Polymer Molecular Weight Alters Properties of pH-/Temperature-Sensitive Polymeric Beads

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Purpose. To study the physical and release properties of different molecular weight (MW) pH- and temperature-responsive statistical terpolymers and beads of N-isopropylacrylamide (NIPAAm), butylmethacrylate (BMA) and acrylic acid (AA).

Methods. Random terpolymers of varying MW were synthesized with NIPAAm/BMA/AA of feed mol ratio 85/5/10. Polymeric beads were formed by dropping a polymer solution into an oil bath kept at a temperature above the lower critical solution temperature (LCST) of the polymer. The release profile of cytochrome c was investigated as a function of the polymer MW and pH of the release medium at 37°C.

Results. The weight average MW ranged between 49,000 and 3 million. The LCST at pH 2.0 and pH 7.4 was 22°C and 60°C respectively. SEM studies showed that the size of the pores decreased as the MW increased. Irrespective of MW, the polymeric beads did not swell or dissolve at pH 2.0 and 37°C and showed minimal drug release. At pH 7.4 and 37°C, the rate of bead dissolution/swelling decreased as the MW of the polymer increased.

Conclusions. By modulating polymer MW, it was possible to vary the physical properties of beads. Dissolution/swelling characteristics were dependent on the MW of the polymer. Such unique dissolution/swelling properties are useful for delivering drugs to different sites in the intestine.

KEY WORDS: pH-/temperature-sensitive polymer; modulated drug delivery; poly(N-isopropylacrylamide); swelling; molecular weight; polymeric beads.

INTRODUCTION

Stimuli-sensitive polymers have been studied for their unique properties in response to changes in their environment. These polymers have the ability to change their structure and physical properties in response to physical or chemical stimuli. These "intelligent" polymers have been investigated for a variety of biomedical and engineering uses, including controlled drug delivery (1–3), molecular separation processes (4,5), enzyme activity controlling systems (6), cell encapsulation (7,8), tissue culture substrates (9), and for improving biocompatibility (10). Among intelligent polymers, temperature and pH responsive polymers are the most frequently studied. Temperature-sensitive polymers are characterized by their lower critical solution temperature (LCST). These polymers are soluble below their LCST and precipitate above their LCST. The LCST is also dependent on pH for thermosensitive polymers with ionizable groups in side chains (11). Moreover, the hydrophilic/hydrophobic balance of the polymer has a significant impact on the LCST (12).

Previously, we synthesized a series of low molecular weight (MW) (<50,000) pH-/temperature-sensitive terpolymers of N-isopropylacrylamide (NIPAAm), butylmethacrylate (BMA) and acrylic acid (AA) using NIPAAm/BMA/AA of feed mol ratio 90-x/10/x, by varying AA content (13). These polymers were used for the aqueous fabrication of pH/thermosensitive beads loaded with polypeptide drugs (13,14). Peptide/protein drug loading was achieved in an aqueous environment by exclusively utilizing the LCST properties of the polymer. For example, an aqueous solution containing the polymer and drug below the LCST is dropped in an environment above the LCST, whereby most of the drug is entrapped in the polymer matrix when the bead is formed. Below the LCST, the polymer chain is soluble and hydrated in the buffer, the hydrophilic interactions among the polymer, water and protein predominate. Once the polymer/protein solution is placed in an environment above the LCST, hydrophobic interactions between the polymer-polymer chains dominate. The polymer chains entangle the protein while phase separation takes place whereby, the drug is entrapped into the polymer matrix. However, in previous studies (13,14) we found that only fast release could be achieved with the low MW polymer. Also, the characteristics of the polymers and formed beads need to be thoroughly investigated, to provide better understanding of the bead formation process, protein drug loading and release. Thus, we examined the physical characteristics of the polymeric beads prepared from these polymers of varying MW.

Specifically, three different pH-/temperature-sensitive terpolymers were synthesized, namely low (MW ~ 49,000), intermediate (MW ~ 900,000) and high (MW ~ 3×10^6) MW polymers using NIPAAm/BMA/AA of feed mol ratio 85/5/10. The characteristics of these polymers were investigated by gel permeation chromatography (GPC), cloud point determination, differential scanning calorimetry (DSC), elemental analysis and titration. The physical properties of the polymeric beads were studied by scanning electron microscopy (SEM) and optical microscopy. The dissolution/swelling behavior of the beads of varying MW was studied by optical microscopy and by proton-nuclear magnetic resonance (^{1}H-NMR). The dependency of the release profile of the model protein drug cytochrome c was investigated as a function of the polymer MW and pH of the release medium at 37°C. These new polymeric beads have potential applications for controlled drug release in the intestine as a result of their wide-ranging physical properties based on their MW.

MATERIALS AND METHODS

Materials

N-isopropylacrylamide (NIPAAm) was obtained from Fisher Scientific Inc. (Fair Lawn, NJ) and was recrystallized from hexane. Butylmethacrylate (BMA) and acrylic acid (AA) were purchased from Aldrich Chemical Company (Milwaukee, WI). BMA and AA were purified by vacuum distillation at 57°C and 17 mm Hg and at 39°C and 10 mm Hg respectively.

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2,2′-azobisisobutyronitrile (AIBN) was purchased from Eastman Kodak Company (Rochester, NY) and was recrystallized from methanol. Heavy white mineral oil and decane were purchased from Aldrich Chemical Company (Milwaukee, WI). Bovine cytochrome c was purchased from Sigma Chemical Company (St. Louis, MO). Acetonitrile, HPLC grade, was purchased from Fisher Scientific Inc. (Fair Lawn, NJ). All other chemicals were of reagent grade. They were purchased from Sigma Chemical Company (St. Louis, MO) and were used without purification.

Polymer Synthesis

The synthesis of linear terpolymers of NIPAAm, BMA and AA with NIPAAm/BMA/AA of feed mol ratio 85/5/10 was carried out in varying volume percent of benzene and tetrahydrofuran (THF) as solvent (10 wt.% monomer concentration). AIBN was used as free radical initiator (7.41 mmol AIBN per mol of monomer). The solution was polymerized for 24 hours at 60°C under N_2 atmosphere. The synthesized terpolymers were precipitated in diethylether and recovered at about 95% yield. The polymer synthesized in 100% benzene was dried under vacuum, dissolved in dimethylformamide (DMF) and recovered by precipitation in diethylether with a 90% yield. The polymers were filtered and dried under vacuum overnight. The polymers were further purified by dialysis against deionized and distilled water at 4°C which was replaced every 8 hours for 4 days. A Spectra/Por membrane with a molecular weight cut-off (MWCO) of 1,000 was used for the polymer synthesized in 100% THF, a MWCO of 12,000 was used for the polymer synthesized in 90/10 volume % ratio of benzene/THF and a MWCO of 50,000 was used for the polymer synthesized in 100% benzene. The retentate solutions were lyophilized and stored desiccated for subsequent experiments.

Polymer Characterization

Molecular Weight/Polydispersity

Gel permeation chromatography (GPC) was used to estimate the number average MW (M_n) and weight average MW (M_w) of the polymers. MW and MW distribution were obtained relative to poly(N-(2-hydroxypropyl)methacrylamide) (poly(HPMA)) polymer standards (synthesized, fractionated and MW was determined by classical light scattering, MW varied between 50,000–1 million). A Sephacryl S-500 HR (high resolution) 10/30 column (Pharmacia) was used. A 0.05 M Tris buffer at pH 8.0 with 0.5 M NaCl was used as eluent. A fast protein liquid chromatography (FPLC) system (Pharmacia) equipped with a refractometer was used for obtaining the data. A flow rate of 0.4 ml/minute was used.

Lower Critical Solution Temperature

The LCST of the polymers was determined by cloud point measurement and by differential scanning calorimetry (DSC). Polymer samples (1% w/v) were prepared at 4°C, in 20 mM glycine buffer, pH 2.0, 0.15 M NaCl and in 10 mM phosphate buffer, pH 7.4, 0.15 M NaCl. Cloud point determination was performed on a Perkin-Elmer Lambda 7 UV/VIS spectrophotometer. The temperature was raised from 10°C to 90°C in 2°C increments every 10 minutes and the absorbance was measured at 450 nm. The LCST was defined as the temperature at the inflection point in the absorbance versus temperature curve. DSC experiments were performed from 3°C to 105°C at a scanning rate of 1°C/minute using a Differential Scanning Calorimeter, Hart Scientific, Model 5207.

Elemental Microanalysis

The content of carbon, hydrogen and nitrogen was determined by Atlantic Microlab, Inc., Norcross, Georgia. Samples were weighed on an electronic microbalance, Perkin-Elmer, Model AD4 and introduced in an auto-analyzer which was maintained under a positive pressure with the carrier gas helium. Combustion took place at approximately 1400°C in an oxygen atmosphere. Quantitative combustion was achieved by passing the mixture over oxidizing agents and then through a gas chromatographic capillary column containing Perapak PQS. The individual components were separated and eluted as N_2, CO_2 and H_2O. They were measured by a thermal conductivity detector whose signal was fed to a computer for data processing. The content of NIPAAm in the polymer was calculated based on the nitrogen content.

Acrylic Acid Analysis

The content of AA was determined by titration. A 1% w/v polymer solution at pH 2.0 was dialyzed against deionized and distilled water at 4°C which was replaced every 8 hours for 4 days, using a Spectra/Por membrane with a MWCO of 1,000. The samples were freeze-dried and used for AA content determination. One hundred mg of the polymer was dissolved in 10 ml of distilled water. Five drops of phenolphthalein indicator (5% w/v phenolphthalein in ethanol) were added and the solution was titrated against a standard 0.1 M NaOH solution. The amount of AA in each polymer was calculated by establishing the amount of NaOH required for neutralization by a transition point.

Butylmethacrylate Analysis

The content of BMA was determined by hydrolysis of the ester and subsequent titration of the acid. A 1% w/v polymer solution at pH 11.0 was hydrolyzed for 5 days. The pH was then adjusted to pH 2.0 and the solution was dialyzed against deionized and distilled water at 4°C replaced every 8 hours for 4 days, using a Spectra/Por membrane with a MWCO of 1,000. The samples were freeze-dried and used for total acid content determination as described above. One hundred mg of the polymer was dissolved in 10 ml of distilled water. Five drops of phenolphthalein indicator (5% w/v phenolphthalein in ethanol) were added and the solution was titrated against a standard 0.1 M NaOH solution. The amount of BMA in each polymer was calculated by the difference in the amount of NaOH required to neutralize the total acid and the AA.

Polymer Deswelling Kinetics

The rates of deswelling of the different polymers were studied as the polymers collapsed to form beads. A Nikon Eclipse E800 microscope was used and images were processed with Image ProPlus software. A 1% w/v polymer solution was prepared in 20 mM glycine buffer at pH 2.0 containing 0.15

M NaCl at 4°C. A drop of the polymer solution was placed in a mineral oil bath at 35°C covered with decane by means of a syringe. At regular time intervals, pictures of the collapsing polymer were taken and the optical density was processed using Image ProPlus software. The time at which the optical density reached a plateau was taken as the time at which the polymer had collapsed completely.

Bead Preparation and Characterization

Polymeric beads were prepared from an aqueous solution (20 mM glycine buffer, pH 2.0, 0.35 M NaCl) containing the pH-/temperature-sensitive terpolymer (7 or 10% w/v) and 0.5% w/v of cytochrome c. The solution was kept at 4°C overnight to allow the solubilization of the polymer. The polymer solution at 4°C was added dropwise using a syringe and 25G needle into 50 ml of mineral oil kept at a temperature of 35°C which is above the LCST of the polymers. The mineral oil was covered with 5 ml of decane to reduce the surface tension and to aid in the penetration of the solution drop at the air/oil interface. The formed beads were washed with hexane and air dried at 35°C for 1 hour and then dried in a rotary evaporator with aspiration for 30 minutes.

Bead Size

Bead size was determined by optical microscopy using a Nikon microscope, S&M Microscopes Inc., Conifer, CO. An average of 20 beads were used for each category of beads.

Scanning Electron Microscopy

The structural morphology of the beads was evaluated using scanning electron microscopy (SEM). Samples were coated with gold and the surfaces and cross-sectional surfaces were examined with a scanning electron microscope (Cambridge, S240 SEM), using a LaB_6 filament and a secondary electron (SE) detector at 15–25 kV acceleration voltage. Cross-sectional surfaces were obtained by breaking the beads in liquid nitrogen.

Dissolution/Swelling Studies

Beads were placed in 20 mM glycine buffer, pH 2.0, 0.15 M NaCl at 37°C or in 10 mM phosphate buffer, pH 7.4, 0.15 M NaCl at 37°C to study the dissolution and swelling behavior of the beads under different pH conditions. At different time intervals, images of the beads were taken using a Nikon Eclipse E800 microscope and the images were processed with Image ProPlus software.

The kinetics of dissolution and swelling were also studied by proton-nuclear magnetic spectroscopy (^{1}H-NMR). Ten mg of beads were placed in 1 ml of 10 mM phosphate buffer prepared in deuterated water, pH 7.4, 0.15 M NaCl at 37°C. The change in the area of the peak at δ = 1.2 ppm (signal corresponding to methyl groups in NIPAAm) was monitored with time for the different MW beads. The percent of polymer dissolved was normalized per gram of beads.

Loading Efficiency

The content of cytochrome c in the beads was determined after complete dissolution of the beads at 4°C. Ten mg of the dried beads containing cytochrome c were dissolved in 2 ml of 20 mM glycine buffer, pH 2.0, 0.15 M NaCl at 4°C overnight. The resulting solution was heated to 50°C for 10 minutes. After the polymer solution precipitated, the supernatant was collected and assayed for cytochrome c by reversed phase high performance liquid chromatography (RP-HPLC). The HPLC apparatus consisted of a Waters gradient system (an automated gradient controller, model 680 and HPLC pump, model 501, Waters Corporation, MA). One hundred µl of the samples were injected via an intelligent sample processor (WISP, model 712, Waters) connected to a UV detector (model 484, Waters) and an integrator (model 745, Waters). The C_4 column (5 µm, 4.6 × 250 mm, Vydac, Hesperia, CA) was equilibrated at a flow rate of 1 ml/minute with 80% eluent A (water, 0.1% trifluroacetic acid) and 20% eluent B (acetonitrile, 0.1% trifluroacetic acid). Cytochrome c was eluted with a gradient starting at 80% eluent A and 20% eluent B and ending at 40% eluent A and 60% eluent B over 20 minutes. The absorbance of the eluent was recorded at 380 nm. The column was calibrated with cytochrome c solutions of known concentration. The loading efficiency was calculated as the ratio of drug per gram of beads to the total amount of drug used per gram of polymer during bead fabrication.

Release Studies—In Vitro

One hundred mg of cytochrome c-loaded beads were placed in 10 ml glycine buffer with 0.15 M NaCl, pH 2.0, at 37°C for 2 hours and then in 10 ml of 10 mM phosphate buffer with 0.15 M NaCl, pH 7.4, at 37°C so as to mimic in vivo conditions in the gastrointestinal (GI) tract. The beads were pre-washed before the studies so as to remove surface-bound drug. At different time points, 1 ml of the release medium was collected and replaced by the same volume of buffer. Cytochrome c released was assayed by RP-HPLC as described above.

RESULTS AND DISCUSSION

Polymer Characterization

Molecular Weight/Polydispersity

The MW and polydispersity (M_w/M_n) of the polymers prepared using NIPAAm/BMA/AA of feed mol ratio 85/5/10 were estimated by GPC and the results are shown in Table I. The MWs were estimated using poly(HPMA) standards. M_w varied from 49,000 to 3×10^6 and the results were confirmed by static light scattering. The low MW polymer (MW = 49,000) was synthesized in pure THF. THF is known to act as a chain transfer agent, thus limiting the size of the polymer chains (15,16). However, as the volume percent of benzene in the solvent mixture increases, the chain transfer reaction is prevented and thus, the MW of the polymer increases. The polydispersity increases with increasing benzene content in the solvent mixture, being 3.1, 8.0 and 16.9 for 0%, 90% and 100% benzene content respectively.

Lower Critical Solution Temperature

To determine the LCST of the terpolymers at pH 2.0 and pH 7.4, cloud point and DSC experiments were performed. Results are shown in Table I and Fig. 1. The cloud point method

Table I. MW, Polydispersity, and LCST of NIPAAm/BMA/AA Polymers of Feed Mol Ratio 85/5/10

Polymer type	Solvent	M_w[b,c]	M_n[b]	Polydispersity (M_w/M_n)	LCST by cloud point (°C)		Maximum of endothermic peak by DSC (°C)
					pH 2.0	pH 7.4	pH 2.0
Low MW	THF	49,000	16,000	3.1	22.5	56.8	24.4
Intermediate MW	Benzene/THF[a]	900,000	113,000	8.0	23.6	56.3	24.9
High MW	Benzene	3×10^6	178,000	16.9	23.9	53.8	25.4

[a] Benzene/THF volume ratio: 90/10.
[b] Sephacryl S-500 HR 10/30 column using poly(HPMA) standards.
[c] M_w was also independently determined by static light scattering (SLS) and results of GPC and SLS corroborated.

shows a sharp transition at pH 2.0 and a more gradual transition at pH 7.4 (data not shown). At pH 2.0, the LCST of the polymers is around 22°C. At pH 7.4, the LCST of the polymers is around 60°C. The LCST at pH 2.0 or pH 7.4 does not vary much with change in MW as expected due to the statistical nature of the terpolymers.

Figure 1 shows the heat capacity versus temperature curve obtained by the DSC method. At pH 2.0, a sharp transition is seen and the LCST of all three polymers is around 24°C. The endothermic heat of phase separation (Fig. 1A) is related to the breaking of hydrogen bonds between water molecules surrounding hydrophobic moieties on the polymer (17). At pH 7.4, no phase transition is observed by DSC.

The LCST at pH 2.0 is lower than that at pH 7.4. This observation can be explained by the effect of ionizable groups on thermosensitive polymers as investigated by Feil *et al.* (11). At low pH, the AA is uncharged permitting greater interaction between the polymer chains through hydrogen bonding, leading to shifting of the LCST to a lower temperature (22°C)(3). At high pH, the AA is charged and consequently such polymers have high solubility/swelling or strong hydrophilicity, and thus, a significantly higher LCST (60°C).

Another interesting point is the sharpness of the phase transition at pH 2.0 as observed by both cloud point and DSC measurements and the more gradual transition at pH 7.4. At pH 2.0, the deswelling process is sudden as can be seen by the sharp change in absorbance or by the narrow endothermic DSC peaks, suggesting that all the polymer chains collapse simultaneously. At pH 7.4, AA increases the LCST due to the increased hydrophilicity of the charged carboxylic groups, thereby causing the polymer chains to collapse slowly, which is reflected in a gradual change in absorbance. The deswelling process is gradual at pH 7.4, such that DSC cannot detect any change in enthalpy (Fig. 1B), and hence, we do not see any peak on the endotherm, but a change in absorbance can still be detected by the cloud point method. Similar observations have been made in other studies (11,18). The incorporation of hydrophilic groups in NIPAAm polymers led to a broadening or disappearance of the endothermic peak under pH conditions where the hydrophilic group was ionized.

Elemental, Acrylic Acid, and Butylmethacrylate Analysis

The theoretical and actual content of NIPAAm, AA and BMA in the polymers is close. The theoretical content of NIPAAm, AA and BMA is 0.87 g, 0.065 g and 0.064 g per gram of polymer respectively. The actual content of NIPAAm was 0.84 ± 0.005 g, 0.86 ± 0.004 g and 0.85 ± 0.005 g per gram of polymer for the low MW, intermediate MW and high MW polymer respectively. The actual content of AA was 0.051 ± 0.001 g, 0.060 ± 0.001 g and 0.059 ± 0.002 g per gram of polymer for the low MW, intermediate MW and high MW polymer respectively. The actual content of BMA was 0.055 ± 0.003 g, 0.056 ± 0.001 g and 0.053 ± 0.002 g per gram of polymer for the low MW, intermediate MW and high MW polymer respectively. The incorporation of monomers is close to feed values. Thus, the difference in properties observed for

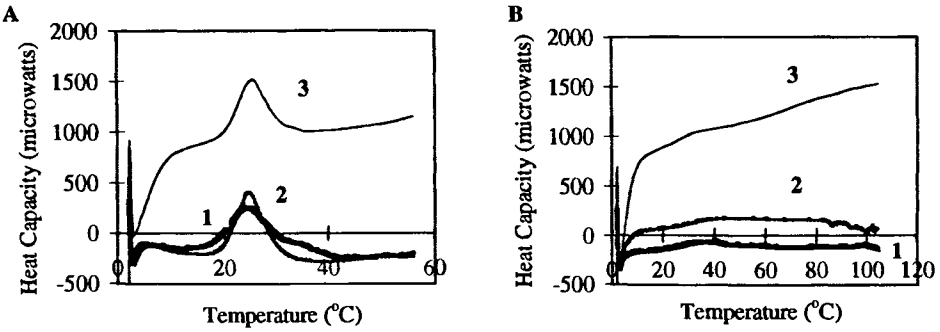

Fig. 1. LCST of different MW NIPAAm/BMA/AA polymers of feed mol ratio 85/5/10 determined by the DSC method at a scan rate of 1°C/minute. A: pH 2.0, B: pH 7.4. 1% w/v solutions at a certain pH were studied. (1) Low MW, (2) Intermediate MW, (3) High MW.

the different MW polymers cannot be attributed to a change in composition of the polymers with MW.

Deswelling Kinetics

The rate of deswelling of the different MW polymers were determined by measuring the change in optical density of the collapsing polymer with time. The low MW polymer takes 21 seconds to collapse, the intermediate MW polymer takes 28 seconds and the high MW polymer takes 38 seconds to collapse. We also observe that the low MW polymer shows a sharp change in optical density with time and as the MW of the polymer increases, the change in optical density with time becomes more gradual.

These observations can be attributed to the change in mobility of the polymer chains of varying MW. Initially, the polymer chain is extended and the chain has to fold on collapse. It is a well known fact that as the MW of the polymer increases, the mobility of the polymer chain decreases (19). The macroscopic diffusion coefficient D of a polymer chain varies with MW M of the polymer as $D \sim M^{-2}$ (20). Thus, as MW of the polymer increases, we should observe a decrease in the rate of collapse of the polymer chain. This behavior is reflected in the slow and gradual deswelling kinetics of the high MW polymer as compared to the faster deswelling kinetics of the low MW polymer and the intermediate deswelling kinetics of the intermediate MW polymer.

Bead Preparation and Characterization

Previously, we have shown that bead formation using low MW terpolymer with NIPAAm/BMA/AA of feed mol ratio 80/10/10 was affected by factors such as temperature, ionic strength and pH (13,14). In this study, optimum bead formation was in 20 mM glycine buffer and 0.35 M NaCl at pH 2.0 and 35°C. Figure 2 illustrates the process of bead fabrication. The formation of beads is explained by the formation of an outer thin skin layer due to polymer collapse (13). The spherical drops shrink at the outer edge first and the inside of the sphere undergoes a phase change more gradually. The diameter of the beads was determined by optical microscopy. The beads have a diameter ranging from 1 to 2 mm, the low MW beads have a diameter of 0.99 ± 0.06 mm, the intermediate MW beads are 1.27 ± 0.07 mm in diameter and the high MW beads are 1.80 ± 0.16 mm in diameter.

Scanning Electron Microscopy

The SEM pictures of the surface and interior of the beads are shown in Fig. 3. It can be seen that pores are present on the surface of the beads, the low MW polymeric beads having larger pores than the high MW polymeric beads. Analysis of the interior section of the beads again confirms the larger pore size of the low MW polymeric beads compared to the high MW polymeric beads.

Based on well established thermodynamic rules, it is known that as the MW of a polymer increases, its solubility decreases. An increase in the solubility of the polymer in the aqueous phase would favor the formation of poorly porous matrices under the precipitation conditions. Since, the opposite is observed in this case, it implies that a different mechanism is involved in the formation of the pores.

The shrinking process of poly(NIPAAm)-based gels in water from the swollen state has been investigated in detail by several authors (2,21). When the temperature is increased from the swelling temperature to the shrinking temperature, the surface of the polymer shrinks before the bulk material leading to the formation of a dense, less permeable surface layer (skin). It has been well documented that in the case of NIPAAm/BMA copolymers, a skin type barrier forms at the surface of the polymer when it collapses (2). The formation of the skin has been confirmed by the presence of water pockets on the surface of the gel by optical microscopy (2). This layer on the surface

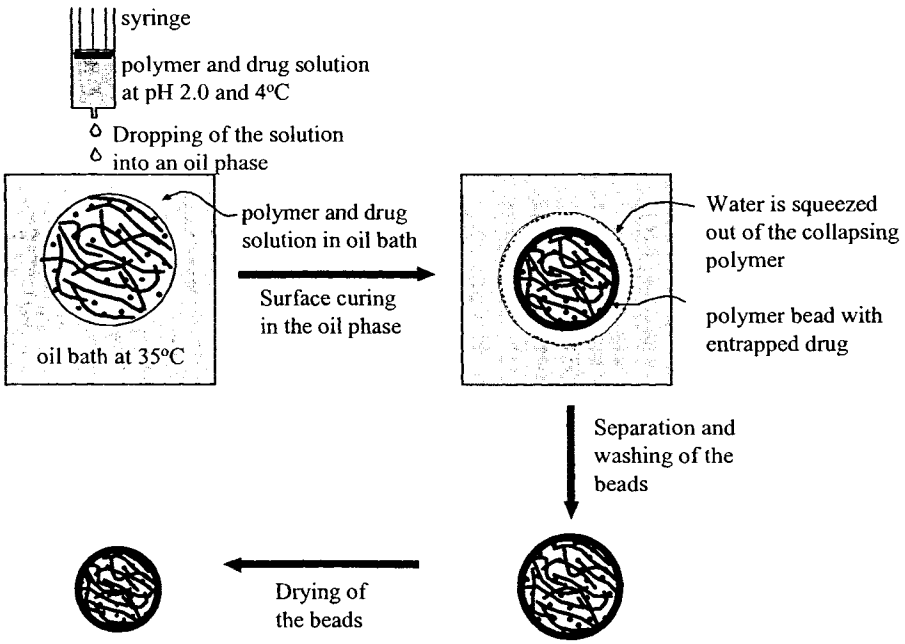

Fig. 2. Scheme illustrating the process of fabrication of beads.

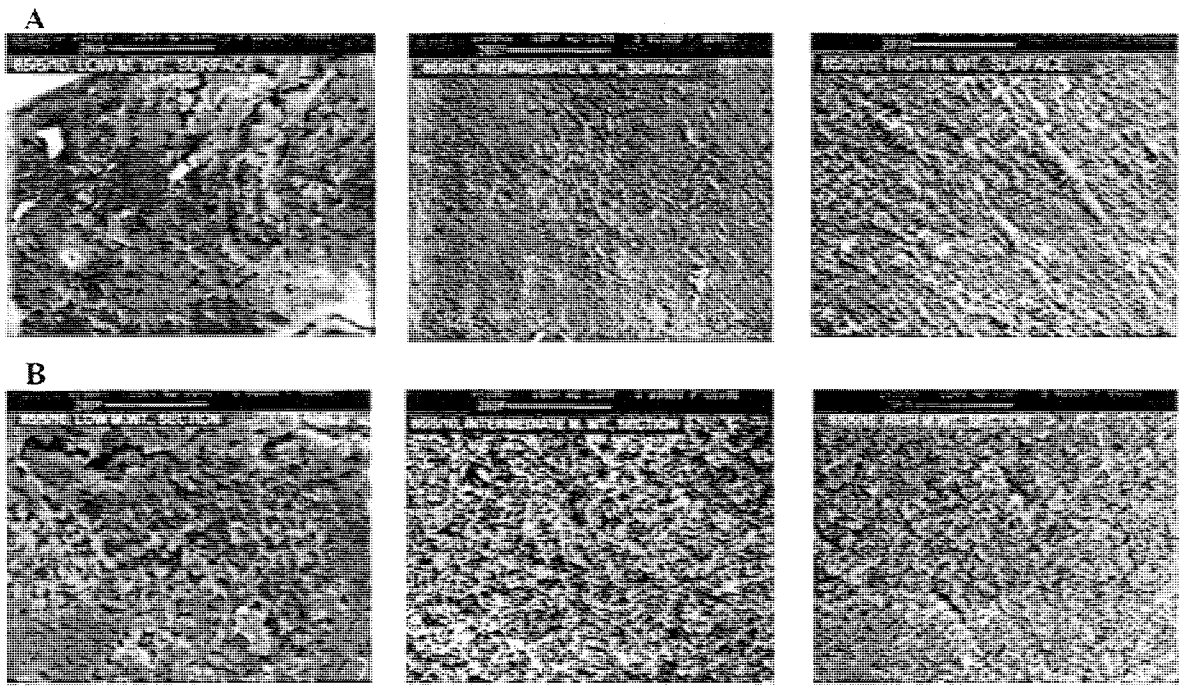

Fig. 3. A: Scanning electron microscope surface views at 1,610, 1,580 and 1,600 magnification of low MW (left), intermediate MW (center) and high MW (right) polymeric bead. B: Scanning electron microscope cross-sectional views at 1,580, 1,560 and 1,530 magnification of low MW (left), intermediate MW (center) and high MW (right) polymeric bead. NIPAAm/BMA/AA polymer of feed mol ratio 85/5/10 was used.

of the gel is dense enough to retard the outflow of water from the inside, preventing the gel from shrinking and stopping the shrinking process for a certain time period (21). Hydrostatic pressure is induced within the gel in the process of shrinking. This internal pressure induces the outward convection of water through the surface membrane that forms (21). The phenomenon of water convection has been seen by optical microscopy during the bead fabrication process (a water layer is squeezed out of the low MW, intermediate MW and high MW polymers respectively during collapse). The time required to see this water layer increases as the MW of the polymer increases. It implies that it takes longer for the pressure to build up within the polymeric bead as polymer MW increases. The pressure that builds up has an impact on the membrane (skin) that forms. The faster the pressure builds up inside the collapsed polymer, the more defects there are on the outer membrane because the water is squeezed out before the outer surface has time to form completely. Thus, in the case of the low MW polymer, the polymer collapses so fast, that there is no contiguous skin formation which is seen as large pores or defects on the surface and inside the bead. As the MW of the polymer increases, the rate of polymer collapse and water flux out of the surface layer decreases leading to fewer defects on the skin and smaller pores.

Dissolution/Swelling Studies

Dissolution/swelling studies again show MW-dependent behavior of the beads (Figs. 4 & 5). At pH 2.0 and 37°C, no obvious volume changes were observed for any of the beads, low, intermediate or high MW beads. No bead disintegration or swelling was seen since these conditions of pH and temperature are above the LCST of the polymers and hence, the polymers are insoluble. At pH 7.4 and 37°C, the low MW polymeric beads did not swell, but rather disintegrated and dissolved in about 2 hours (Fig. 4A) and the intermediate MW beads showed some swelling and had dissolved within 4 hours (Fig. 4B). The high MW beads instead swelled and disintegrated slowly (Fig. 5). After a period of 8 hours, the high MW beads were still in the process of swelling and slow degradation. The pictures show swelling of the high MW beads only for a period of 3 hours. After 3 hours, the beads had swollen considerably. With time, the swelling front (transparent gel, Fig. 5) keeps increasing whereas the size of the dry bead (inner portion, Fig. 5) keeps decreasing.

^{1}H-NMR studies corroborate with the observations made by optical microscopy (Fig. 6). Low MW polymeric beads disintegrate and dissolve within 2 hours and the intermediate polymeric beads take 4 hours to dissolve. The high MW beads remain swollen after 8 hours and the intensity of the peak is still gradually increasing, indicating that the polymer chains are dissolving slowly. The kinetics of swelling/dissolution is rate-limiting in the case of the high MW polymeric beads.

Loading Efficiency

The loading efficiency of cytochrome c was 37.6% for the low MW polymer, 50.2% for the intermediate MW polymer and 58.6% for the high MW polymer. The increase in loading efficiency with increase in polymer MW indicates that polymer network entrapment has an effect on the loading efficiency.

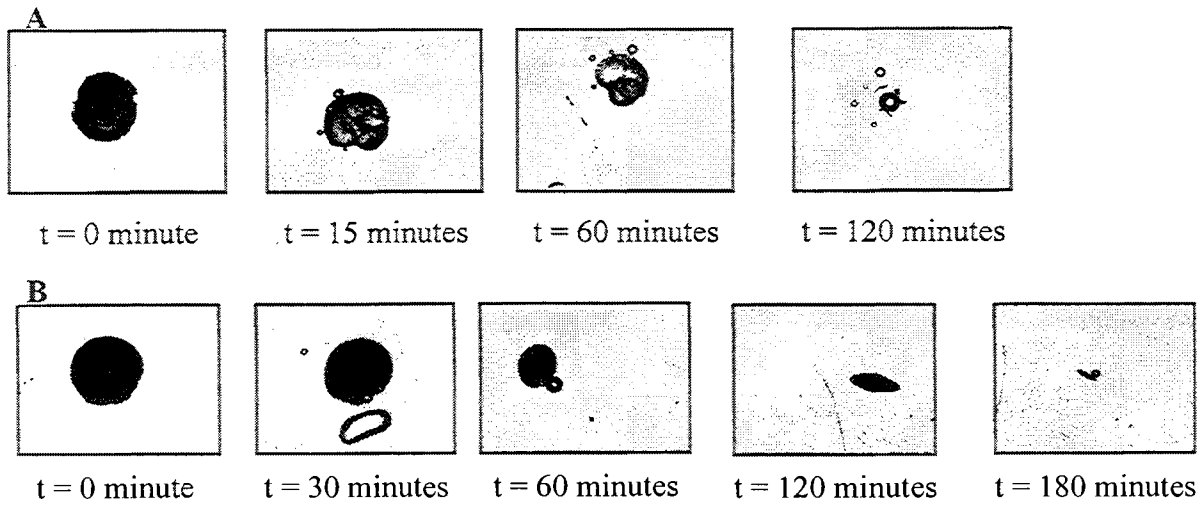

Fig. 4. Time dependence of A: low MW, B: intermediate MW polymeric bead dissolution at pH 7.4 and 37°C studied by optical microscopy. NIPAAm/BMA/AA polymer of feed mol ratio 85/5/10 was used.

Insulin has a very high loading efficiency (>90%) in all the polymers, indicating that specific polymer-insulin interactions are responsible for this high loading efficiency (22). Cytochrome c seems to interact with the polymers only through nonspecific interactions.

Release Studies—In Vitro

The release profile of cytochrome c from different MW polymeric beads at 37°C placed for 2 hours at pH 2.0 and then at pH 7.4 is shown in Fig. 7. A small fraction of cytochrome c (less than 5%) was released from all the polymers at pH 2.0. The low MW polymeric beads released 98% of the cytochrome c at pH 7.4 and 37°C after 2 hours, the intermediate MW polymeric beads released 95% of the drug within 4 hours, while the high MW polymeric beads released most of the drug in 8 hours. At pH 2.0 and 37°C, the polymers did not swell or dissolve and hence, there is minimal drug release. At pH 7.4 and 37°C, the beads degraded or swelled, depending on the MW of the polymer and accordingly, drug release was observed from all the polymers. The release profiles correlate with the dissolution and swelling properties of the different MW polymeric beads as discussed above (Fig. 6).

For a polymer to dissolve, polymer-polymer interactions have to be replaced by polymer-solvent interactions (23). The dynamics of macromolecules is complicated by the size of these molecules. The mobility of polymer chains is greatly affected by the MW of the polymer. The concept of entanglement is

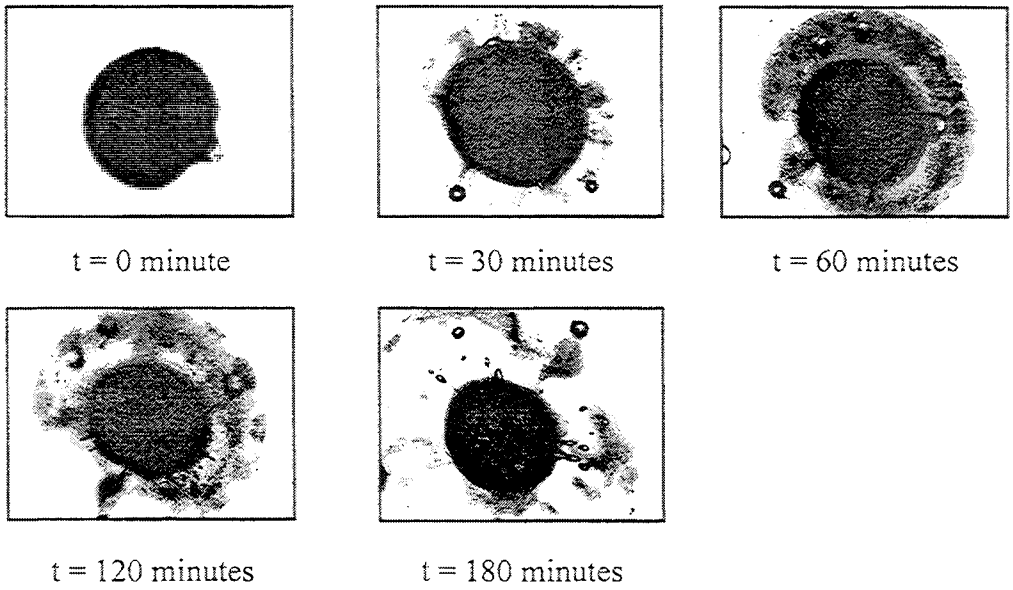

Fig. 5. Time dependence of high MW polymeric bead swelling and disintegration at pH 7.4 and 37°C studied by optical microscopy. NIPAAm/BMA/AA polymer of feed mol ratio 85/5/10 was used.

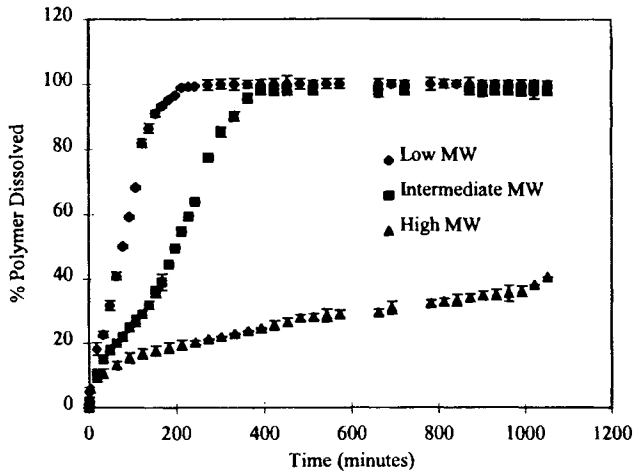

Fig. 6. Dissolution/swelling kinetics of the different MW polymers in 10 mM phosphate buffer at 37°C studied by ^{1}H-NMR (n=3). NIPAAm/BMA/AA polymer of feed mol ratio 85/5/10 was used.

widely used to explain the dynamics of macromolecules. Entanglement is a phenomenon whereby contact points between polymer chains are formed and these act like pseudo-crosslinks (24,25). Entanglement interactions dominate all properties which depend primarily on large-scale chain motions. They increase in importance with chain length and concentration (25). A broad MW distribution might allow entanglement at a lower polymer concentration (26). The average lifetime of a particular entanglement is proportional to the combined length of the two chains from which it is formed (27).

The concept of polymer chain mobility and entanglement cannot be separated. An entanglement MW M_e was calculated to be approximately 6,500 for the melt of the polymer used in this study by analogy to other polymers (28). During the dissolution studies, the polymer beads are similar to a highly concentrated polymer solution. It seems reasonable to assume that there is entanglement in all the three types of polymers studied. However, the number of knots due to entanglement

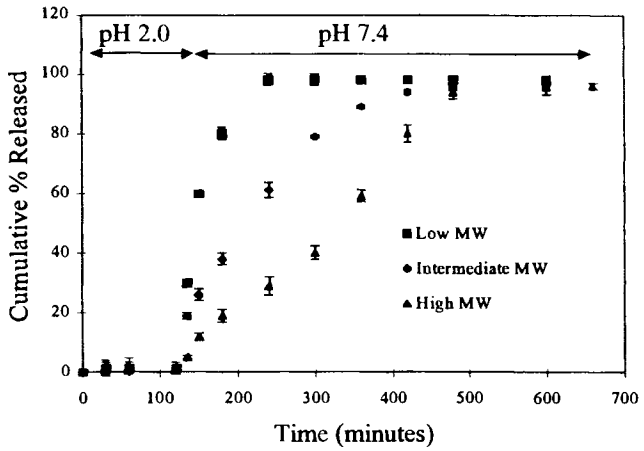

Fig. 7. Modulating cytochrome c release profile from pH-/temperature-sensitive beads made of terpolymers of NIPAAm/BMA/AA of feed mol ratio 85/5/10 and increasing MW. Beads were placed at pH 2.0 and 37°C for 2 hours and then at pH 7.4 and 37°C for the remainder of the release studies (n=6).

within a chain increases as the MW increases and this leads to an increase in the number of pseudo-crosslinks (19). This has a dramatic effect on the kinetics of dissolution of a polymer. The reptation time T_{rep}, of a polymer chain is proportional to the cube of the degree of polymerization N (19). Thus, as the MW of the polymer increases, the reptation time of the polymer will increase and hence, the rate of dissolution of the polymer will decrease, because it will take longer for polymer-polymer chains to disentangle and for polymer-solvent interactions to form (24). The MW distribution significantly affects the diffusion process of a polymer chain and in turn would affect the dissolution process (29). Currently, we are investigating the effect of MW and polydispersity on the disintegration and dissolution behavior of polymers.

Thus, the different deswelling, dissolution/swelling behavior observed by the polymers is mainly due to the effect of MW on the mobility of the polymer chains. Due to the extremely high MW, the high MW polymer has very low mobility which accounts for the slow deswelling and dissolution/swelling and release kinetics as compared to the lower MW polymers.

CONCLUSIONS

pH-/temperature-sensitive terpolymers of NIPAAm/BMA/AA of different MW were prepared by free radical polymerization for modulating the physical properties of polymeric beads which are designed to be used as an oral drug delivery system. These polymers were used to prepare beads by dropping an aqueous polymer solution at pH 2.0 and 4°C into an oil phase at 35°C. Bead formation proceeded with an initial skin layer around the solution droplet by interfacial precipitation. LCST was found to vary with pH because of the presence of ionizable groups on the polymer. Phase transition was sharper at pH 2.0 than at pH 7.4. It was shown by SEM and deswelling/swelling studies, that the physical characteristics of the polymeric beads differed based on the MW of the polymer. Irrespective of MW, the beads did not dissolve at pH 2.0 and 37°C. At pH 7.4 and 37°C, however, dissolution and swelling kinetics were a function of polymer MW. The different dissolution/swelling patterns observed were governed by the mobility of the polymer chains which is a decreasing function of MW. These polymers were used for aqueous loading of the protein drug cytochrome c, through physical interaction between the polymer and the drug and no chemical modification of the drug was involved. The different MW polymeric beads loaded with macromolecular drugs were used to modulate the drug release rate. Low MW polymeric beads led to a rapid polymer disintegration and dissolution, accompanied by a fast drug release rate. Intermediate MW polymeric beads dissolved slower and released drug over a slightly longer period of time. High MW polymeric beads swelled and dissolved gradually and released drug over a much longer period of time. This polymeric drug delivery system has the ability to release drugs in different regions of the intestine based on the rate of polymer swelling and dissolution.

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