Ionic Strength/Temperature-Induced Gelation of Aqueous Poly(N-Isopropylacrylamide-co-Vinylimidazole) Solution

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Summary: A high molecular weight copolymer of N-isopropylacrylamide (NiPAAm) and vinyl imidazole (VI) was synthesized and its phase transition behavior in aqueous solutions (5 wt%) by simultaneous changes of ionic strength and temperature was investigated. At low ionic strengths, the copolymer solution showed two phases (clear and opaque solutions), which were freely mobile, as increasing temperatures up to 65 °C due to repulsion interaction of positive charges developed by basic imidazole group on the polymer aggregates. However, at the physiological condition (I=0.15, T=37 °C), four distinctive phases (clear solution, opaque solution, gel and shrunken gel) were observed because of charge shielding effect by added salts. The gel state was stable and maintained from 32 °C to 55°C. In particular, the phase transition from opaque solution to gel rapidly occurred by the change in ionic strength (from ~ 0 to 0.15) at 37 °C. This characteristic can be utilized as a liquid embolic agent.

Introduction

Embolotherapy has become a routine part of malignant tumor surgery and treating arteriovenous malformations due to the pronounced therapeutic effectiveness of endovascular embolization.[1] A battery of synthetic and natural materials in various forms has been investigated as embolic agents during the past several years.[2] Among them, autologous materials, oxidized cellulose, polyvinyl alcohol (PVA), silicon and metallic spheres, liquid agents involving absolute ethanol and cyanoacrylate derivatives have been used as materials for practical embolotherapy. However, for transcatheter embolotherapy in clinical situations, these materials face various technical diffulties.[3-5] For example, in spite of well-established bio- and blood compatibility, autologous blood clot has now been abandoned because of unpredictable duration of occlusion. Oxidized cellulose also failed to produce a permanent occlusion. Although PVA is physiologically inert and causes minimal inflammatory responses, it is different to infect PVA via a small diameter catheter due to its high viscosity. As an alternative, liquid embolic agents composed of responsive-polymers that transform to a gel in response to changes in the

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external environmental conditions of temperature, pH and ionic strength, can be considered. Such agents may offer some advantages, over conventional agents, of easy injection (low viscosity in a sol or colloid state), rapid and permanent occlusion (stable gel formation), and the reduction of potential side effects arising from the use of organic solvents.

It has been reported that N-isopropylacrylamide based copolymers showed reversible sol-gel transition by increasing temperature around 32 °C.[6,7] When we tested one of these copolymers for the feasibility for an embolic material, it was found that the temperature-induced gelation blocked the catheter pathway during injection due to thermal equilibration before reaching to a target site. For a more effective embolic material, we synthesized a copolymer of N-isopropylacrylamide (NiPAAm) and vinyl imidazole (VI) (poly(NiPPAm-co-VI), feed mole ratio = 95:5). Vinyl imidazole (VI) is a weak basic monomer and its polymer has been used for a metal chelating agent and for stabilization of colloidal suspension.[8-10] In this paper, we report the gelation behavior of aqueous poly(NiPPAm-co-VI) solution is response to changes in temperature and ionic strength with the emphasis on the role of a weak basic imidazole group in the gelation process.

Synthesis of poly(NiPAAm-co-VI)

A high molecular weight copolymer of NiPAAm (95 mol%) with VI (5 mol%) was synthesized by free radical polymerization in benzene (10 wt% monomer concentration) with AIBN as an initiator (7×10^3 moles/moles of monomer) (Fig. 1). The polymerization was performed at 60°C for 16 h under dried nitrogen gas. The polymer became phase-separated in the solvent as polymerization proceeded. The precipitated product was dissolved in acetone/methanol mixture (90/10 v/v%) by warming. This polymer solution was precipitated in an excess amount of diethyl ether and then dried in vacuum for 3 days. The product was dialyzed using dialysis membrane (MW cut off; 15,000) against distilled water for at least 7 days to remove unreacted monomers and low molecular weight compounds. Finally, the polymer in a powder form was obtained by freeze-drying with additional drying under P_2O_5. The molecular weight of the polymer determined by light scattering was approximately 1.5×10^6 Dalton. In order to get the information of copolymer composition, non-aqueous titration using ASTM D 974; solvent (methanol, 30ml), pH indicator (phenophtalate), standard base (0.5 N KOH) and standard acid (0.01 N $C_8H_5O_4K$) was tried. The calculated result indicated

that the mole ratio of N-isopropylacrylamide to vinyl imidazole in the copolymer is 94.73 to 5.27.

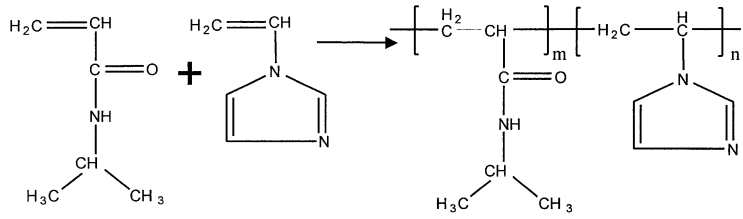

Figure 1. Synthesis scheme of poly (NiPAAm-co-VI) (m:n= 95:5 in mole ratio)

Effect of ionic strength on gelation of poly(NiPPAm-co-VI)

The phase transition behaviors of poly(NiPPAm-co-VI) in an aqueous solution (5 wt%) were investigated by monitoring the change in transmittance and by inversion test at various ionic strengths and temperatures. The temperature of the solutions was raised from 5 to 65 °C at a rate of 0.1 °C/min and the transmittance at 500 nm was measured by a Varian UV-VIS spectrometer (CARY IE). The cloud temperature was defined as the temperature at which the transmittance drops 50 % of the initial value at 5 °C. In the inversion test, the polymer solutions (5 wt%) at various ionic strength in glass vials were kept in a thermostated water bath and observed at 1 ^{o}C intervals. The gelation temperature was that at which the polymer solution did not flow by vial inverting. The shrinking temperature was also determined visually at the temperature when the polymer gel began to expel entrapped water and a vial was not fully filled with the polymer gel (syneresis).

Fig. 2 shows the resulting phase diagram as a function of ionic strength adjusted with NaCl. The clear solution (phase A) at low temperature became cloudy as temperature increased (defined as cloud temperature, phase B). The phases A and B were freely mobile. With further increase in the temperature, the opaque polymer solution subsequently became motionless (defined as the gelation temperature, phase C). Upon cooling, the gel turned mobile at the same temperature as the gelation temperature. The gel started to shrink by expelling water at the shrinking temperature (phase D). Once the gel was formed, it did not dissolve nor change its water content in the presence of an excess amount of water. In particular, the time required for gelation was apparently of the same order as the thermal induction time and the gel had a tendency to adhere to the wall of the glass vial. At an ionic strength close to zero, the solution did not form phases

C or D. Increasing the ionic strength produced the gel and shrunken gel phases. This result suggests that it requires a critical ionic strength to form a stable gel phase at the given polymer concentration (5 wt%).

In our previous report, aqueous poly(NiPAAm-co-acrylic acid) solutions above a critical polymer concentration showed a similar phase transition pattern as the increasing temperature. This polymer showed distributions in polymer composition as well as molecular weight, thus forming a mixture of polymer chains having different lower critical solution temperatures. The chain entanglement and the formation of physical associations of collapsed chains at 32 °C seemed to be responsible for the gelation[7]. In the case of poly(NiPAAm-co-VI) used in this study, the polymer solution at lower ionic strength did not develop physical domains of collapsed polymer chains to act as physical crosslinking junctions. Around the physiological ionic strength (I = 0.1-0.2), the stable gel was maintained in a wide temperature range. It is interesting to note that without further increasing in the ionic strength, the temperature range for the gel became narrower and the upper temperature for the gel region sharply decreased. In the phase B formed at low ionic strengths, the opaque polymer solution was able to flow through a small-diameter (1 mm) tube without noticeable flow resistance. The gelation occured immediately when the polymer solution came in contact with the body fluid where the ionic strength is kept at 0.15. This feature of the polymer solution offers high potential for use as an embolic agent.

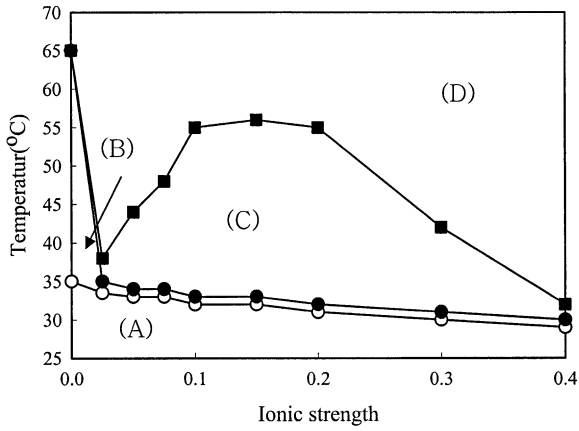

Figure 2. Phase diagram of poly (NiPAAm-co-VI) aqueous solutions (5 wt%) as a function of ionic strength by increasing pH. (A) clear solution; (B) opaque solution; (C) gel phase; and (D) shrunken gel

To partially examine the effect of ionic strength on the solution behavior, the temperature-dependent transmittance of the polymer solution having a low polymer concentration of 0.2 wt% at two different ionic strengths (~ 0 and 0.15) was measured and compared (Fig.3), because the light transmittance reflects the degree of the association or aggregation of the polymer chains. when the temperature was increased from 28 to 50 °C at I = ~ 0, only a slight decrease in transmittance was observed until the temperature reached 35 °C and from 35 °C, which coincided with the clouding temperature, it started to drop much sharply in a 10 °C range and reached slightly below 70 % at 50 °C. However, at I = 0.15, no measurable transmittance changes were observed below 32 °C; but at 32 °C, the transmittance of poly(NiPPAm-co-VI) solution drastically decreased from 100 % at 32 °C to almost 0 % transmitance at 33 °C. This change appeared in a few min at temperature equilibrium state. These results suggests that at I = ~ 0, the polymer could not form high aggregates most likely due to electrostatic repulsive forces between charged imidazole groups which prevent further aggregation of the polymer, while at I = 0.15, the destabilization of polymer dispersion became accelerated, thus forming high aggregates.

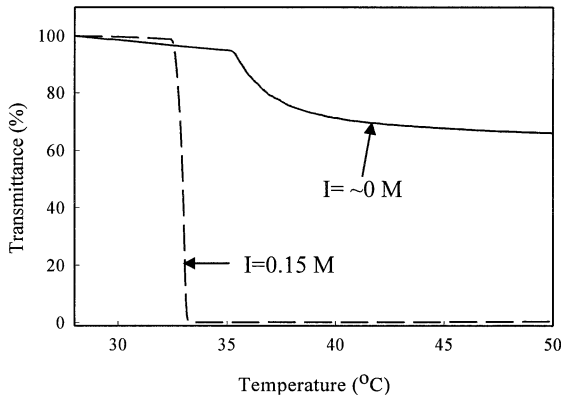

Figure 3. Temperature-dependent transmittance of poly(NiPAAm-co-VI) solutions (0.2 wt%) at different ionic strength (I = ~ 0 M and 0.15 M)

This result indicated that poly(N-isopropylacrylamide-co-vinyl imidazole) is more appropriate compared with poly(N-isopropylacrylamide-co-acrylic acid) for embolization in a deep site in the body by delivering the solution via a long capillary

catheter because the temperature-induced gelation of poly(N-isopropylacrylamide-co-acrylic acid) leads to clogging a capillary catheter in the body (37 °C) because the gelation occurs by temperature unless the solution is thermally insulated.

To confirm the charge effect on aggregation, zeta potential measurement was performed at the two ionic strengths. The influence of temperature on the zeta potential of the polymer solution at I = ~ 0 and 0.15 is presented in Fig. 4. The polymer dissolved in deionized water developed a weak positive surface charge (Fig. 4(a)), which was attributed to the ionization of basic imidazole groups. Above the clouding temperature, the surface positive charge was greatly enhanced, most probably due to reorientation and aggregation of polymer molecules above the lower critical solution temperature (LCST), resulting in the redistributing of charged imidazole groups on the surface of the collapsed polymer chains, while some of the polymers remained uncollapsed. This might have led to the two peaks in the zeta potential profile at 37 °C. This positive surface charge seems to be responsible for inhibiting further hydrophobic aggregation between collapsed polymer chains, leading to even lower viscosity. In the case of I = 0.15 solution (Fig. 4(b)), the polymer showed a strong negative surface charge because of adsorbed counter ions. It notes that at 27 °C, only one peak appeare at –270 mV in zeta potential profile, but as the temperature was increased up to 32 °C, a small new peak at -220 mV appeared, just below the LCST.

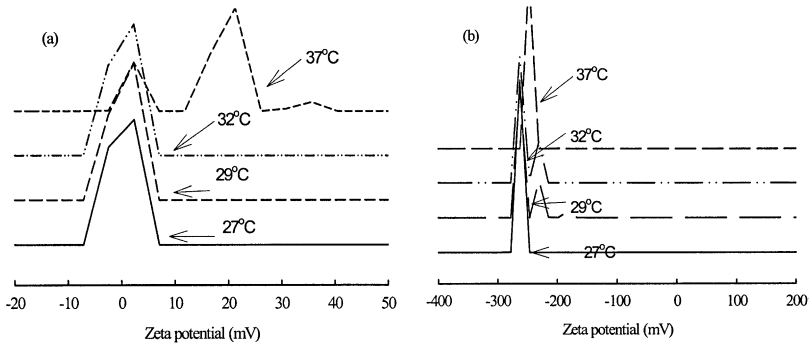

Figure 4. The changes of ζ-potential values as a function of temperature (0.2 wt%, I = ~ 0 (a) and 0.15 (b))

Above LCST, the two peaks combined to form a single peak at –240 mV. This observation is not clearly understood, but it may suggest some salt effects on this particular polymer, possibly charge shielding and specific interactions with imidazole

groups. The added salt may contribute its role in the gelation of polymer solution at higher concentration. Further elucidation is needed to understand the salt-temperature induced gelation process of the polymer solution.

Conclusion

A high molecular weight poly(N-isopropylacrylamide-co-vinylimidazole) formed a gel phase at physiological ionic strength and at a temperature range of 34-56 °C. The polymer dissolved in deionized water became opaque at 37 °C but freely flowed through small diameter tubing. When this opaque solution was injected into a phosphate buffered saline solution with an ionic strength of 0.15 adjusted with NaCl and 37 °C, immediate gelation was observed, forming a stable hydrogel. This unique property may offer a new liquid embolic material with injection convenience through a small diameter catheter and the least side effects. The role of ions in the gelation process is not clearly understood and need further investigation. The ions may participate in charge distribution on the collapsed polymer chains at high temperature and polymer chain reorientation possibly via charge shielding effect and/or specific interaction with imidazole rings.

Acknowledgements

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