Macromolecular Research, Vol. 13, No. 2, pp 147-151 (2005)

Modulation of Poly(β-amino ester) pH-Sensitive Polymers by Molecular Weight Control

Min Sang Kim and Doo Sung Lee*

Department of Polymer Science and Engineering, Sungkyunkwan University, Suwon, Kyungki 440-746, Korea

Eun-Kyung Choi

Department of Radiation Oncology, University of Ulsan, Ulsan 680-749, Korea

Heon-Joo Park

Department of Microbiology, College of Medicine, Inha University, Incheon 402-751, Korea

Jin-Seok Kim

College of Pharmacy, Sookmyung Women's University, Seoul 140-742, Korea

Received January 5, 2005; Revised February 2, 2005

Abstract: The main objective of this study was to modulate pH-sensitive polymers (poly(β-amino esters)) by controlling their molecular weight during their synthesis. These pH-sensitive and biodegradable polymers were synthesized by Michael-type step polymerization. 1,4-Butane diol diacrylate was used as the unsaturated carbonyl compound and piperazine as the nucleophilic compound. Various molecular weight polymers were obtained by varying the mol ratio of piperazine/1,4-butane diol diacrylate. The synthesized polymers were characterized by ^{1}H-NMR and their molecular weights were measured by gel permeation chromatography(GPC). The dependence of the molecular weight on the mol ratio was evaluated by the titration method. Also, the pH dependent turbidity of the polymers was determined by UV-Vis spectrophotometry. This pH dependent property of the polymers could be very useful for preparing drug carriers that are sensitive to pH.

Keywords : pH-sensitive polymer, pK_b, molecular weight control.

Introduction

Polymeric materials have received a great deal of attention in the biomedical field. For example, they have been used in long-term and temporary therapeutic devices, drug formulations and delivery systems, aids in the improvement of diagnostic examinations, and devices for bioengineering. In particular, there has been a steadily increasing interest and demand for the development of novel drug delivery systems that are both highly efficient and specific to a target site.[1-3]

Stimuli-sensitive polymers, which are affected by such factors as the pH, temperature, light, electric fields, etc., have attracted increasing interest in the field of science and technology. Especially, pH sensitive polymers are of particular interest, because of their potential use in targeted drug delivery systems. There has been continued interest in the potential applications of pH-sensitive polymeric systems, including linear polymers, grafted polymers and hydrogels, in the pharmaceutical arena. The ionizable groups like a base or an acid are important at pH-sensitive behavior. Ionizable groups act as hydrophilic part or hydrophobic part of the polymer. Then reversible soluble-insoluble transition is appeared as hydrophobicity change of polymer. An acidic group such as carboxylic acid is ionized at pH above pK_a and deionized at pH below pK_a. But a basic group such as amine is deionized at pH below pK_b and ionized at pH above pK_b. Representative pH-sensitive materials are sulfonamide[4] and L-histidine,[5] whose pH-responsive solubility or property originates from the character of the ionizable group.

In their study, Langer *et al.* synthesized poly(β-amino esters) by Michaeal type polymerization, and the degradability and cytotoxicity of this material, which is biodegradable and pH-sensitive, were investigated.[6] In addition to microspheres[7] and nanoparticles[8] were also fabricated, and their properties were analyzed. In the case where these

*e-mail: dslee@skku.edu

1598-5032/04/147-05

microspheres were used in targeted drug delivery systems, the drugs were released below pH 6.5, whereas drug loaded state was maintained above pH 6.5.

In the present study, the molecular weights of the poly(β-amino esters) were controlled in order to modulate the solubility of polymer by pH change. There are two main methods of controlling the molecular weight in step polymerization. The first method relies on the establishment of a nonstoichiometric reaction mixture, by allowing the polymerization reaction to proceed to a point at which one reactant is completely used up, while the other reactant is present in excess. The other method of achieving the desired molecular weight is through the addition of a monofunctional monomer. The monofunctional monomer controls and limits the polymerization of the bifunctional monomers because its reaction with the growing polymer yields chain ends devoid of a functional group and therefore incapable of further reaction.

The piperazine, which is added material in excess, was used as pH-sensitive and the 1,4-butandiol diacrylate was used as hydrophobic and biodegradable material. The polymer is soluble when the piperazine is ionized, and conversely polymer becomes a hydrophobic and then insoluble when piperazine was deionized.

Experimental

Materials. Piperazine and 1,4-butane diol diacrylate were purchased from Sigma-aldrich.

Synthesis. Piperazine and 1,4-butane diol diacrylate were each dissolved in THF. Then, the piperazine solution was added to the 1,4-butane diol diacrylate solution dropwise, so as to obtain mixtures with mol ratios of piperazine to 1,4-butane diol diacrylate of 1.0, 1.1, 1.3, 1.5 and 4. The reaction was carried out for 48 hrs at 50 °C. Polymers with various molecular weights were acquired after precipitation in *n*-hexane.

^{1}H NMR Characterization. The synthesized polymers were characterized by ^{1}H NMR spectroscopy. The ^{1}H NMR spectra were recorded by means of a 500 MHz FT-NMR (Utility Inova 500NB, Varian) spectrometer. $CDCl_3$ was used as the solvent.

GPC Analysis. The molecular weights of the synthesized polymers were measured by gel permeation chromatography(GPC). THF was used as the eluant at a flow rate of 1 mL/min. KF-803L and KF-802.5(Shodex) columns were used in series. The data were analyzed by means of an RI detector (RI-101, Shodex). Poly(ethylene glycol) standards (Waters) were used to determine the molecular weights.

Titration. The pK_b values of the various molecular weight polymers were measured by the titration method. In each case, 50 mg of the polymer was dispersed in 50 mL distilled water and the pH adjusted so as to be less than 5.0, and then the polymer was dissolved. 0.1 mL of 1 N NaOH solution was added and measured the pH, and then the titration profile was obtained.

The Light Transmittance. The light transmittance of the solutions was measured by UV-Vis spectrophotometry. In each case, 1 mg/mL of the polymer was dissolved in pH 5 buffer solution(0.1 N Borax/photassium dihydrogen phosphate) and the pH adjusted, in order to obtain polymer solutions with various pH values. The turbidity change of the polymer solutions was determined from the light transmittance at λ=500 nm.

Results and Discussion

^{1}H NMR Characterization. The ^{1}H NMR spectra of all five polymers were similar. The ^{1}H NMR spectra of the monomer and polymers are shown in Figure 1. The characteristic peaks of the methylene group ($-CH_2$) in the ^{1}H NMR spectrum of piperazine, that is appeared at 2.84 ppm, were shifted to 2.63 ppm in the ^{1}H NMR spectrum of the poly(β-amino ester). The peaks at 2.84 ppm in the ^{1}H NMR spectrum of poly(β-amino ester) correspond to the methylene proton of the end group piperazine. The methylene peaks (H_2=CH-) in the ^{1}H-NMR spectrum of 1,4-butane diol diacrylate situated between 5.8 and 6.4 ppm disappeared in the spectrum of poly(β-amino ester) and were shifted around 2.5 ppm.

GPC Analysis. The molecular weights of polymers were controlled by the addition of piperazine in excess. The mol

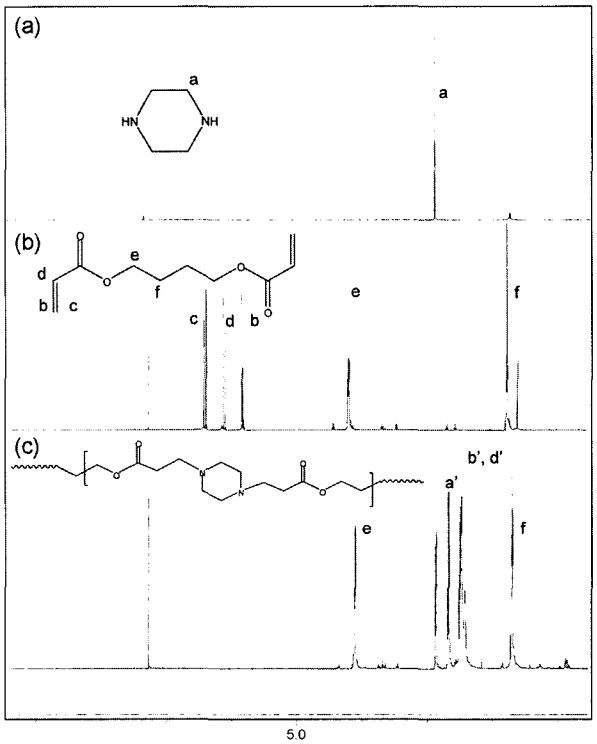
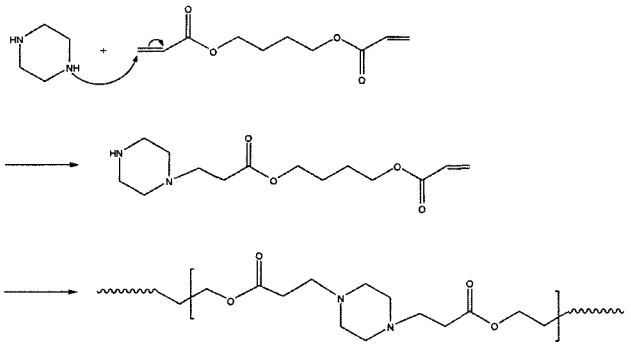


Figure 1. ^{1}H NMR spectra of (a) piperazine, (b)1,4-butane diol diacrylate, and (c) poly(β-amino ester).

Table I. Molecular Weight of Synthesized Polymers

Mol Ratio of Piperazine/ 1,4-Butane Diol	M_p	M_n	M_w	PDI
1	9,287	4,624	10,814	2.3
1.1	2,465	2,376	3,881	1.6
1.3	415	465	687	1.5
1.5	274	265	403	1.5
4	271	213	346	1.6



Scheme I. Step polymerization by Michael-type addition.

ratios of piperazine to 1,4-butane diol diacrylate were 1.0, 1.1, 1.3, 1.5 and 4. The molecular weights of polymers are listed in Table I.

Figure 2 shows the dependence of the molecular weight

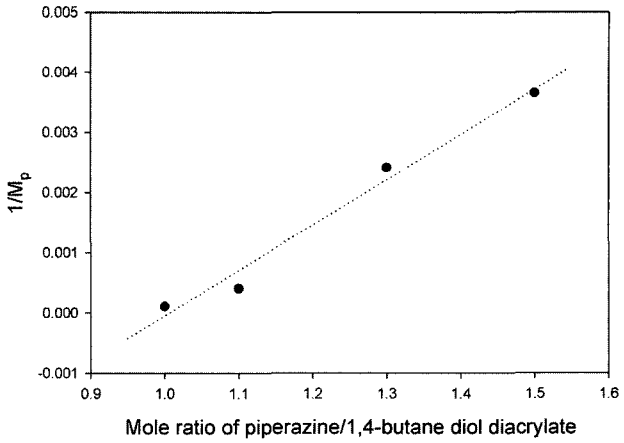


Figure 2. Dependence of the molecular weight on the mole ratio of piperazine/1,4-butane diol diacrylate.

on the mole ratio. The molecular weight decreased as the proportion of piperazine increased. The relationship between $1/M_p$ and the mole ratio shows the linear relationship by the increasing addition of piperazine in excess. It would be possible to prepare a desired molecular weight of the polymers by using this plot. In Michael-type addition polymerization, the reactants which are present in excess affect the molecular weight of the resulting polymer in the same manner as in the more common step polymerization.

Titration Curve and pK_b. The pK_b values of the polymers were obtained by the titration method, by calculating them

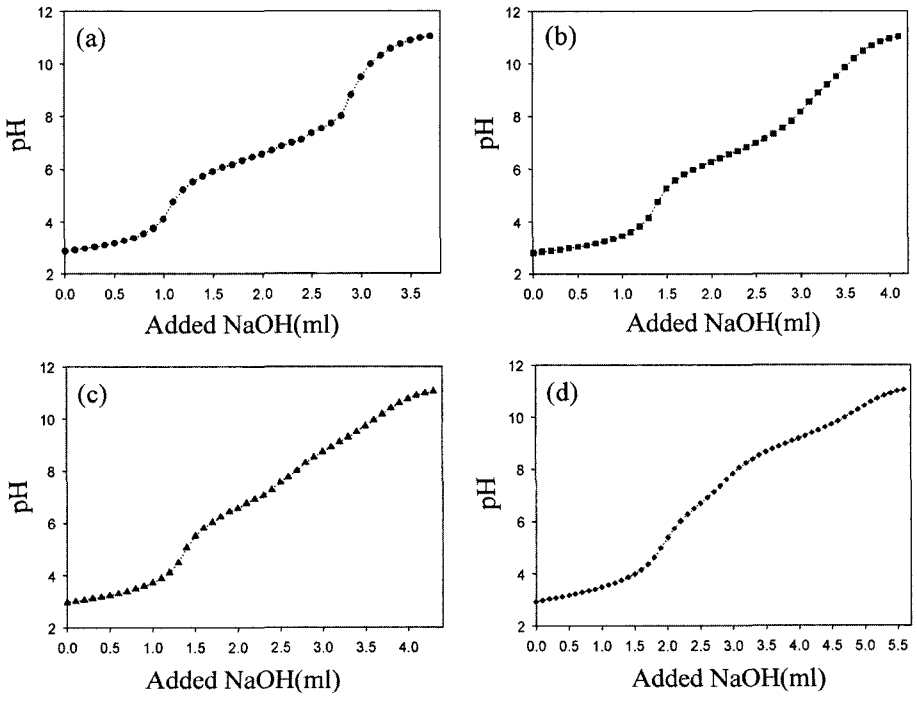


Figure 3. Titration curves of the polymers ; (a) M_p=9,287, (b) M_p=2,465, (c) M_p=415, and (d) M_p=271.

from the derivative values of the titration curves, that means inflection point. The titration curves of the polymer solutions are presented in Figure 3. The buffering region varied from pH 6 to pH 10 according to the molecular weight of the polymer. The buffering region appeared at lower pH as the molecular weight increased. The pK_b value of the highest molecular weight polymer(M_p=9,287) is 6.78 and this value is increased in the case of the lower molecular weight polymers. The pK_b values of the polymers with M_p values of 2465, 415 and 271 are 6.94, 7.51 and 9.88, respectively.

pH Sensitivity. The pH sensitivity was measured from the transmittance change of the polymer solutions at various pH values by UV-Vis spectrophotometry at λ=500 nm. The turbidity change is shown in Figure 5. The low molecular weight polymers are soluble and transparent in wide pH range, but the high molecular weight polymers(M_p=9,287, M_p=2,465) show a pH dependent turbidity(Figure 5). The turbidity transitions of these polymers occur depend on their molecular weight as expected from pK_b values. The transition region of polymers of M_p=9,287 and M_p=2,465 were in

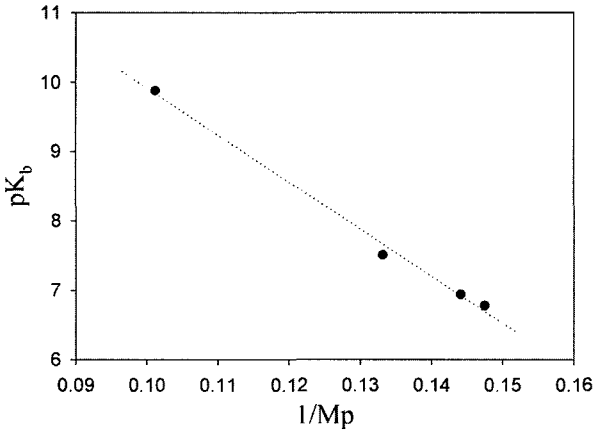


Figure 4. Relationship between pK_b value and molecular weight.

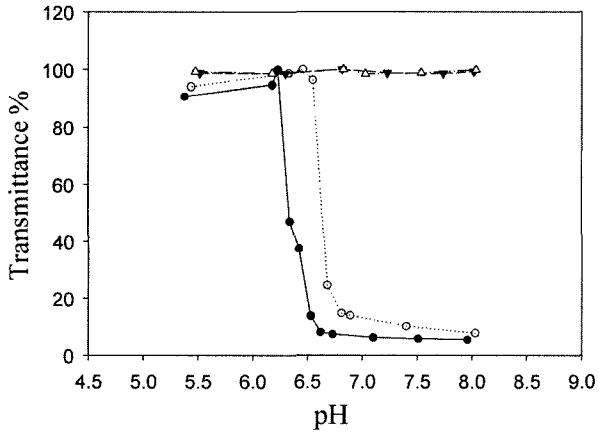


Figure 5. The light transmittance of the polymers solution; M_p=9,287(●), M_p=2,465(○), M_p=415(▲), M_p=271(▽).

the range of pH 6.23-6.34 and pH 6.55-6.88, respectively. But low molecular weight poly(β-amino esters) has no transition point because that is always soluble at any pH. This phenomenon results from the fact that the solubility of high molecular weight polymer is lower than the low molecular weight polymer due to the entropic effect of the polymer. Because the high molecular weight polymer has less solubility, the more degree of ionization at low pH is needed to dissolve the polymer.

The pK_b value of low molecular weight poly(β-amino esters) is similar with that of piperazine(pK_b=9.8) from literature,[9] because low molecular weight poly(β-amino esters) having M_p=271 contains secondary amine(chain end group) like piperazine. But higher molecular weight polymer has much more tertiary amine than low molecular weight polymer. Finally, pK_b value is suddenly increased at certain molecular weight, because of increasing secondary amine. In case of high molecular weight, molecular weight affects the pK_b value due to entropic effect, and in case of low molecular weight, the number of secondary or tertiary amine affects the pK_b value.

The environment around tumor cell has a weak acidity, about 6.0~7.2, then the polymers from this study could be useful as targeting drug carrier in cancer therapy and be useful in gene therapy because the cationic character helps polymer form a complex with anionic DNA.

Conclusions

Michael type addition polymerization follows the procedure of step polymerization. The nonstoichiometric method is effective in controlling the molecular weight. We were able to control the molecular weight of the polymers and the characteristics of the polymers were analyzed. The molecular weight is an important factor, affecting both the pK_b value and pH sensitivity of the polymers. The pH sensitivity was not observed for those polymers with a low molecular weight. Therefore, it is necessary for the polymer to have a specific molecular weight in order for it to exhibit pH sensitivity. Polymers buffering at approximately pH 6.0 to 7.0 are useful in drug delivery systems and therefore, the ability to control the molecular weight of the polymers is highly valuable. In the future work, more studies will be progressed about nanoparticle for cancer therapy because it is expected that microparticle or nanoparticle can release a drug below about pH 6.5.

Acknowldgements. The authors wish to thanks, to The Korea Institute of Science and Technology Evaluation and Planning for financial assistance.

References

(1) N. Sato, T. Okada, H. Horiuchi, N. Murakami, J. Takakashi,

M. Nawata, H. Ota, K. Nozaki, and K. Takaoka, *Nat. Biotechnol.*, **19**, 332 (2001).
(2) K. E. Uhrich, S. M. Cannizzaro, R. S. Langer, and K. M. Shakesheff, *Chem. Rev.*, **99**, 3181 (1999).
(3) M. N. V. Ravikumar and N. Kumar, *Drug Dev. Ind. Pharm.*, **27**, 1 (2001).
(4) S. Y. Park and Y. H. Bae, *Macromol. Rapid Commun.*, **20**, 269 (1999).
(5) E. S. Lee, H. J. Shin, K. Na, and Y. H. Bae, *J. Controlled Release*, **90**, 363 (2003).
(6) D. M. Lynn and R. Langer, *J. Am. Chem. Soc.*, **122**, 10761 (2000).
(7) D. M. Lynn, M. M. Amiji, and R. Langer, *Angew. Chem. Int. Ed.*, **40**, 1707 (2001).
(8) A. Potineni, D. M. Lynn, R. Langer, and M. M. Amiji, *J. Controlled Release*, **86**, 223 (2003).
(9) A. Warshawsky, N. Kahana, V. Kampel, I. Rogachev, E. Meinhardt, R. Kautzmann, J. L. Cortina, and C. Sampaio, *Macromol. Mater. Eng.*, **283**, 103 (2000).