Colloid Polym Sci (2006) 284: 1121–1129
DOI 10.1007/s00396-006-1489-4

ORIGINAL CONTRIBUTION

Nurettin Sahiner
W. T. Godbey
Gary L. McPherson
Vijay T. John

Microgel, nanogel and hydrogel–hydrogel semi-IPN composites for biomedical applications: synthesis and characterization

Received: 24 January 2006
Accepted: 21 March 2006
Published online: 9 May 2006

N. Sahiner · W. T. Godbey · V. T. John
Department of Chemical & Biomolecular Engineering, Tulane University,
New Orleans, LA 70118, USA

G. L. McPherson
Department of Chemistry, Tulane University,
New Orleans, LA 70118, USA

N. Sahiner (✉)
Materials Science and Engineering,
University of Delaware,
201 DuPont Hall,
Newark, DE 19716, USA
e-mail: sahiner@udel.edu
Tel.: +302-831-6194
Fax: +302-831-4545

Abstract Quaternary ammonium salt hydrogels from a cationic monomer, (3-acrylamidopropyl)-trimethylammonium chloride (APTMACl), in a variety sizes such as bulk, micro- and nano- has been prepared. The synthesis of micro- and nanogels were carried out in the microenvironment of water-in-oil microemulsions using two types of surfactants, namely, L-α-phosphatidylcholine (lecithin) and dioctyl sulfosuccinate sodium salt (AOT). Additionally, hydrogel–hydrogel composite semi-interpenetrating polymer networks (semi-IPN) was synthesized by dispersing previously prepared micro/nanogel into neutral monomers such as acrylamide (AAm) or 2-hydroxylethyl methcarylate (HEMA) before network formation. Hydrogel swelling and pH response behaviors have been investigated for bulk gels. Morphology, structure, and size of nano-, micro- and bulk materials were explored utilizing transmission electron microcopy (TEM), scanning electron microscopy (SEM) and atomic force microscopy (AFM). It was confirmed with gel electrophoresis that completely charged nanogel form a strong complex with DNA.

Keywords Nanogel · Nanocomposite · Hydrogels · Polymeric biomaterials · pH-sensitive nanohydrogel

Introduction

Hydrogels are an extremely important class of materials with tremendous application potential in biology and pharmaceutical sciences [1–6]. Synthetic hydrogels are polymeric networks of hydrophilic groups containing chains that are swollen in water. Polyelectrolyte hydrogels are especially useful as they either carry, or are able to develop charges on the chain. Hydrogels can be designed to exhibit significant volume changes in response to small changes in their environment such as pH, ionic strength, temperature [4, 7, 8] electric field [5], solvent [9], or magnetic field [10].

Many biological applications of polyelectrolytes are due to their ability to bind oppositely charged species to form complexes. Recently, numerous studies have utilized cationic systems for gene and antisense therapies and bile acid sequestrants [11] and have devoted to the development of viral and nonviral vectors for DNA and oligonucleotide delivery [12, 13]. Among the synthetic carriers, major attention is paid to cationic polymers which are able to do both condense large structure into smaller ones and mask the negative DNA charges, which is necessary for transfecting most types of cells [14]. A good delivery system should provide resistance to premature enzymatic degradation and aggregation, be able to target specific

tissues, cross the cell membrane, facilitate the nuclear uptake, and supply controlled release of the genetic material, while inducing no toxicity or immune response [15, 16]. For these reasons, suitable coating of delivery system is very important for certain applications [17].

Micro- and nanosized hydrogels are faster in responding to changes in their environment than their macroscopic or bulk counterparts and can be used more efficiently in medical and sensor applications [18, 19]. Due to their much higher interfacial area per unit mass, they have greater exchange rates [20].

In this work, we determine the feasibility of preparing highly charged cationic hydrogels in the micro- and nanometer size range. Our approach has been to use self-assembling surfactant systems that serve as confined media to restrict the growth of hydrogels. An additional aspect is the use of phospholipids in synthesis so that the hydrogels are synthesized with phospholipid coatings for enhanced biocompatibility. This paper, therefore, reports the synthesis and characterization of a cationic hydrogel in various sizes using the templating environment of surfactant microstructures. We have also examined the complexation of such cationic hydrogel particles to anionic DNA to substantiate the concept that such particles can be used as gene delivery vehicles. Finally, we show that novel hydrogel–hydrogel composites can be obtained by embedding cationic hydrogel particles into matrices of neutral or anionic hydrogel.

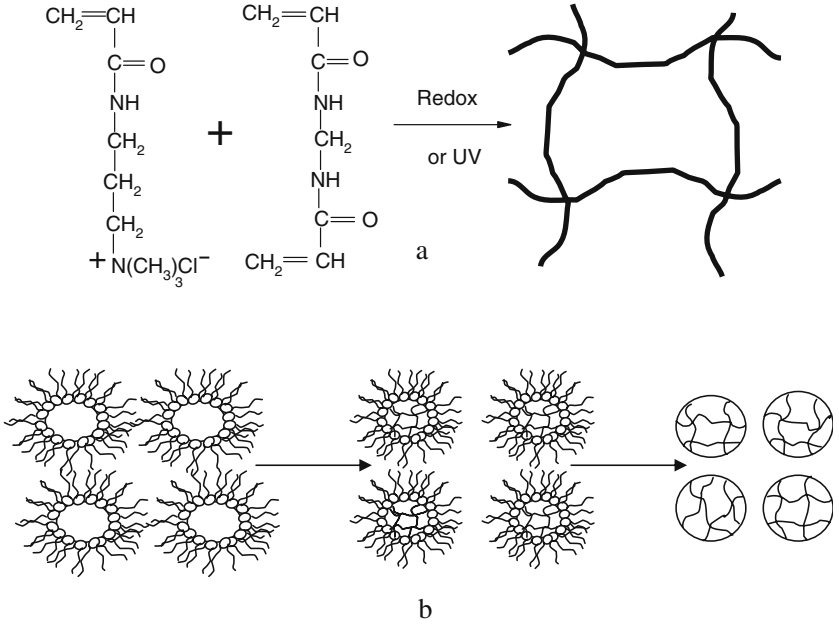
Materials and methods

Materials

(3-Acrylamidopropyl)-trimethylammonium chloride (APTMACl), acrylamide (AAm) and 2-hydroxyethyl methacrylate (HEMA) as monomers, and *N*,*N*′-methylene-bisacrylamide (Bis) as a cross-linker (X), ammonium persulfate (APS) as redox initiator, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) as an accelerator, dioctyl sulfosuccinate sodium salt (AOT) and L-α-Phosphatidylcholine (lecithin) as surfactants, 2,2,4-trimethylpentane (isooctane) and cyclohexane as organic solvents, 2,2′-azobisisobutyronitrile (AIBN) as a radical initiator, and fluorescein sodium salt (FSS) as fluorescence dye were used, and are all products of Aldrich Chemical Company (Milwaukee, Wisconsin).

Bulk hydrogel preparation

Typical hydrogels of APTMACl, AAm, and HEMA were prepared using the following steps. One gram monomer was dissolved in 0.5-ml water, containing the specified amount of X, and 100-μl TEMED (the accelerator) was added. To this solution, 0.5 ml of 1 mol % (with respect to the monomer) of APS solution (the redox initiator) was added. After mixing thoroughly, the solution was injected into plastic straws (for bulk synthesis) and/or between sealed two glass slides (for bulk films) and kept at least 24 h to complete the polymerization/cross-linking reaction at room temperature. Following the cutting procedure for the preferred size and shape (ca 0.5 mm in length and 0.3 mm radius for cylindrical hydrogels), hydrogels were



Scheme 1 Schematic representation of hydrogel formation. **a** The chemical structure of cationic hydrogel synthesis as bulk. **b** Monomer swollen inverse micelles, to form nanogel and removal of surfactant

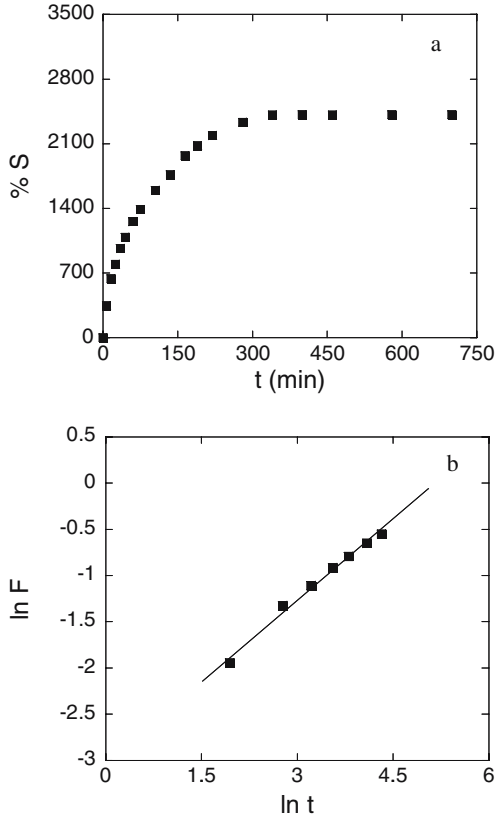


Fig. 1 Swelling kinetic curves of **a** 1 % cross-linked *APTMACl* hydrogel water. **b** ln F vs ln t for determination of diffusion type

kept in distilled water for 2 days with frequent replenishment of the water to remove any impurities and unreacted species. With the UV irradiation method, the same procedure was employed except that TEMED and APS were replaced with 5–10 mg of AIBN (the free radical initiator) and the solution was irradiated in a photochemical reactor operating with 120 V, 50/60 Hz. (The Southern New England Ultraviolet Company, CT, USA, Model # RPR-100).

Microgel/nanogel preparation

The cationic microgel was synthesized in lecithin (Lec) solution in cyclohexane via UV irradiation technique of different amounts of Xs containing APTMACl aqueous solution and AIBN as initiator with w_0=7.5 (w_0=[H_2O]/[Lec]) value. Upon mixing of 0.1 ml of APTMACl precursor solution with 15 ml, 0.1 M of Lecithin solution, a yellowish transparent gel-like solution was formed. This gel (surfactant-hydrogel precursors) was irradiated for 8 h, followed by a washing process to remove/partially remove the surfactant. Acetone and ethyl alcohol at a volume ratio of 1:1 was chosen as the solvent as the surfactant is fully soluble in this mixture of solvents and does not stabilize the hydrogel which precipitates out of solution. To completely remove the surfactant, the obtained mixture was centrifuged at 2,500 rpm for 10 min several times.

Polymeric nanogels of APTMACl were also synthesized by means of inverse microemulsion system at room temperature by redox polymerization method. The polymerization was carried at w_0=10 medium with various amounts of X ratios. In a typical route for nanohydrogel synthesis, the following steps were employed: (1) 0.5 ml of APTMACl is diluted with the required amount of distilled water and transferred to a 150-ml, one-necked flask containing 75-ml, 0.1-M AOT solution in isooctane. This solution was vortexed to obtain clear water-in-oil microemulsions. (2) In a separate vial, varying amounts of X, 0.5–2 mol % were dissolved in distilled water with 50-μl TEMED. (3) In another vial, the initiator solution, APS, was prepared, which is 1 % (with respect to monomer amount) in 0.5-ml distilled water. The water level of the solution was adjusted to bring the final water content to w_0=10 condition. After mixing all these three solutions in the sealed flask, the system was purged with nitrogen for at least 1 min to remove the dissolved oxygen, followed by

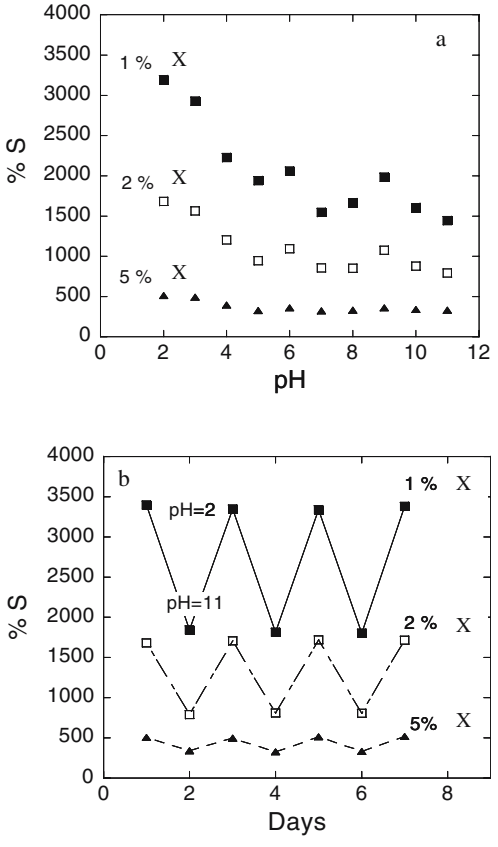


Fig. 2 **a** pH sensitivity of different % cross-linked (X) hydrogel. (*squares*: 5 %, *open squares*: 2 %, and *triangles*: 1 % X p(APTMACl) cationic hydrogels, respectively.) **b** The reversible pH response of different % X p(APTMACl). Hydrogels were alternatively kept in pH values of 2 and 11 for a day

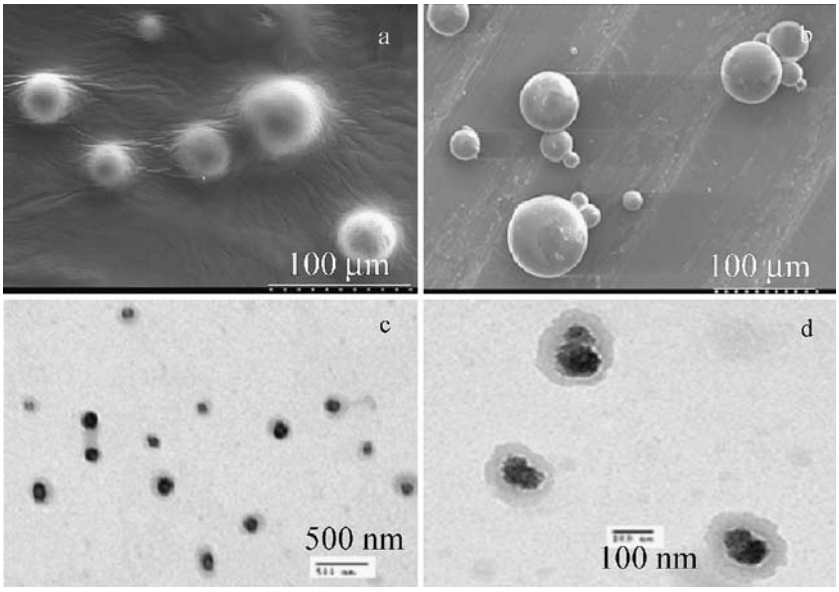


Fig. 3 SEM images of freeze dried 2 % X cationic microgels **a** with phospholipid, and **b** after removing phospholipid, and TEM images, **c** and **d** after filtering and partially removing the phospholipid (*scale bars* are 500 and 100 nm, respectively)

the addition of another 50-μl TEMED. The reaction was carried out for 24 h with stirring on a magnetic stirrer set up, at room temperature. After the reaction, the mixture remains clear indicating that any polymer formed remains sustained in the microemulsion droplets. The polymer was then precipitated using excess acetone to destabilize the micelles. Repeated centrifugation and washing with acetone removes virtually all surfactant.

Hydrogel–hydrogel semi-interpenetrating network (IPN) composites

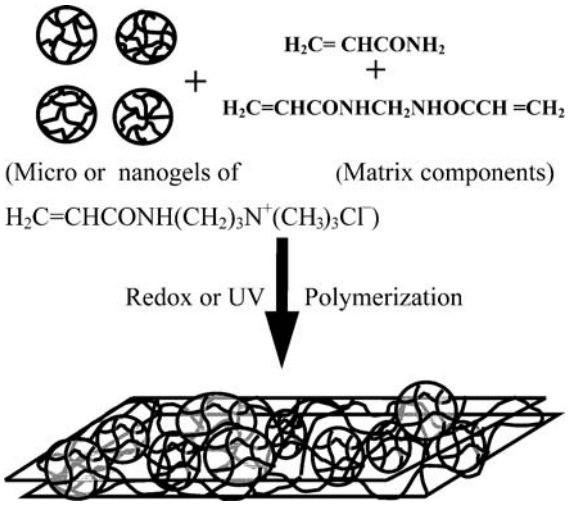
The hydrogel–hydrogel composite semi-IPN synthesis was carried out in the form of hydrogel films between the two glass slides using AAm, HEMA, or APTMACl as matrix material and the APTMACl cationic microgel/nanogel as the second composite materials. In semi-IPN synthesis, 1-g AAm (or matrix monomers) and the X, Bis (0.5 mol %) are dissolved in 1-ml water and mixed with 25-μl TEMED and the desired amount of the different percentages of X cationic microgel/nanogel. This was followed by the addition of 0.25 ml of APS initiator solution (0.5 %). After mixing thoroughly, the mixture is spread on a precleaned glass slide and is covered with more glass slides and is reacted for 12 h. After cutting the semi-IPN hydrogel–hydrogel composite to appropriate sizes, they were immersed in distilled water for the washing procedure, as described previously, to remove unreacted species such as AAm, APS, Bis, or unbind microgels/nanogels.

Hydrogel imaging

Dry hydrogels were imaged using scanning and transmission electron microscopy (Hitachi S3000 N (SEM) and JEOL 2010 (TEM), respectively). SEM imaging of the microgels was obtained with the particles freeze-dried on SEM stubs and gold sputtered before imaging. For TEM imaging of nanoparticles, a drop of aliquot is taken after completion of the polymerization/cross-linking reaction and diluted 60- to 80-fold with isooctane and placed on formvar-coated copper grids and kept in a closed environment at ambient temperature for 12 h before imaging. Atomic force microscopy (AFM) (I-MAC Molecular Imaging) was used to characterize hydrogels. The powdery nanohydrogel was first swollen in distilled water before placing on mica surface, and was dried in the closed environment at an ambient temperature for 24 h before imaging. It was found that the intermittent contact mode (tapping mode) technique was capable of imaging the polymeric nanoparticles.

Gel electrophoresis

Nanohydrogels were complexed with 4,460 bp containing plasmid DNA by lyophilizing with 100 to 1 charge ratio (+: −) in Tris Acetic acid Ethylene diamine tetra acetic acid (TAE) buffer solutions. After lyophilization, the complexes were resuspended in TAE solution at different times, 2, 1 and 0 days, respectively, and analyzed by electrophoresis on 0.6 % (w:v) agarose gel containing ethidium bromide.



Scheme 2 Schematic representation of hydrogel–hydrogel semi interpenetrating (*semi*-IPN) hydrogel synthesis

Results and discussion

The water-soluble acrylamide derivatives as monomers were used for cationic and neutral hydrogel syntheses. All the monomers used in this study can be polymerized either by UV irradiation or by redox polymerization methods [21, 22]. Generally, for micro/nano-material synthesis, reverse micelle technique is employed [23–25]. The microemulsion systems were formed using different surfactants such as Lec and AOT. With both surfactants, the swollen inverse micelles were obtained containing aqueous monomer solution, cross-linker, and accelerator dispersed in continuous organic phase, cyclohexane, and isooctane respectively. Upon polymerization with the addition of initiator or irradiation, micro- or nanogel materials were obtained. Scheme 1 summarizes both bulk (a) and nanomaterial (b) synthesis. Although the conversions were almost complete for bulk hydrogels, the microgels or nanogels have lower polymer recoveries as the multiple washing and centrifugation steps to remove the surfactant lead to loss of the polymer.

Bulk hydrogel swelling characteristics

For bulk hydrogel characterization, the most common methods, swelling and sensitivity experiments, were carried out. The swelling experiments of 1 % X cationic bulk hydrogel of APTMACl were performed in distilled water at room temperature, and water diffusion coefficient was calculated. The certain amount of dried gel placed in distilled water and the water intake of initially dry hydrogel was followed in terms of weight increase with time. The hydrogel was removed periodically from the swelling medium and after blot drying and reweighing, was replaced back into the same media. The percentage swelling value (%S) is calculated applying the following equation;

$$\%S = \frac{w_t - w_0}{w_0} \times 100 \quad (1)$$

Where w_0 and w_t are the weights of the hydrogels initially and at time t. Figure 1a shows the %S values of 1 % X APTMACl hydrogel with time. As can be seen from the figure, the percentage of the equilibrium swelling value is about 2,400 and was reached in about 6 h. As soon as the glassy hydrogel is in contact with water, the hydrogel starts to uptake water and begins to swell. Diffusion involves transport of water into preexisting or dynamically formed spaces between hydrogel chains. Swelling of a hydrogel requires a larger scale of segmental motion, resulting in an increase in the distance of separation among the hydrogel chains. Due to the importance of cross-linked swellable hydrogel in many fields such as biomedical, pharmaceutical [26–28], diffusion of water into or out of the hydrogel

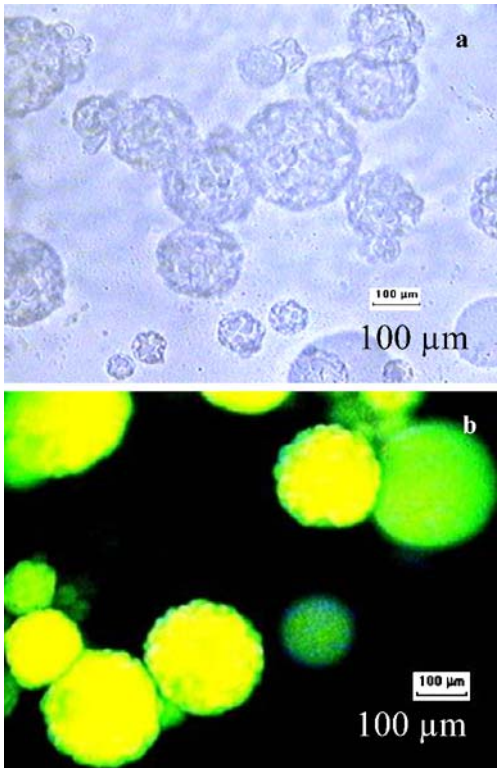


Fig. 4 The microscopy images of 0.5 % X AAm film containing 2 % X cationic microgel (hydrogel–hydrogel *semi*-IPN) under white light **a**, and **b** fluorescence microscopy image after absorption of fluorescein dye(FSS)

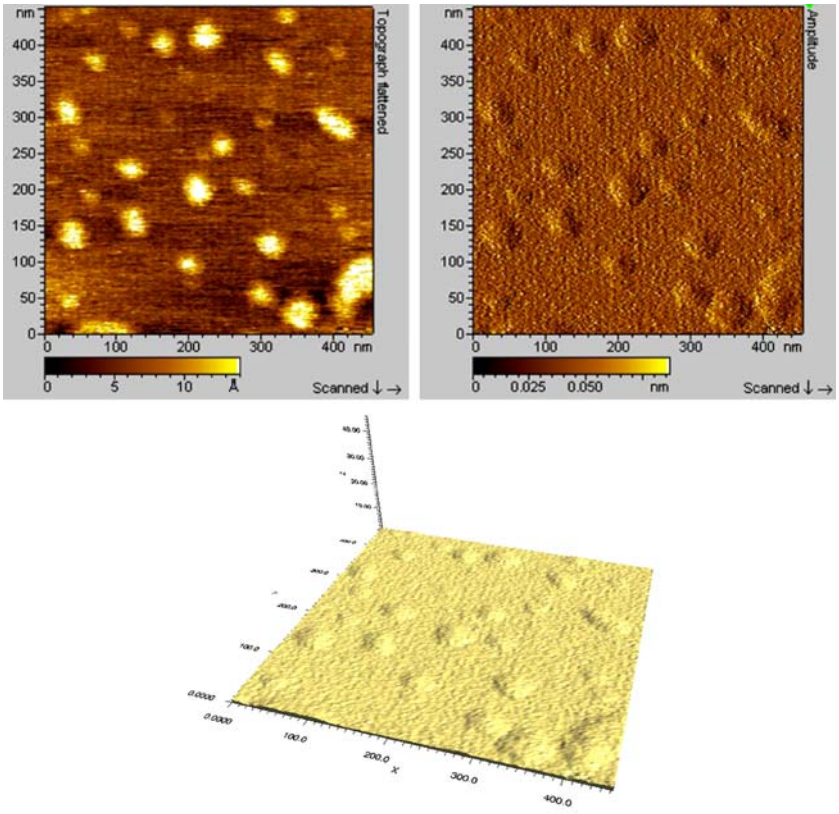


Fig. 5 *AFM* images of 1 % X cationic hydrogel obtained with tapping mode

is important, and the equation below is used for the calculation of the diffusion parameters [29].

$$F = \frac{M_t}{M_\infty} = kt^n \tag{2}$$

Here, F is the fractional water uptake, M_t and M_∞ is the amount of water absorbed at time t and at infinity [(at equilibrium) (time in sec)], respectively, and k is a constant incorporating the characteristic of the macromolecular network and the diffusing species. The n is the diffusion exponent that elucidates the transport mechanism. Eq. 2 is applied only to the initial 60 % of the normalized solvent absorption of the swelling curve. To determine the diffusion coefficient, the plot of ln F vs ln t is constructed and shown in Fig. 1b. The two limit values for n are 0.5 and 1, which corresponds to Fickian (case I) and non-Fickian (case II) type of diffusions, respectively. From the slope of Fig. 1b, it was found that the value of n is between 0.5 and 1 (n=0.58), indicating that the water diffusion into p(APTMACl) hydrogel is non-Fickian anomalous or case II in character implying that the relaxation rate to polymer chains is slow.

To investigate the pH sensitivity of the different % X cationic APTMACl hydrogel, a known amount of dried bulk hydrogels were immersed into 100 ml of different pH buffer solutions (Hydrion Chemvelope Assorted Buffers—Aldrich Chemical Company) at room temperature, and their percent swelling values at equilibrium (%S) were calculated after 2 days. The constructed %S vs pH graph is shown in Fig. 2a, which is characteristic of the behavior of cationic hydrogels [17]. In the figure, the most noticeable feature is the sharp change in the %S values between pH 2 and 5, which corresponds to a transition from charged to uncharged state on the network. Additionally, Fig. 2a shows that the pH sensitivity diminishes with increase in the cross-linker levels. This is a consequence of the increased cross-linking density that limits the hydrogel from swelling. The feature is also noticeable in Fig. 2b, which depicts an alternating swelling behavior of the two extreme pH values. Known amounts of different % X hydrogels were placed in a 100-ml buffer solution of pH values 2 and 11 and alternated daily by blot drying before placing the sequential displacement.

Hydrogels synthesized in surfactant systems

Our initial experiments were done using lecithin, a fully biocompatible zwitterionic phospholipid. At very low water contents (w_0 values of 5 and lower), lecithin forms inverse microemulsions in hydrocarbon solvents such as cyclohexane. As the water content is increased to around 10, the viscosity of the solution dramatically increases as a

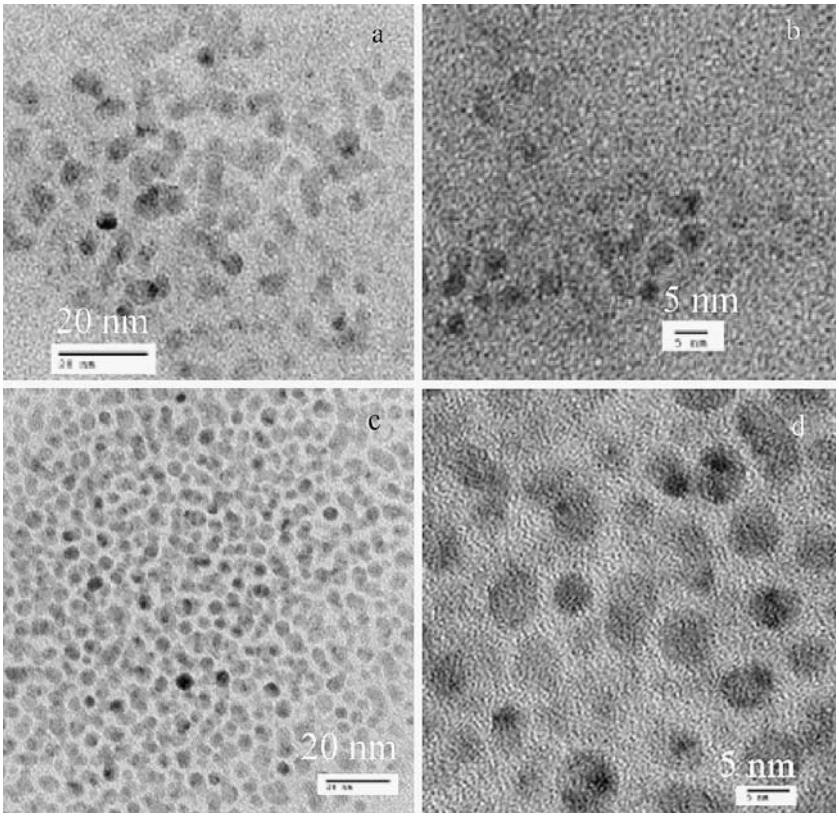


Fig. 6 TEM images of 2 % X p(APTMACI) nanohydrogels **a** *scale bar*: 20 nm, **b** 5 nm, and copolymeric APTMACl:AAm nanohydrogels (1:1 mole ratio) **c** *scale bars* 20 nm **d** 5 nm, respectively

consequence of the wormlike water channels in solution leading to entanglements and the formation of a gel phase. For microhydrogel synthesis, this zwitterionic phospholipid surfactant which commonly occur in biological membranes [30], was used. The SEM images in Fig. 3a,b depict the freeze-dried 2 % X p(APTMACl) cationic hydrogels, obtained in lecithin system before and after the removal of surfactant, respectively. Although lecithin is phosphatidylcholine, it gives the broad particle size distributions when used for material synthesis as can be seen from SEM images in Fig. 3a,b. However, lecithin makes it possible to obtain the phospholipid-coated nanohydrogel by partially removing the lecithin with the appropriate washing procedure as shown in Fig. 3c,d, which refers to these types of phospolipid-coated nanohydrogels. TEM images were obtained by moderately removing the surfactant and filtering the sample with 0.2-μm syringe filter. In Fig. 3c, the haze around the nanoparticles ascribed to the surfactant coating, lecithin, and the close-up images in Fig. 3d demonstrates that the nanohydogels are made up of smaller aggregates of hydrogel particles with phospholipid coatings. These types of phospholipid-coated cationic nanoparticles are very useful due to their bio-compartmental structures, which are able to load a variety of molecules. The cationic core act as an ion exchange matrix, carrying ionic compounds while the external layer can adsorb amphiphilic or hydrophobic compounds providing a cell-like membrane and insert membrane proteins [23, 31]. These kinds of coated material has a wide range of application from cosmetic to drug carries or for gene therapy purposes as they facilitate cell internalization and provide advantageous over noncoated systems [32, 33]. Fenart et al. has shown a three- or fourfold increase in the crossing of the blood brain barrier (BBB) of lipid-coated, ionically-charged, 60-nm particles in comparison with uncoated particles [34].

We also prepared microgel- and nanogel-containing polymeric hydrogel–hydrogel semi-IPN in which a functional (charged) micro/nanogel was dispersed in nonfunctional (neutral) (AAm or HEMA) or a charged (anionic or cationic) matrix before polymerization. Scheme 2 is the schematic representation of the hydrogel–hydrogel semi-IPN network synthesis. We call this semi-IPN because the micro/nanogel is synthesized initially, and the second hydrogel matrix is formed in the presence of the first network. The microscopy images of the hydrogel–hydrogel semi-IPN is shown in Fig. 4a under white light and Fig. 4b after FSS dye absorption. As can be seen from Fig. 4b, only the cationic microgel is imaged, as the matrix AAm or HEMA is neutral in character and does not absorb the FSS dye, while the oppositely charged microgel is strongly binding through electrostatic interactions. The cationic nanogel containing p(AAm) or p(HEMA) hydrogel–hydrogel semi-IPN was also prepared as composites materials. However, a clear image could not be obtained via

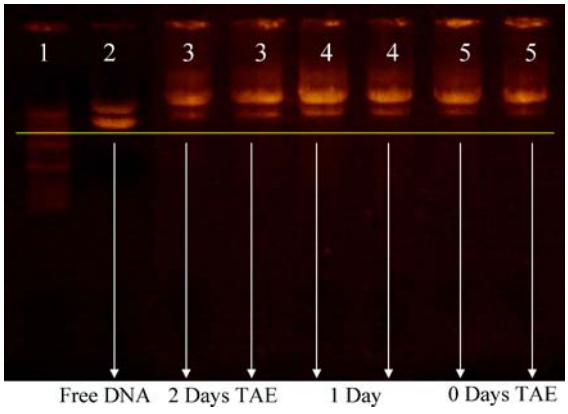


Fig. 7 Gel electrophoresis analysis of complexes formed by DNA–cationic nanohydrogel with 1:100 charge ratios

fluorescence microscopy because the resolution of fluorescence microscopy is well above the size of the cationic nanogels. However, after FSS dye absorption of a relatively high amount of nanogel loaded p(AAm)-IPN, the matrix has shown the scatter of fluorescence light images throughout the matrix implying the nanogel presence (image is not shown). As a result, it is probable to impart a functionality to a nonfunctional matrix, as the microgels are pH sensitive, whereas, the matrix material is neutral. These types of hydrogel semi-IPN materials have a potential in prolonged and sustained release of active agents [11]. For example, in ophthalmologic applications [35], instead of dispersing drug particles inside contact lenses, drug loaded nanogel can be incorporated into contact lenses that can provide continuous and extended delivery of active agents.

Furthermore, we have directly prepared a completely charged nanohydrogel by means of water-in-oil microemulsion utilizing another surfactant such as AOT. The nanogel precursors were dissolved in inverse micelles of AOT in isooctane continuous phase, and upon addition of a redox initiator (APS) in the presence of an accelerator, polymerization was done at room temperature. Figure 5 illustrates the AFM images of 1 % X-ed cationic nanohydrogels obtained with tapping mode. As can be seen from Fig. 5, the sizes of the particles are about 20–50 nm that are bigger than expected. Due to the smaller amount of X (1 %), the swollen hydrogels spread out on the mica surface, and it is also known that there is an interaction between the charged nanogel and the tip of the AFM. This is in accordance with the result of McAllister et al., in which the hydrogels they prepared have relatively low amounts of cationic charge in a copolymeric structure [36]. As the nanogels were loosely cross-linked (1 %), they posses a flattened spherical morphology when dried onto a charged surface (mica). To further examine the particle size, TEM images of nanogels were taken and illustrated in Fig. 6a,b which present the 2 % X nanohydrogel taken from the reaction medium and diluted with isoctane and placed on a formwar-coated cupper grid. As these particles are ultrasmall and highly charged, it is very difficult to obtained clear images. So, to render rigidity to the structure, the copolymer of APTMACl with AAm was synthesized with equal mole ratios and corresponding TEM images are shown in Fig. 6c,d. AAm was chosen as a comonomer because it is neutral and has the same chemical repeating units; therefore, the reactivity ratios of the monomers were assumed to be not very different, and additionally, the presence of a neutral comonomer can provide the ability of distribution of a positive charge along the network by just adjusting the stoichiometry. As can be seen from Fig. 6, the nanogel structure is more rigid and homogenous in size (Fig. 6c,d). It is also noteworthy to mention that to the best of our knowledge, these nanohydrogel particles are the smallest hydrogel particle imaged in the literature considering the highly charged nature and relatively low X extent.

To investigate whether these nanohydrogels can interact with DNA (for possible future gene delivery opportunity), a gel retardation assay was performed. After freeze-drying the nanohydrogel–DNA complex in 100 to 1 charge ratio, the complexes were resuspended in TAE buffer solutions at different times and analyzed by electrophoresis on 0.6 % (w:v) agarose gel, and the corresponding image is depicted in Fig. 7. From the figure, number (#) 1 denotes the MW ladder while # 2 is showing the free DNA; all the other sequential numbers were assigned as duplicates for the days that polycation–DNA complexes were placed in TAE solution before running the electrophoresis. From the agarose gel analysis, it can be said that these nanogels form strong complexes with DNA because the DNA band wave shifted as an indication of the interaction of APTMACl nanoparticles with DNA. The light color in the wells is due to the nanoparticle–DNA complex. Besides, the smearing is also a further evidence of DNA binding. The result obtained here is consistent with the results of Giammona et al. for polyaminoacidic copolymers as nonviral gene vectors [16]. Hence, these nanogel particles pose a great potential for gene therapy applications.

Conclusions

With this investigation, it was shown that cationic hydrogel can be prepared in a variety of sizes utilizing inverse microemulsion polymerization technique. Due to a very small size and a highly charged nature of the prepared micro/nanohydrogel, it can be assumed that their response time is much faster in comparison with the bulk counterparts to external stimuli such as pH, ionic strength, or a solute molecule. These materials, including semi-IPN hydrogel–hydrogel composites, have potential applications in many fields as carriers for sustained and uniform drug delivery devices, gene therapy, and biosensor applications. Additionally, it was also shown that a nonfunctional

material can be made functional, i.e., pH sensitive by inserting a micro/nanohydrogel to a neutral matrix. This could have compelling applications in the field of ophthalmology. For instance, nanogel-containing contact lenses (semi-IPN) can be loaded with different drugs to be used for treatment of the eye while not hindering the vision, or contact lenses can be further functionalized for absorption/adsorption of some fluid species or afford more porosity for transport features. The nanogels of different sizes and with phospholipid coatings are also very useful as devices for therapeutic purposes to facilitate cell internalization, and it can be shown that these particles can be attached to DNA. As a result, hydrogels of nanosized materials have many and viable applications in biotechnology and biomedicine.

Acknowledgements The authors would like to thank Tulane Institute for Macromolecular Engineering and Science (TIMES) and Coordinated Instrumentation Facility (CIF) at Tulane University.

References

1. Janusz MR, Yoshii F (1999) Nucl Instrum Methods Phys Res B 151:56
2. Nicholas AP, William L (2004) J Biomater Sci Polym Ed 15:125
3. Karlsson OJ, Gatenholm P (1997) Polymer 38:4727
4. Jing Z, Nicholas AP (2000) Macromolecules 33:102
5. Yoshihito O, Jian-ping G (1998) Adv Mater 10:827
6. Jun C, Haesun P, Kinam P (1999) J Biomed Mater Res 44:53
7. Zhao B, Moore SJ (2001) Langmuir 17:4758
8. Karlsson JO, Andersson M, Berntsson P, Chihani T, Gatenholm P (1998) Polymer 39:3589
9. Fumiyoshi I, Naoki M, Takeshi K, Sachio N, Kimio K, Mitsuhiro S (2003) Langmuir 19:2568
10. Zrínyi M (2000) Colloid Polym Sci 278:98
11. Marieta N, Zhu XX, Wilms B, Doina C, Adrian C (2001) J Pharm Sci 90:681
12. Vladimir GS, Olga AN, Olga AP, Anatoly AZ, Valentina BR, Alexander BZ, Kenichi Y, Victor AK (2002) J Am Chem Soc 124:11324
13. Rosso AB, Barbarisi M, Petillo O, Margarucci S, Calarco A, Peluso G (2003) Mater Sci Eng C 23:371
14. Stefaan CDS, Joseph D, Wim EH (2000) Pharm Res 17:113
15. Jaroslav B, Ignác C (2000) Macromolecules 33:5353
16. Giammona G, Cavallaro G, Pitaressi G, Pedone E (2000) Colloid Polym Sci 278:69
17. Sergey K, Marian K, Iwao T, Kalle L (2002) Macromolecules 35:1911
18. Norton LV, Tengell E, Toporek SS, Reichert WM (2005) Biomaterials 26:3285
19. Jason EM, Matthew JS, Scott MG, Benjamin MR, David MJ, Romy K, Ramakrishnan K, Kalpana M, Benjamin H, Zachary LH, Andrew DE, Michal VP, Grant CW (2004) Chem Mater 16:5574
20. Bouillot P, Vincet B (2000) Colloid Polym Sci 278:74
21. Isamu K, Atsushi S, Hideo N (2004) J Colloid Interface Sci 175:450
22. Andreas R, David H (1999) Polymer 40:3545
23. Essler F, Candau F (2001) Colloid Polym Sci 279:405
24. Braun O, Selb J, Canadu F (2001) Polymer 42:8499
25. Sanjeeb KS, Tapas KD, Ghosh PK, Amarnath M (1998) J Colloid Interface Sci 206:361
26. Hennik WE, Nostrun VCF (2002) Adv Drug Deliv Rev 54:13
27. Soppimath KS, Aminabhavi TM, Dave AM, Kumbar SG, Rudzinski WE (2002) Drug Devel Ind Pharm 28:957
28. Daoji G, Andrew LL (2001) J Am Chem Soc 123:7511
29. Saraydin D, Karadag E, Guven O (1995) Polym Adv Technol 6:719
30. Donald V, Judith GV, Charlotte WP (1999) Fundamentals of biochemistry. Wiley, Ch 9:219–238
31. Ignacio de M, Laurent I, Valerie R, Michel M, Roger K, Didier B (2000) Pharma Res 17:817
32. Maria JV, Rutger WHMS, Willem JMM, Arno SD, Bart AJJ, Ben de K, Anton IPMde K (2004) Biochim Biophys Acta 1663:135
33. Koert NJB, Rutger WHM, Hanke CDV, Maria JV, Paul HB, Peter MF, Ben DK (2002) Nat Med 8:81
34. Fenart L, Casanova A, Dehouck B, Duhem C, Slupek S, Cecchelli R, Betbeder D (1999) J Pharmacol Exp Ther 291:1017
35. ElJarrat-Binstock E, Raiskup F, Frucht-Pery J, Domb AJ (2004) J Biomater Sci Polym Ed 15:397
36. McAllister K, Peter S, Mirielle A, Moo JC, Michael R, Richard JS, Joseph MD (2002) J Am Chem Soc 124:15198