A Facile Method for the Preparation of Poly(4-vinylpyridine) Nanoparticles and their Characterization

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Submicron size particles were synthesized from 4-vinylpyridine (4-VP) using an oil-in-water emulsion system. Divinyl benzene (DVB) and ethylene glycol dimethacrylate (EGDMA) were utilized as crosslinkers for the synthesis of p(4-VP) hydrogel nanoparticles in sodium dodecyl sulfate (SDS) surfactant systems. Characterization of the particles was made by transmission electron microscopy (TEM) and dynamic light scattering (DLS) methods. To render a positive charge, p(4-VP) particles were reacted with 1-bromo butane (1-BB).

Key Words: Microgel, nanogel, nanoparticles, nanotechnology, core-shell structure, biomaterials, bactericidal, antibiotic polymers.

Introduction

Due to their appealing characteristics, hydrogels have been studied for several decades. The ability of hydrogel materials to respond to the environment with such characteristics as hydrophilicity (aqueous inner environment), a porous network structure, compatibility with biological systems, and facility for conjugations of active agents (i.e., drugs and biomolecules), has made them indispensable in biomedical applications as targeted drug delivery vehicles and as matrix materials, e.g. scaffolds for tissue regeneration, and as templates for composite material synthesis.[1−3] Charged hydrogels attract special attention due to their polyelectrolyte characteristics in relation to ionizable functional groups. These hydrogels can impart network responsive properties, such as pH responsiveness and the ability to bind oppositely charged species to form complexes. Most charged hydrogels used for metal ion removal have or are able to develop negative charges to form complexes with positively charged

metal ions.[4−6] Nevertheless, there are relatively few investigations regarding positively charged polymers and their hydrogels. It is known that positively charged structures are useful for gene delivery purposes.[7−9]

The preparation of monodisperse polymer particles in the submicron range has been accomplished only from relatively hydrophobic monomers (solubility less than 2 wt% in water, i.e. styrene and methylmethacrylate). On the other hand, the preparation of submicron-size uniform particles from hydrophilic monomers via dispersion polymerization has been very difficult.[10] Although emulsion polymerization has been widely used for polymer particles or latex syntheses from hydrophobic monomers, there are relatively few studies on hydrogel particle syntheses, which generally requires reverse emulsion polymerization.[11−13]

Considering such novel chemical properties as acidity, basicity, and hydrophilic-hydrophilic balance, 4-VP is of great importance.[14] The poly- or copolymers of 4-VP are intriguing materials with many applications, such as in sensors and actuators, host/ligand of metal-containing chromophores, antimicrobial materials, and so on.[15−19]

Herein a facile method for the synthesis of p(4-VP) submicron hydrogel particles using emulsion polymerization is reported, and it is shown that the positively charged form of p(4-VP) can be readily prepared via the quaternization reaction.

Experimental Section

Materials

The monomer 4-vinyl pyridine (4-VP) (95%) and the crosslinkers, divinyl benzene (DVB) (80%) and ethylene glycol dimethacrylate (EGDMA) (98%), as well as the initiator ammonium persulfate (APS) and a quaternization agent, 1-bromo butane (1-BB), were obtained from Sigma-Aldrich Chem. Co. (Milwaukee, Wisconsin) and used as received. The surfactant, sodium dodecyl sulfate (SDS), was purchased from Bio-Rad. The distilled water was 18.3 Mohm.cm and was obtained with a NANOpure Diamond system (Barnstead) for SDS solution preparation, and was used throughout the experiments. Acetone (HPLC grade) and ethyl alcohol (absolute, 200 proof) were purchased from Fisher Scientific.

Synthesis of p(4-VP) Particles

Different quantities of crosslinkers containing p(4-VP) particles were prepared using an SDS surfactant system. For particle preparation DVB and EGDMA were used as crosslinking (X) agents. In a typical experiment, 0.4 mL of 4-VP was dispersed in 15 mL (0.1 M) of SDS aqueous solution. To this solution, different types (DVB or EGMA) and quantities (3%-5% based on monomer mole ratio) of crosslinkers were added. The mixture was vortexed until a clear solution was obtained. The simultaneous polymerization and crosslinking reaction was started by the addition of 1 mL of 1% APS aqueous solution, based on the quantity of 4-VP. The reaction proceeded under constant mixing (750 rpm) at 70 °C for 6 h. The particles were then washed by adding a large quantity of acetone and centrifugation at 4000 rpm for 10 min. Following centrifugation, the obtained yellow precipitate was dissolved in ethanol and a large quantity of acetone was added; following another centrifugation, a white suspension containing p(4-VP) particles was collected and used for characterization.

Derivatization of p(4-VP) Particles

p(4-VP) particles were reacted with 1-BB in an ethanol-acetone mixture (50:50) to quaternize the particles at ambient temperature. Briefly, the suspension of p(4-VP) in acetone, which was obtained after the final precipitation procedure described in the previous section, was mixed with an equal amount of ethyl alcohol and then with an excess amount of 1-BB (at least 4 times), based on the 4-VP repeating unit under continuous mixing (750 rpm) at room temperature for 24 h. Then, the reaction mixture was repeatedly washed with acetone, assuming that all excess 1-BB was eventually removed from the p(4-VP) particles.

Particle Characterization

The sizes of the synthesized p(4-VP) particles were measured via transmission electron microscopy (TEM) and dynamic light scattering (DLS) techniques. After synthesis and the removal of surfactant, the particles were suspended in ethyl alcohol and a drop of this suspension was placed on a formwar-coated 200 mesh cupper TEM grid. TEM images were obtained using a JEOL 2010 TEM under vacuum, with an operational voltage of 200 keV.

DLS studies were performed with a 90 Plus particle size analyzer (Brookhaven Instrument Corp.) using a diluted solution of p(4-VP) particles in water and acetone. The DLS experiment was conducted with a detector angle of 90°, with a Lexel 95 ion laser operating at a wavelength of 614 nm and 100 mW as the light source.

Zeta potential measurements were performed using a ZetaPals Zeta Potential Analyzer BIC (Brookhaven Inst. Corp.) A diluted aqueous solution of p(4-VP) particles was measured before and after the quaternization reaction ($\sim$1.5 mg/ml).

FT-IR spectra of p(4-VP) particles were recorded using a Perkin-Elmer FT-IR System Spectrum GX. A drop of p(4-VP) particles in acetone was placed on a CaF_2 window. After drying with a heat gun, the obtained thin film of particles was recorded against the background of the CaF_2 window, and same procedure was repeated for the quaternized p(4-VP) particles.

Result and Discussion

The reaction scheme shown in Figure 1 represents the p(4-VP) submicron particle formation in an SDS emulsion system. Due to the unique properties of p(4-VP) interest in 4-VP-based polymers and particles has been increasing;[19,20] however; the synthesis of homo-p(4-VP) particles has always been a challenging task, requiring a high boiling point, toxic solvents, and various emulsifiers for suspension polymerization.[12,13] In the present study a facile, one-step synthesis method for the preparation of p(4-VP) particles is described. Although the yield was > 50% (determined gravimetrically), the ease and simplicity of the synthesis method offers an alternative for the preparation of p(4-VP)-based nanoparticles and their further functionalization. Additionally, the use of high purity reagent for p(4-VP) particle synthesis may increase overall conversion. Figure 2 shows TEM images of the p(4-VP) particles obtained using 3% DVB (2a and b), and 4% EGDMA as crosslinkers (2c and d), respectively. Both crosslinkers gave particles having more or less the same morphology. Although the hydrophilicity of the crosslinkers were different, both crosslinkers were relatively hydrophobic; thus, the monomers inside the SDS micelles crosslinked more readily than the monomers outside of the SDS micelles

(in the aqueous environment). As depicted in Figure 1, 4-VP monomers partitioned in the oil phase of the micelles and in the aqueous phase because of their amphiphilic character; however; it can be presumed that the concentration of 4-VP was higher in and at the periphery of the SDS micelles than in the continuous aqueous phase. At elevated temperatures, i.e. the reaction temperature (70 °C), the oil phase (SDS micelles) became even richer in 4-VP and X concentrations than the continuous phase (water phase); therefore, it can be assumed that the polymerization and crosslinking reaction inside the micelle can occur more rapidly upon collision with initiator (APS) molecules than it can outside of the micelle, which most probably forms linear and/or slightly crosslinked 4-VP polymer chains. Nonetheless, when these linear or slightly crosslinked/branched polymers run into micelles containing a growing crosslinked network of p(4-VP) during continuous mixing, they can produce a core-shell-like architecture. Additionally, polymerization and the simultaneous crosslinking reaction inside the micelles can be faster than on the outside because of the relatively higher abundance of monomers and crosslinkers. As can be seen from the TEM images, both particles, whether DVB (Figure 2a and b) or EGDMA crosslinked (Figure 2c and d), have a core-shell-like morphology. Although the core-shell-like architecture was more pronounced in the EGDMA-crosslinked p(4-VP) particles; this could have been due to the relatively greater hydrophilic character of EGDMA in comparison to DVB, and the artifacts of both particles. The irregularities on and around the particles can also be attributed to the presence of impurities and/or the existence of linear and/or sparsely crosslinked p(4-VP) structures.
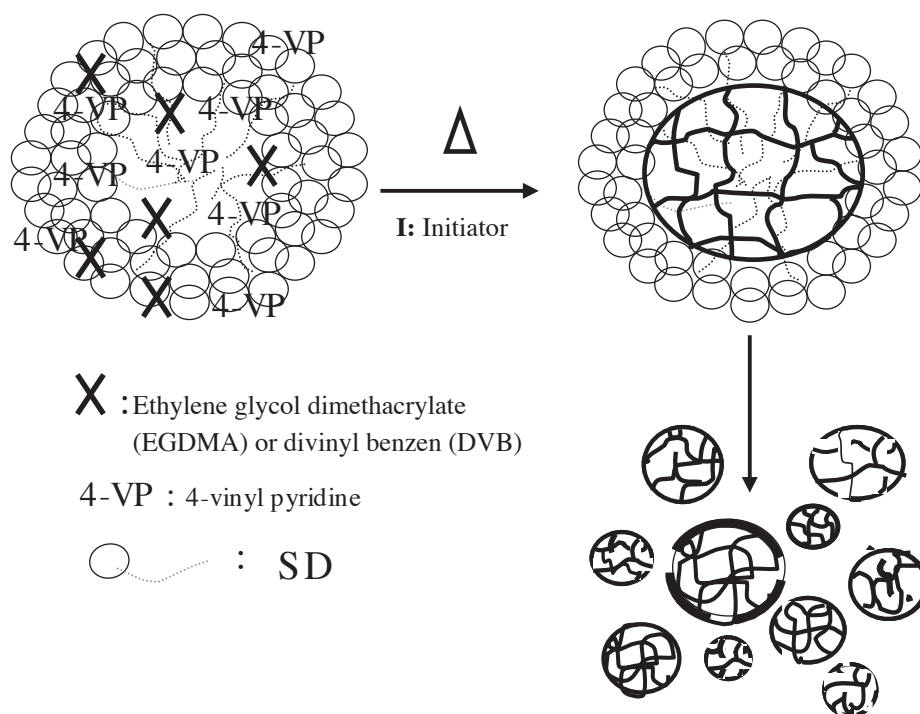
Figure 1. Reaction scheme for the simultaneous polymerization and crosslinking of 4-VP in SDS micelles.

For further particle size characterization DLS measurements were performed in 2 solvents; water and acetone. These solvents were chosen as DLS measurement media because acetone is a good solvent for p(4-VP), whereas; water is not. Water mixtures with alcohols were reported as solvents for p(4-VP).[21] Figure

3a shows the results of DLS measurements on the analysis, in terms of the cumulants of the autocorrelation function and lognormal particle size distribution based on the Stokes-Einstein relationship. In this technique the hydrodynamic diameter of a particle is determined based on the relationship between the time-dependent fluctuations in the intensity of the scattered light and the rate of diffusion of the particles in a solvent.[22] The integration time for each sample was at least 15 s. Figure 3a shows that the diameter of the particles in water was 256 nm, with relatively low polydispersity (0.059). On the other hand, measurements made in a good solvent, acetone, showed that average particle size was larger (292 nm) and polydispersity was almost doubled (0.112). This is conceivable, as water is a relatively poor solvent for p(4-VP) particles and they do not swell in water to the same degree as they do in acetone.
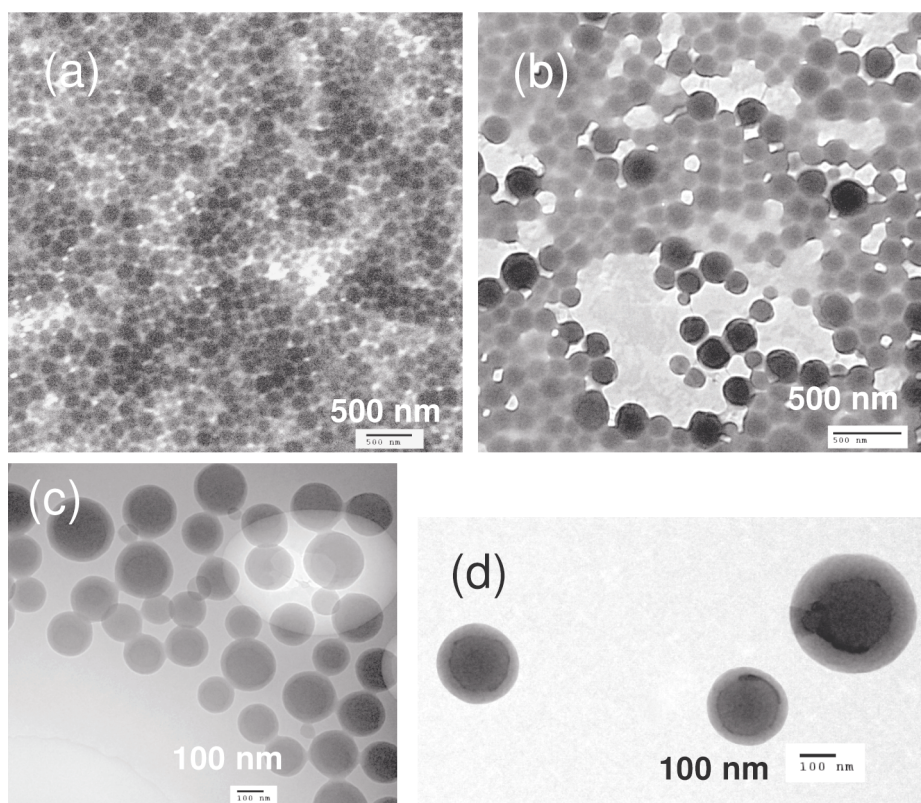

Figure 2. TEM images of 3% DVB-crosslinked (a and b), and 4% ethylene glycol dimethacrylate–crosslinked p(4-VP) (c and d).

One of the intriguing applications of p(4-VP) is the ability of its quaternized forms to kill bacteria.[14,23] To quaternize p(4-VP) particles, diluted particles were reacted with 1-bromobutane; the corresponding reaction scheme is shown in Figure 4. With the introduction of positive charge to the network, these types of particles can also be very useful in other biological and environmental applications.[6]

Figure 5 shows the FT-IR spectra of p(4-VP) (a) and functionalized p(4-VP) (b) particles. The most important distinction between these 2 spectra is the development of a band at 1640 cm^{-1} following the functionalization of p(4-VP) particles with 4-BB upon reaction with 1-BB. This is in agreement with reports

of the quaternization reaction by the introduction of a positive charge to form a pyridinyl ring in the polymer structure.[13,17] Additionally, the intensity of the band at about 1560 cm^{-1}, as shown in Figure 5, corresponds to positively charged nitrogen atoms.
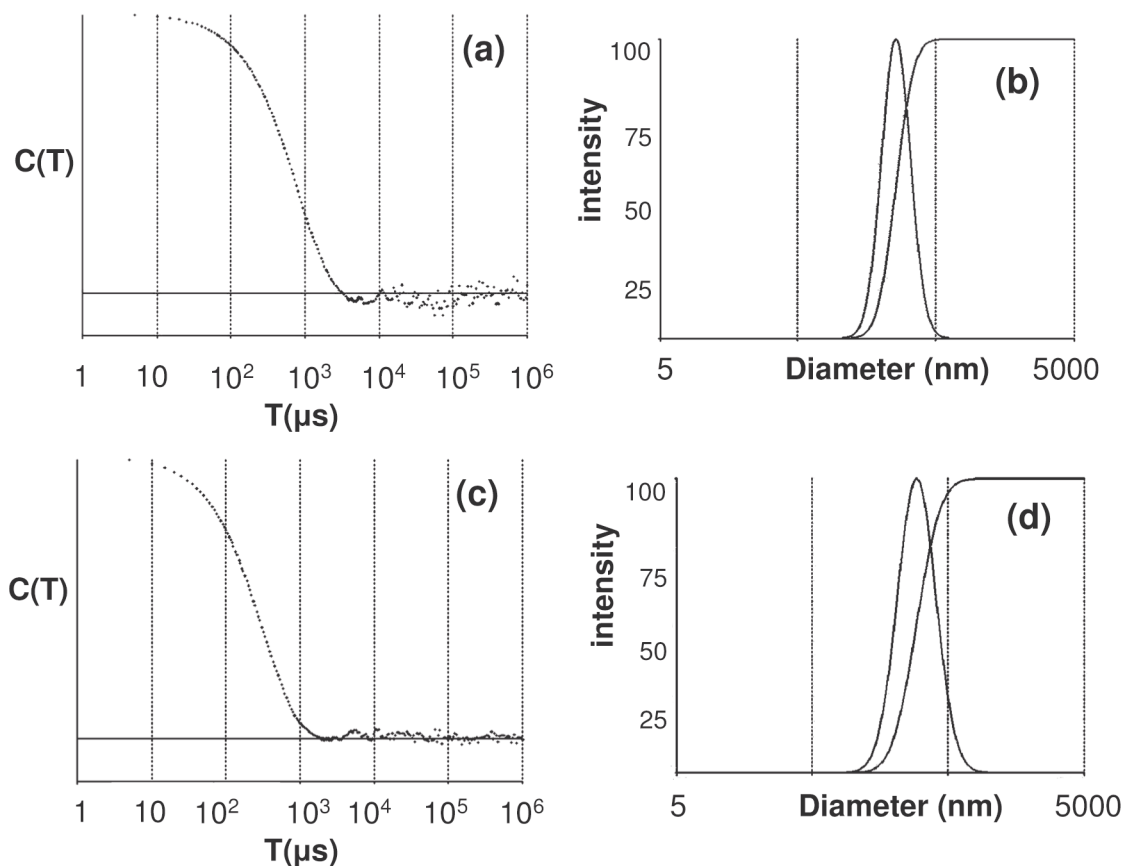
Figure 3. DLS measurement of p(4-VP) particles. (a) Correlation function decay with time and (b) lognormal particle size distribution in water (c) (mean particle diameter: 256 nm; polydispersity: 0.059). (c) Correlation function decay with time and (d) lognormal particle size distribution in acetone (mean particle diameter: 292 nm; polydispersity: 0.112).

Zeta potential measurements further confirm the positive charge formation on the p(4-VP) particles, as illustrated in Figure 6. As can be seen from Figure 6a, before the introduction of positive charges onto p(4-VP) particles they were almost neutral (slightly positive (2.58 ± 0.2 mV). These slight positive charges can be attributed to the basic character of 4-VP, which can abstract hydronium ions from water (the reaction is performed in an aqueous environment, and it is known that the slight dissolution of atmospheric carbon dioxide in water forms carbonic acids). After the reaction of p(4-VP) particles with 4-BB, the particles' charges increased significantly (83.80 ± 1.02), as shown in Figure 6b.

The positive charge can also be simply introduced by contacting p(4-VP) particles with acids, such as hydrochloric acid (neutralization reaction), to create positively charged particles that are very useful for the removal of metallic oxy-anions in aqueous environments.[24]
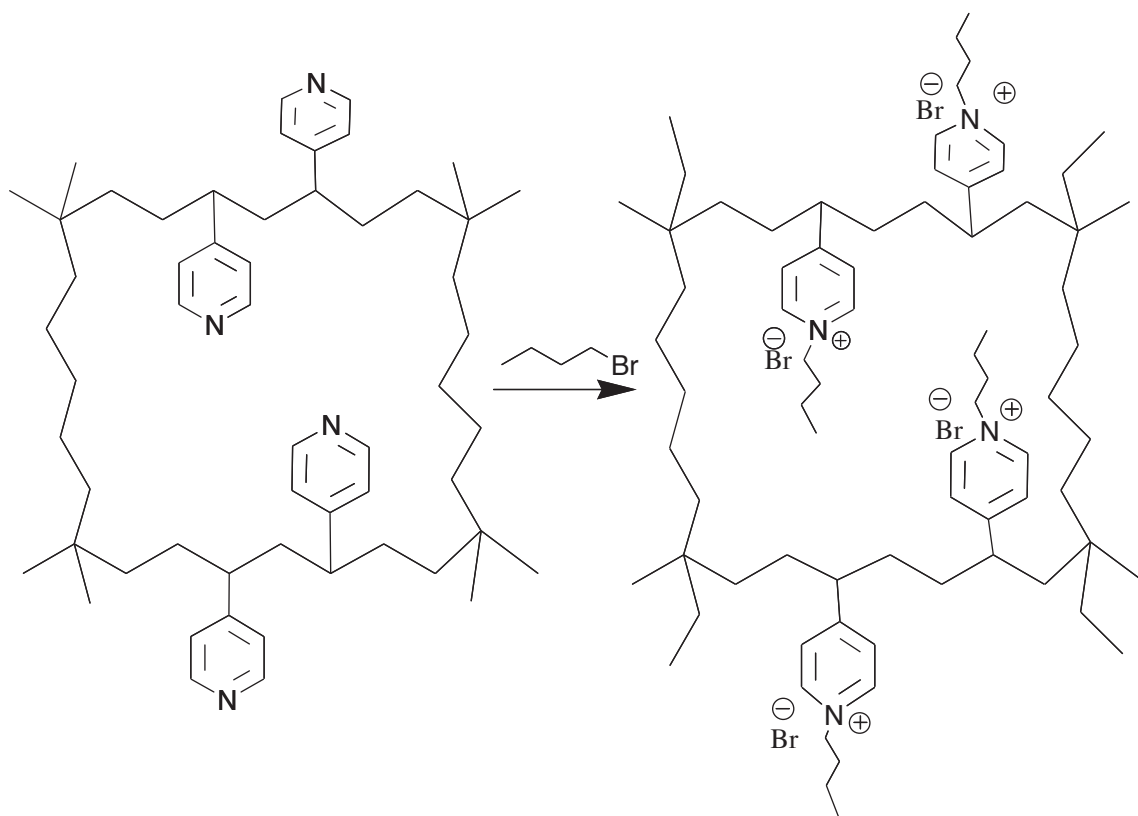
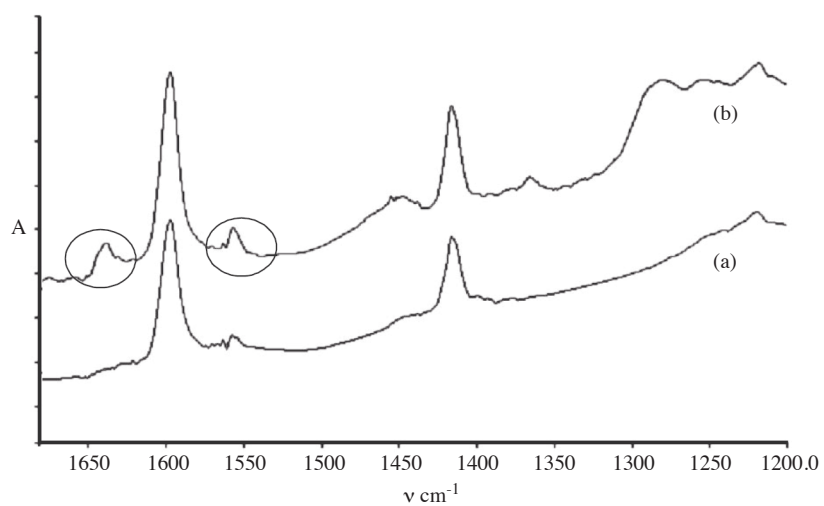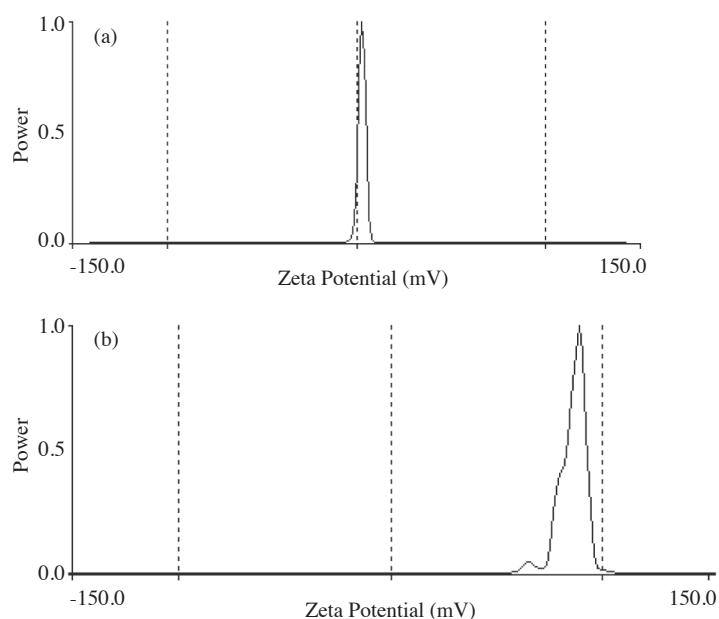**Figure 4.** Quaternization reaction mechanism of p(4-VP) nanonetwork with 1-bromobutane.

Figure 5. FT-IR spectra of (a) p(4-VP) and (b) quaternized p(4-VP) particles.

Figure 6. Zeta potentials for (a) 3% crosslinked p(4-VP) (2.58 ± 0.2 mV) and (b) after the quaternization reaction (83.80 ± 1.02).The graphs represent the average of 10 measurements.

Conclusion

This investigation demonstrated the facile, one-step synthesis of p(4-VP) nanoparticles. Furthermore, using this method it may be possible to prepare particles with different morphologies in the presence of different co-monomers with various crosslinkers/initiators. It was also shown that a positive charge can be introduced into the network by means of a simple quaternization reaction with alkyl halides. These kinds of functional materials have many potential biological and environmental applications. In fact, more detailed reaction kinetics, and precise control of particle size and shell thickness, as well as quaternization reactions with different alkyl chain lengths are currently under investigation for the synthesis of antimicrobial hydrogel nanoparticles for use in various applications.

Acknowledgements

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