copolymers, abbreviated as PADMA50, PADMA72, PADMA80, PADMA86 and PADMA90, were synthesized with monomer feed ratio (AAC/DMAPMA) ranging from 50/50–90/10 (mole). The reactions were carried out at 41 ± 1 °C in aqueous medium using APS and TEMED as the redox initiator. Each of the five PADMAs was prepared in four different sets of reaction conditions (set I–set IV), where amount of water in feed and reaction time being the system variables (Table 1).

In set I and II, the reactions were carried out for short period (30 min). In a typical reaction, the dilute monomer mixture was taken in 100 ml three-neck round-bottom flask, equipped with a magnetic stir bar, a thermometer and a nitrogen line. The flask was kept sub merged in thermostated water bath at 41 ± 1 °C over a magnetic stirrer. Nitrogen gas was purged into the monomer for 15 min with constant stirring followed by the addition of APS and TEMED. After 30 min of reaction, the polymers were precipitated from 2-butanol. The polymers were then washed three times by reprecipitation in order to remove unreacted monomers. These were then dried in vacuum to constant weight.

In set III, the first 30 min of polymerization was carried out same as set I. The reactions were then continued for 24 h in nitrogen atmosphere without stirring. The polymers were extracted as in the previous sets.

The reactions in set IV were done in two steps as described in our previous article [21]. Briefly, the first step was carried out in round-bottom flask, similar to set II, to initiate the polymerizations uniformly all over the mixtures. After 5 min, the individual reaction mixtures were transferred to respective molds to accomplish the second step of the reaction. The rectangular mold, 70 cm/80 cm/0.08 cm in dimension, was prepared using Teflon spacers along the three edges of a pair of glass plates. The open side along the remaining edge was partially closed by a smaller spacer, leaving two orifices at the corners. Nitrogen gas was purged through one of the orifices, while the other was used to inject the reaction mixture under gentle steam of nitrogen. The molds were then placed vertically in a thermostated water bath at 41 ± 1 °C and dipped up to the height of reaction mixtures. Nitrogen purging was stopped after 15 min and the orifices were closed using paraffin grease. After 24 h, a set of five PADMA membranes were removed from the molds, cut into pieces, washed in regularly changed distilled water for 3 days to remove the unreacted monomers, and dried in vacuum.

The homopolymers of AAC and DMAPMA (PAAC and PDMA) respectively were prepared maintaining the conditions similar to those of set IV PADMA membranes. PAAC was obtained as a translucent solid monolith. PDMA, however, could not furnish a solid monolith, and hence was precipitated from 2-butanol after 24 h of reaction.

Two different poly(MAAC-co-DMAPMA) membranes were prepared with MAAC/DMAPMA feed ratio of 50/50 and 80/20. The reactions were carried out in rectangular glass mold, similar to set IV PADMA membranes.
2.4. Solubility study

Solubility of PADMA copolymers, the homopolymers and pol-
y(MAAc-co-DMAPMA) membranes was studied at room temperature in saline water and/or buffered solution of pH 1–12. Approximately 10 mg polymer was suspended over 20 mL of chosen solvent and the solubility was verified after 2 h. Exact formulations of saline water and pH buffers are given as supplementary materials (Table S1, S2 and Normal saline).

2.5. Viscosity measurement

Intrinsic viscosity, [η], of the set I PADMA copolymers in 0.5 N NaCl solution at 25 ± 0.5 °C was measured using a Ubbelhode viscometer. The molar concentration of NaCl in the polymer solution was maintained constant during the dilution process. [η] was determined using Huggins equation, maintaining the criteria of dilute solution.

2.6. Differential scanning calorimetry (DSC)

DSC measurements were performed using a PerkinElmer Differential Scanning Calorimeter (Pyris 6 DSC). Samples were scanned in a gentle flow of moisture-free N2. Thermal history of the polymers was removed by heating the samples in N2, at a rate of 10 °C/min. The glass transition was measured from the trace of second cycle according to standard Tg measurement and the criterion of Tg at ΔCp/2.

2.7. Nuclear magnetic resonance (NMR) spectroscopy

1H NMR and 13C NMR spectra were recorded on a 300 MHz Bruker Spectrospin DPX FT NMR. Chemical shifts were reported as δ values (ppm) relative to internal standard Me4Si.

2.8. Fourier transform infrared (FTIR) spectroscopy

IR measurements were carried out in a Bruker Alpha-P ATR—F- TIR spectrometer, and the spectra were collected in the wave number range of 4000–500 cm⁻¹, at 4 cm⁻¹ resolution.

2.9. Wide-angle X-ray diffraction (WAXD)

Dry PDMAPMA homopolymer and the PADMA membranes were ground to fine powder. X-ray powder diffraction data were collected on a Bruker D8 Advance diffractometer using Ni-filtered CuKz radiation. The scanning 2θ range was from 5° to 60°.

2.10. Atomic force microscopy (AFM)

Surface morphology of the PADMA membranes and poly(MAAc-co-DMAPMA) membranes was investigated by scanning in contact mode using Nanoscope III (Vecco Digital Instruments, USA), a multimode atomic force microscope. Micrographs were captured at a scan rate of 1 Hz and resolution of 256 number of samples/line.

2.11. Scanning electron microscopy (SEM)

Morphology of swollen PADMA membranes was investigated by electron microscopy, using an EVO 50 SEM. The membranes (set IV) were swollen in buffered solution of pH 7, lyophilized, and kept in vacuum till silver sputtering treatment.

2.12. Mechanical testing

TA-XT2i Texture Analyzer (Stable Micro Systems, UK) with a 5 kg load cell was used in our experiments. PADMA membranes were equilibrated in buffered solution pH 1.0 and 7.0. Circular discs of 15 mm in diameter were punched off the swollen membranes. Uniaxial compression experiments were performed on the discs at room temperature with a cylindrical aluminum probe of 35 mm diameter (P35). The pre-test speed and test speed were set up at 2.00 mm/s, 100 mm/s respectively, with an acquisition rate of 200 points/s. The force necessary for compressing the discs at 0.8 mm was recorded in order to determine compressive elastic modulus (E) and system hardness (Fmax). The stress values (σ) were determined using equation (1) [22].

\[ \sigma = \frac{F}{A} \]  \hspace{1cm} (1)

where, F is the force and A is the cross-sectional area of the strained specimen. All data were obtained in triplicate.

The parameters generated by the instrument were force and time/displacement. Those information were then converted to elastic modulus, E, by using following equation.

\[ \sigma = E (\lambda - \lambda^{-2}) \]  \hspace{1cm} (2)

E was determined from the slope of the stress—strain relationship. The macroscopic deformation ratio (λ) was calculated as λ = Ld/L0. Here, Ld and L0 are the length of the deformed and undeformed specimen respectively.

2.13. pH-sensitivity and dynamics of reversible swelling

To investigate the pH responsive swelling behavior, the dry PADMA discs of ~5 mm diameter were weighed and placed in buffered solution of various pH values ranging from 1 to 10 at 37 ± 1 °C. The ionic strength of each buffer solution was adjusted to 0.2 M by the addition of potassium chloride. Degree of swelling was determined by gravimetric method. The discs were removed from the solutions at regular intervals, the surface water was carefully soaked, and the weight was recorded till equilibrium swelling. The swelling ratio (Qs) was then calculated as \( \frac{(W_t - W_0)/W_0 \times 100} \), where \( W_0 \) and \( W_t \) are the final weight of the swollen discs and the initial weight of the dry discs respectively. Similarly, deswelling experiments were carried out with PDMAP86 and PADMA90 in buffered solutions of pH 1.0 and 3.5 at 37 ± 1 °C. Dynamics of deswelling was determined with the discs equilibrated in buffered solutions of pH 7.0.

3. Results and discussion

3.1. Appearance of MM50 and a hypothesis

Densities of AAc and DMAPMA at 25 °C are 1.048 g/ml and 0.94 g/ml respectively. DMAPMA, with a molecular mass of 170.26 u, is a dull straw colored liquid, apparently having slightly higher viscosity than AAc, which is colorless with characteristic pungent smell. During drop wise addition of DMAPMA into AAc, the viscosity than AAc, which is colorless with characteristic pungent smell. During drop wise addition of DMAPMA into AAc, the viscosity was determined with the discs equilibrated in buffered solutions of pH 1.0 and 7.0.
be explained as follows. N-substituted secondary amides like DMAPMA, having a bulky N-substituent, predominantly exist in polymeric form through H-bonding in trans conformation as shown in Scheme 1a. DMAPMA molecules may form complex through H-bonding and/or coulombic attraction with AAc as shown in Scheme 1b.

Coulombic attraction arises from simple acid–base reaction, whereas, H-bonding may be considered to be the penultimate step towards it. Probability of formation of the H-bonded complex and electrolytic complex should depend on reaction temperature. Salt formation is less likely to predominate at ice-cold condition due to the lack of activation energy for the deprotonation of AAc molecules. In practice, salt formation was experienced visually when the monomers were mixed rapidly at room temperature. The temperature rose gradually with the progress of mixing and innumerable tiny particles were found floating in the thick mixture. The white particles felt like rocky sand on rubbing. The particles slowly disappeared on standing at 4 °C.

3.2. Solubility of the polymers

Effect of system variables (reaction time and amount of feed water) on the solubility of the products in saline water is summarized in Table 1.

The results as shown in Table 1 indicated that monomer/water ratio plays an important role in the solubility and, hence, on the nature of the PADMA copolymers. All of the set IV PADMA copolymers (PADMA membranes) were insoluble in saline water, as well as in buffered solution of pH 1–12. All PADMA membranes were found to be dimensionally stable even at very high swelling in the buffered solutions (see Fig. 10) and behaved like crosslinked polyelectrolyte gels. It is worth mentioning here that PADMA membranes have been prepared without any additional crosslinker. Chemical crosslinking due to impurity, if any, was overruled as the monomers were distilled before use. Again, thermal crosslinking was unlikely as the reactions were carried out in thermostat using molds of thin glass plates. IR spectra of the PADMA membranes, as shown in Fig. 5, also made it evident that the membranes do not have thermally crosslinked moiety. Role of AAc-DMAPMA combination in the formation of stable three-dimensional structures became convincing when it was found that the homopolymer of AAc (PAAc) was soluble in alkaline buffers and the homopolymer of DMAPMA (PDPMAPMA) found to be fairly soluble in distilled water. Poly(MAAc-co-DMAPMA) membranes were insoluble in saline water but soluble in buffered solution of pH 7.0. Insolubility in saline water may be due to the presence of H-bonding.

3.3. Viscosity and glass transition

Intrinsic viscosity of the set I PADMA copolymers in saline water and the variation of the respective Tg through set I-set IV is summarized in Table 2 along with the yield of various PADMA copolymers in different sets.

Yield of PDMAPMA and PAAc were found to be 6% and 100% respectively. Corresponding Tg values were 137 °C and 110 °C.

Yield of PADMA copolymers in set I-III increased in the order of PADMA90-PADMA50. This may be explained as follows. AAc and DMAPMA molecules were expected to form ionic complex through acid–base reaction. This intermolecular association reduces the mean free path of the reacting monomers and, thus, increases the collision frequency. Higher the extent of complex formation, higher would be the collision frequency which, in turn, proportionally increases the reaction rate and yield. MM mixtures approach stoichiometry in the order of MM90-MM50. Therefore, extent of complexation should increase in the same order.

Amount of water in feed also determined the extent of complexation, hence, the yield of the product varied accordingly. The copolymerization reactions of AAc and DMAPMA were carried out for 30 min in set I and set II. Monomer–water feed ratios in all the set II PADMA copolymers were higher than set I. Therefore, the higher yield of set II PADMA over set I is justified. Extended reaction time further increased the yield in set III. Finally, the reaction time and the amount of feed water were in favor of maximum yield in set IV.

3.4. 1H NMR analysis

DMAPMA (CDCl3): δ = 1.64–1.72 (2H, m, J = 6 Hz), 1.93 (3H, s), 2.24 (6H, s), 2.41–2.45 (2H, t, J = 6 Hz), 3.38–3.44 (2H, q, J = 6 Hz, 3.5 Hz), 5.29 (1H, s), 5.74 (1H, s), 7.93 (1H, s), AAc (CDCl3): δ = 5.83–5.88 (1H, dd, J = 10 Hz, 6 Hz), 5.98–6.07 (1H, dd, J = 17 Hz 10 Hz), 6.37–6.45 (1H, dd, J = 17 Hz 6 Hz), 11.12 (1H, s).
MM50 was partially soluble in CDCl₃ and completely soluble in D₂O. ¹H NMR spectra of MM50 in CDCl₃ is shown in Fig. 1A. The protons labeled as a, b and c are directly associated to the tertiary N(N(CH₃)₂) atom in DMAPMA. Chemical shift of these protons is expected to move downfield when the nitrogen atom is protonated or undergoes H-bond formation as shown in Scheme 1.

The methylene protons of DMAPMA (labeled as a) were found to resonate in DMAPMA at δ (centre) = 2.47, 2.73 and 1.88 ppm respectively. Fig. 1A indicates that these protons moved downfield and resonate at δ (centre) = 3 ppm when the nitrogen atom is protonated to form quaternary ammonium salt [23–25]. In the spectra of MM50, a new peak appeared at δ = 3.0 ppm and was labeled as x in Fig. 1A. Relative intensity of peak x to peak a increased considerably when the spectra was taken in D₂O (figure not shown). Again, summation of integrals of peak a and x was always found to account for six protons (in CDCl₃ as well as in D₂O). These results indicated that AAc and DMAPMA undergo complexation in both the forms as shown in Scheme 2A and B.

Peak a was due to the H-bonded complex; whereas, peak x was due to the electrolytic complex. The latter was most probably weakly soluble in CDCl₃ and completely soluble in D₂O. Another interesting observation was that the chemical shift of the peak labeled as “y” in Fig. 1A varied considerably. MM50 was prepared several times to ensure the chemical shift of y. It was observed that the peak appeared anywhere in between δ = 10.37 and 4.60. The peak was always observed to be much broader and flatter than that of the COOH proton of AAc. These results clearly indicated that the concerned proton was not solely linked to AAc in MM50 but was shared with DMAPMA.

¹H NMR spectra of PDMAPMA and PADMA50 in D₂O are shown as Fig. 1B and C respectively. The protons directly associated to the nitrogen of the 3⁺ amine of DMAPMA units (proton a, b and c) were once again found to move to downfield in PADMA50. As observed in MM50, the methyl protons of DMAPMA (labeled as a) resonate in two different chemical shift positions (labeled as peak a and x). Relative intensity of these two peaks was found to vary from batch to batch. The methyl protons of DMAPMA (labeled as b) should also resonate in two different frequencies due to two different types of complexes. The second peak other than δ = 3.20 was most probably buried under the intense peaks at δ = 2.75–2.98. These results indicated that the two forms of complex as shown in Scheme 2 also exist in the PADMA50 polymer.

### 3.5. ¹³C NMR analysis

¹³C NMR spectra of PDMAPMA and PADMA50 in D₂O are shown in Fig. 2A and B respectively. The methyl (CH₃) and methylene (CH₂) carbon atoms, directly associated to the 3⁺ N atom of PDMAPMA, resonate at 44.1 ppm and 30.4 ppm respectively. The methyl protons of PDMAPMA (labeled as b) should also resonate in two different frequencies due to two different types of complexes. The second peak other than δ = 3.20 was most probably buried under the intense peaks at δ = 2.75–2.98. These results indicated that the two forms of complex as shown in Scheme 2 also exist in the PADMA50 polymer.

### Table 2

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Polymer</th>
<th>P50⁺</th>
<th>P72⁺</th>
<th>P80⁺</th>
<th>P90⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (% wt)</td>
<td>set I</td>
<td>67 ± 6.1</td>
<td>56 ± 4.5</td>
<td>46 ± 4.5</td>
<td>38 ± 5.0</td>
</tr>
<tr>
<td>set II</td>
<td>70 ± 5.7</td>
<td>64 ± 3.0</td>
<td>64 ± 3.0</td>
<td>53 ± 1.5</td>
<td>38 ± 7.0</td>
</tr>
<tr>
<td>set III</td>
<td>89 ± 3.5</td>
<td>82 ± 3.1</td>
<td>70 ± 2.5</td>
<td>57 ± 7.0</td>
<td>47 ± 3.5</td>
</tr>
<tr>
<td>set IV</td>
<td>100⁺</td>
<td>100⁺</td>
<td>100⁺</td>
<td>100⁺</td>
<td>100⁺</td>
</tr>
<tr>
<td>[η] (dL/g)</td>
<td>set I</td>
<td>1.75 ± 0.13</td>
<td>0.73 ± 0.10</td>
<td>0.68 ± 0.08</td>
<td>0.43 ± 0.04</td>
</tr>
<tr>
<td>set II</td>
<td>98.54 ± 1.05</td>
<td>100.08 ± 1.54</td>
<td>97.68 ± 1.29</td>
<td>97.61 ± 1.11</td>
<td>96.32 ± 1.44</td>
</tr>
<tr>
<td>set IV</td>
<td>99.861.03</td>
<td>102.57 ± 1.61</td>
<td>106.7 ± 0.46</td>
<td>111.82 ± 1.81</td>
<td>116.47 ± 0.80</td>
</tr>
</tbody>
</table>

a P stands for PADMA.

b All of these yields were found to be slightly > 100%, most probably due to the presence of bound water, and were considered to be 100%.

c Intrinsic viscosity of PADMA copolymers of set I in saline water at 25°C.

### Scheme 2

Different modes of complexation between AAc and DMAPMA molecules: A) Hydrogen bonding; B) Electrolytic complex.
45.4–45.8 ppm (doublet) respectively. Both of these two carbons were found to resonate at two different chemical shift positions in PADMA50. The methyl carbons resonate at 43.0 ppm and 50.9 ppm, whereas, the methylene carbon resonate at 45.4 e 45.7 ppm and 61.6 e 62.3 ppm (doublet). These results again supported the existence of two types of complexes between AAc-DMAPMA units in PADMA50. Chemical shift of the amide carbon of PDMAPMA (C¼ONH−) remained unchanged at 179.5 ppm, which ensured that the amide group would not interact with the AAc units in PADMA50.

3.6. FTIR analysis

IR spectra of AAc, DMAPMA and their mixture MM50 are shown in Fig. 3. Absorption band at 1697 cm⁻¹ in AAc was due to νC = OOH. Intensity of this band reduced considerably in MM50 and appeared as a shoulder at 1710 cm⁻¹. This was due to the fact that majority of the total AAc molecules were engaged in electrolytic complex formation with DMAPMA molecules. Shifting of the absorption from 1697 cm⁻¹–1710 cm⁻¹ might be due to the change in the mode of H-bonding in AAc molecules. It is known that the carboxylic acids like AAc predominantly exist in dimeric form as shown in Scheme 3a.

The carbonyl oxygen of the carboxyl group becomes free when AAc forms H-bond with DMAPMA in MM50. This increases the bond order of the carbonyl group. Therefore, absorption at higher frequency is expected.

Absorption due to the asymmetric stretching of the carboxylate ions (νCOO⁻) was masked by the intense band at 1616 cm⁻¹ due to νC = C of (meth)acrylic double bonds. Amide I (νC = ONH) and amide II (δCON–H) of DMAPMA absorbed at 1655 cm⁻¹ and 1528 cm⁻¹ respectively and remained unchanged in MM50. This again indicated that AAc would not interact with the amide group of DMAPMA molecules.

IR spectra of set I PADMA copolymers and set IV PADMA membranes, as shown in Fig. 4 and Fig. 5 respectively, provided important information regarding monomer distribution in the PADMA copolymers.

Important absorption in Fig. 4 were at 1710 cm⁻¹ (νC = OOH), 1632 cm⁻¹ (νC = ONH), 1583 cm⁻¹ (νCOO⁻, asymmetric), 1531 cm⁻¹ (δCON–H) and 3300 cm⁻¹ (νCON–H). The weak absorption at 1710 cm⁻¹ and the strong absorption at 1583 cm⁻¹ indicated that the AAc and DMAPMA units predominantly remain as electrolytic complex in all the PADMA copolymers. Intensity of the strong band at 3300 cm⁻¹ did not vary noticeably from PADMA50 to PADMA90 copolymers. Again, the intensity of absorptions at 1632 cm⁻¹ and 1531 cm⁻¹, relative to that at 1583 cm⁻¹ varied only to a small extent from PADMA50 to PADMA90. These results indicated that all the set I PADMA copolymers were having almost same monomer composition. Therefore, it can be inferred that all the PADMA copolymers initially propagate in similar fashion, in spite of their different feed compositions, and equally dominated by both the monomers. In contrary to the set I PADMA copolymers, the set IV PADMA membranes showed significant variation in the IR spectra from PADMA50 to PADMA90. Fig. 5 clearly demonstrated that the amount of free AAc units (νC = OOH, 1710 cm⁻¹) progressively increased from PADMA50 to PADMA90. These results indicated that all of the PADMA copolymers, after complete reaction as in set IV, were composed of two types of segments (blocks) along their backbones. The initial propagating steps produced a segment which was dominated by both the monomer units. The latter stage propagation furnished second type of segment which was predominated by AAc units.

![Fig. 2. ¹³C NMR spectra: A) PDMAPMA in D₂O; B) PADMA50 of set I in D₂O.](image)

![Scheme 3. Schematic representation of the mode of H-bonding of AAc: a) dimeric form of AAc; b) with DMAPMA.](image)
3.7. XRD analysis

PAAc is known as amorphous polymer. Wide-angle XRD pattern of PDMAPMA and the five PADMA membranes are shown as Fig. 6.

All the PADMA membranes showed similar diffraction pattern with a blunt peak at 2\(\theta\) ranging in between 18 and 20\(^{\circ}\), which indicated that the membranes were predominantly amorphous. Poly-DMAPMA also showed a similar peak at 2\(\theta = 18^{\circ}\) along with two sharp diffraction peaks at 29.8\(^{\circ}\) and 31\(^{\circ}\). However, low intensity of all the peaks ensured its amorphous nature.

3.8. Compressive property

Compression experiment was done to determine the system hardness (\(F_{\text{max}}\)) and compressive elastic modulus (\(E\)) of the swollen PADMA membranes at pH 1.0 and 7.0. Profiles obtained from a typical experiment for individual PADMA membranes at pH 1.0 and 7.0 are shown in Fig. 7A and B respectively.

Results of all the experiments are summarized in Table 3. Each of the curves in Fig. 7A and B has two halves. The ascending half was due to the stress applied onto the hydrogel membranes. The peak in each curve denoted the maximum stress applied at 0.8 mm compression. The descending half in individual curves was due to the withdrawal of stress from the membranes.

Linear stress–strain relationship was observed at low strain (~10% deformation) in all the samples, and is shown as inset. Corresponding elastic moduli (\(E\)), as given in Table 3, indicated that the flexibility of PADMA membrane at a particular pH vary with composition. This variation was mainly due to the variation in degree of swelling with composition. The voids that were filled with water increased in size with increase in swelling. In other words, the least swollen membrane was the most compact one and, hence, possessed least flexibility or maximum elastic modulus. The elastic modulus increased in the order of PADMA72 to PADMA90 at pH 1.0 and decreased in the same order at pH 7.0. Fig. 7 also revealed that the hardness of PADMA membranes at a particular pH vary in the same order as elastic modulus. System hardness at pH 7.0 ensured that the PADMA membranes retained appreciable strength even at very high swelling.
3.9. AFM analysis

AFM micrographs, as shown in Fig. 8, displayed the nano scale surface morphology of the collapsed PADMA membranes. The micrographs indicated that the surfaces of the membranes were not continuous, and made up of interconnected nano particles. The particles developed in situ during the formation of the monolithic matrices and appeared as if acting as building blocks. The micrographs gave an impression of the way the polymer chains grew during reaction.

3.10. SEM analysis

SEM micrographs of swollen PADMA membranes, as shown in Fig. 9, revealed quite interesting and unique ‘nano-to-macro’ scale morphological features. Surface of PADMA50 found to be macroporous and had honey-comb type architecture.

The macroporous surface morphology, however, was not observed in any of the other PADMA membranes. Rather, a continuous skin enveloped the porous interior. Fig. 9b, c, d, e showed respectively the surface of PADMA72, PADMA80, PADMA86 and PADMA90. An impression of the macroporous skeleton just beneath the skin could be clearly observed in Fig. 9b. Careful observation revealed a gradual change in the surface from PADMA50 to PADMA90. Pores were wide open at the surface of PADMA50, and no sign of surface skin could be noticed. PADMA72 developed a thin skin and hence left a clear impression of the skeleton of macroporous interior. Thickness of the skin gradually increased and, hence, the impression of porous skeleton disappeared through PADMA80, PADMA86 and PADMA90. Fig. 9f and g showed respectively the cross section of PADMA50 and PADMA80. Fig. 9h showed both surface and interior of PADMA90. These figures revealed that the interior of all PADMA membranes were macroporous and had honey-comb type architecture.

The gradual change in surface morphology, but not in bulk morphology, may be explained as follows. Reaction time was increased gradually from PADMA50 to PADMA90. The reaction(s) were relatively fast and took only 2 h for the formation of PADMA50. In case of PADMA72, it took ~6 h. The reaction time gradually increased and reached the maximum in PADMA90, which required 24 h for complete reaction. The heat generated during the exothermic polymerization reaction expected to dissipate through the thin glass mold, which was in contact of thermostated water. PADMA50, however, did not allow the same to happen because of fast reaction. Therefore, the surface and interior developed uniformly (macroporous) and quickly. The slower reactions in other PADMA hydrogels resulted in a temperature gradient between the bulk and the surface. The propagating polymers at the cooler surface condensed and started developing a continuous skin.

Fig. 9k showed that each microscopic part of the surface was made up of interlocked nanogels of ~300 nm. Here the term “interlocked” has been used as the nanogels remained interconnected and do not disintegrate even after very high swelling in phosphate buffered solution of pH 7.0, and acted as stable building blocks. Fig. 9l revealed that the surfaces of the other PADMA membranes were also composed of stable nanogels. In contrary, PAAc and PDMAPMA did not produce such globular structure. Unlike PADMA membranes, these two homopolymers were found to have a continuous morphology at nano scale as shown in Fig. 9i and j respectively.

Following points should be considered before analyzing the mechanism of the formation of interlocked nano globules in situ and the stable macroporous skeleton in PADMA membranes. It is

<table>
<thead>
<tr>
<th>Sample code</th>
<th>$F_{\text{max}}$ (kPa) pH 1.0</th>
<th>$F_{\text{max}}$ (kPa) pH 7.0</th>
<th>$E$ (kPa) pH 1.0</th>
<th>$E$ (kPa) pH 7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>PADMA72</td>
<td>48.14 ± 1.5</td>
<td>77.62 ± 0.9</td>
<td>13.85</td>
<td>28.13</td>
</tr>
<tr>
<td>PADMA80</td>
<td>68.79 ± 0.6</td>
<td>57.13 ± 2.4</td>
<td>16.05</td>
<td>22.56</td>
</tr>
<tr>
<td>PADMA86</td>
<td>85.34 ± 1.9</td>
<td>33.87 ± 1.7</td>
<td>20.44</td>
<td>16.47</td>
</tr>
<tr>
<td>PADMA90</td>
<td>106.21 ± 2.8</td>
<td>23.98 ± 3.1</td>
<td>25.87</td>
<td>15.37</td>
</tr>
</tbody>
</table>

Table 3: System hardness ($F_{\text{max}}$) and compressive elastic modulus ($E$) of the PADMA membranes at pH 1.0 and pH 7.0.
known that coulombic attraction in PAs/PECs leads to aggregation and precipitation/coacervation at optimum condition [25–29]. However, the mechanism based on coulombic interaction could not independently explain all experimental observations. For example, unlike other self-assembled PAs or PECs, PADMA membranes were insoluble in alkaline buffers. Stability of globular structure simply due to oppositely charged polymer-complexes could not be explained in alkaline buffers as the pKa of AAc is \( \approx 4.5 \). In order to ensure any possible role of electrolytic complexation in formation and stability of the building blocks, attempts were made to fabricate poly(MAAc-co-DMAPMA) membranes from DMAPMA and methacrylic acid (MAAc). Two most important observations were: 1) the membranes were fairly soluble in alkaline buffers and 2) the globular building blocks were not observed in these membranes (figure not shown).

Chemically, PADMAs and poly(MAAc-co-DMAPMA) membranes differed only in the structure of AAc and MAAc. MAAc is hydrophobic in comparison to AAc. DMAPMA molecule, on the other

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**Fig. 9.** SEM micrographs: a), b), c), d) and e) shows respectively the surface of PADMA50, PADMA72, PADMA80, PADMA86, and PADMA90 at low magnification (range 100×—1000×) after swelling in buffered solution of pH 7.0. f), g) and h) represents the cross section of PADMA50, PADMA80 and PADMA90 at low magnification (range 100×—200×); i) and j) shows the morphology of PDMAPMA and PAAc at high magnification (50k×); k), l), m) n) and o) shows the building blocks, at high magnification (range 30k×—65k×), at the surface of PADMA50 PADMA72, PADMA80, PADMA86 and PADMA90 respectively.

**Scheme 4.** Hypothetical sketch of the formation of stable PADMA membranes: i) Formation of nano globules during copolymerization of AAc and DMAPMA; ii) Nano globules are interlocked through fusion (a) and common segments (b); iii) Water molecules are being forced out of the growing PADMA globules and trapped inside the matrix; iv) Representative SEM micrograph showing (a) the macroporous interior of swollen PADMA membrane and (b) the building blocks of interlocked nanogels.
hand, is hydrophobic compared to AAc because of the presence of three methyl (CH₃) and methylene (CH₂) groups. Again, IR spectral analysis revealed that PADMA copolymers initially propagated in the form of AAc-DMAPMA complex and then the growing chain progressively became rich of AAc units at the later stage of propagation. Therefore, the individual polymer chains in PADMA membranes consisted of two types of segments (blocks). One of the segments carrying both the monomer units; while the other one was composed of two hydrophilic monomer units, such as PADMA.

Under optimum reaction conditions, PADMA copolymers left the form of AAc-DMAPMA complex and then the growing chain self-assembled in aqueous medium. Self-assembled chains were expected to behave like amphiphiles. The hydrophobic segments forced themselves into a core surrounded by the shell of hydrophilic segments and developed a globular core–shell structure. As the globules in PADMA membranes developed in situ during polymerization, the adjacent propagating globules were most likely to have common propagating polymer chains and, thus, bind each other progressively to build the whole matrix. Once the matrix was formed, hydrophobic core of the innumerable building blocks rendered the matrix insoluble in aqueous media.

The proposed hypothesis became more convincing while analyzing the micro scale morphology of the PADMA membranes (porous interior). AAc, DMAPMA and water remained homogeneously mixed at the beginning of reaction which, ultimately, ended up with the matrices resembled to a honey-comb. It was, therefore, obvious that water did not remain homogeneously mixed with the monomers during the progress of the formation of PADMA matrices. It appeared as if the whole water splits into innumerable tiny droplets, remained trapped inside, and got compartmentalized in order to create cages like honey-comb. This can be explained on the basis of amphiphilicity of the globular building blocks. During membrane formation, the water molecules were forced out of the hydrophobic core of the propagating PADMA globules and accumulated over the hydrophilic shell. With the progress of reaction, the interconnected globules extended in three dimensions. The accumulated water molecules ultimately found themselves trapped inside capsules of the interconnected nano globules. Ultimately, a matrix developed where innumerable tiny water droplets remained trapped inside the skeleton made up of interlocked nano globules. These tiny voids, bearing water droplets, expanded when the matrix was swollen in buffered solution and justiﬁed the macroporous morphology. Hypothetical sketch of the formation of dimensionally stable PADMA matrices with interlocked nanogels as the stable building blocks is shown in Scheme 4.

3.11. pH-sensitivity and dynamics of reversible swelling

Variation in equilibrium swelling of PADMA membrane with pH and monomer composition is shown in Fig. 10.

Swelling behavior of a typical polyampholyte, with an isoelectric point (pI) at ~3.5, was observed. Swelling of PADMA hydrogels at pH < pI was mainly due to the ionization (protonation) of the DMAPMA units [−N(CH₃)₂H⁺]. Whereas, ionization (deprotonation) of the AAc units (−COO⁻) at pH > pI accounted for the swelling at higher pH. Reasonably, increasing DMAPMA content increased the swelling ratio of PADMA membranes at lower pH, and increase in AAc content did the same at higher pH.

Pulsatile swelling—deswelling of PADMA86 and PADMA90 are shown as Fig. 11a and b respectively.

Both the membranes were first swollen in buffer solution of pH 7.0. The membranes reached to the equilibrium swelling of 5000—6000% in ~8 h. The swollen discs were transferred to excess buffer solution of pH 1.0 After 12 h. Rapid deswelling of both the membranes was observed at pH 1.0. The membranes were found to undergo pulsatile swelling at pH 7.0 and deswelling at pH 1.0 and 3.5. However extent of deswelling at pH 3.5 was higher than that at pH 1.0. All the membrane samples retained dimensional stability after successive swelling—deswelling experiments.

4. Conclusions

Taken together, this study provides a first insight on the mechanism of the formation of stable hydrogel out of simple redox copolymerization reaction in aqueous milieu at near ambient temperature. Reaction of AAc and DMAPMA has been investigated step by step with varying conditions, which led to a new hypothesis to explain otherwise an unusual phenomenon of formation of dimensionally stable and robust PADMA hydrogel matrices without any active crosslinker. The hydrogel matrices showed unique nano-to-macro scale hierarchical patterns, which opened a critical window to realize the self-assembly behavior of copolymers composed of two hydrophilic monomer units, such as PADMA. Under optimum reaction conditions, PADMA copolymers left

![Fig. 10. Plot of equilibrium swelling ratio (Rs) of different PADMA membranes against pH of the swelling medium at 37 ± 1 °C (N = 3, ±SD).](image)

![Fig. 11. Swelling—deswelling dynamics: a) PADMA86; b) PADMA90.](image)
impressions to propagate and self assemble in well organized manner, which led to the formation of physically interlocked nanostructured building blocks in situ, and constructed honey-comb type three-dimensional skeleton of superabsorbent hydrogels. PADMA copolymers with AAc/DMAPMA feed ratio ≥ 1 behaved like amphiphilic block copolymers after complete reaction and, thus, developed self-assembled globular building blocks in situ. The porous three-dimensional skeleton resulted from phase segregation of water from the growing amphiphilic polymeric building blocks during the progress of membrane fabrication. The interlocked nanogels most probably acted similar to the sliding crosslink points [30] typical to topological gels and, thus, resulted in considerable toughness in swollen PADMA hydrogels. Ampholytic nature enabled PADMA formulations to bring forth swelling at 3.5 > pH > 3.5 and deswelling at pH = 3.5. The magnitude of pulsate in the swelling—deswelling has been appreciably high and, as such, the robust PADMA formulations may find suitable applications in actuators. The knowledge of the role of specific monomer interactions and feed compositions in developing the complex three-dimensional architecture may prove critical for the development of smart functional hydrogels for biomedical applications. The study on regulation of pulsatile swelling—deswelling characteristics will offer new opportunities to build biologically relevant systems such as pH-sensitive sequential drug delivery system.

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.polymer.2011.05.051.

References