Preparation of Novel Amphiphilic Copolymer Microspheres and Their Drug-Release and Glucose-Sensitive Properties

Yu Tang, Chaoxing Li

Institute of Polymer Science, Nankai University, Tianjin 300071, China

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ABSTRACT: A novel amphiphilic copolymer was prepared by the copolymerization of *N*-acryloyl-3-aminophenylboronic acid with β-cyclodextrin containing maleic anhydride. The copolymer was fully characterized with ^{13}C-NMR, ^{1}H-NMR, IR, and scanning electron microscopy. The self-assembling mechanism of the copolymer in H_2O–CH_3OH cosolvents was studied. Gliclazide as a model drug was loaded inside the copolymer microspheres, and the drug-release behavior of the microspheres was studied. The results of *in vitro* oscillating release tests indicated that the microspheres responded to glucose rapidly in 30 min, and the microspheres exhibited self-regulated on–off release behavior four to six times in 6 h between the solution with 3 g/L glucose and the medium without glucose; this met the clinical requirements of multidrug delivery. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 3848–3852, 2008

Key word: stimuli-sensitive polymers; self-assembly; microencapsulation

INTRODUCTION

Because of their excellent performance and novel functions, stimuli-responsive polymers have been paid more and more attention by scientists in recent years. These polymers undergo fast, reversible changes in microstructure from a hydrophilic state to a hydrophobic state. These changes are triggered by small changes in the environment, such as changes in the pH value, temperature, light, and ionic strength.[1–10] Because of these properties, stimuli-responsive polymers have been studied in diverse application fields, including drug-delivery systems, analytical and preparative separations, and sensor technologies.[11–16] It is well known that phenylboronic acid, which is used in chromatographic studies of sugars and glycoproteins, has been used to study glucose sensing.[17–19] As we know, the self-regulated release systems of insulin and other therapeutic drugs that respond to blood glucose concentration have played important roles in the treatment of diabetes.[20] Phenylboronic acid and its derivatives can form complexes with polyol compounds, which can be dissociated in the presence of a competing polyol compound and form a more stable complex. These particular properties enable phenylboronic acid to have many potential applications as a glucose-sensitive material.

In this field, most of the reports are on glucose-sensitive hydrogels with phenylboronic acid moieties, such as poly[(*N*-isopropylacrylamide)-*co*-(3-acrylamidephenylboronic acid)],[21] poly[(*N*,*N*-dimethylacrylamide)-*co*-(3-acrylamidephenylboronic acid)],[22] and poly[(vinyl alcohol)-*co*-(3-acrylamidephenylboronic acid)] systems.[19,23,24] These reports provide many good modes for the self-regulated releasing systems of insulin. Nevertheless, few investigations have dealt with microspheres, which are the most popular application of controlled release systems. Furthermore, insulin has always been chosen as the model drug for glucose-sensitive polymer materials, whereas many other classic chemical drugs for the therapy of diabetes have been ignored. To fill in these gaps, we synthesized a novel glucose-sensitive amphiphilic copolymer with *N*-acryloyl-3-aminophenylboronic acid (AAPBA) and β-cyclodextrin with maleic anhydride (MAH–β-CD). Gliclazide as a model drug was loaded inside the copolymer microspheres. The drug-release behavior under different conditions was studied, and the results show that the copolymer microspheres exhibited good glucose-sensitive properties.

EXPERIMENTAL

Materials and general methods

3-Aminophenylboronic acid was purchased from JinAo LiWei, Ltd., Co. (Beijing, China). Acryloyl chloride was purchased from The Institute of Chem Agents Co. (Tianjin, China) and was distilled under

Correspondence to: C. Li (lcx@nankai.edu.cn).

reduced pressure before use. 2,2-Azobisisobutyronitrile (AIBN) was supplied by Kehuida Limit Co. (Tianjin, China). ^{13}C-NMR and ^{1}H-NMR spectra were performed in a 500-MHz NMR instrument (Bruker DPX500, Bruker, Germany). IR spectra were measured on a Bruker Vector 22 Fourier transform infrared spectroscopy instrument. Ultraviolet–visible spectra were recorded on a Cary-50 (Varian Australia Pty, Ltd.) spectrophotometer.

Synthesis of the AAPBA-*co*-MAH–β-CD copolymer

Synthesis of AAPBA

AAPBA was synthesized according to a modified literature method.[25] In a typical experiment, 3-aminophenylboronic acid (1.55 g, 10 mmol) was dissolved in a solution of NaOH (20 mL, 2 mol/L) and cooled in an ice bath. Acryloyl chloride (1.6 mL, 20 mmol) was added dropwise to the solution under stirring, and the reaction was run for 3 h at room temperature. The pH value of the reaction mixture was adjusted to about 1 by hydrochloric acid (2 mol/L). The resulting precipitates were filtered and washed with fresh distilled water (50 mL). The precipitates were then dissolved in water (80 mL) at 60°C, and the impurities were filtered off. The solution was kept in a refrigerator overnight. Light violet needle crystals of the product were obtained. The crystals were filtered, washed, and dried *in vacuo* in desiccators over $CaCl_2$. Violet crystals of AAPBA were obtained with a yield of 35%.

Synthesis of MAH–β-CD

MAH–β-CD was synthesized according to the literature method.[26] β-Cyclodextrin (β-CD; 5.68 g, 5 mmol) and maleic anhydride (MAH; 4.90 g, 50 mmol) were dissolved in dry dimethylformamide (DMF; 20 mL) and then stirred for 10 h at 80°C.

The reaction mixture was then cooled to room temperature and poured into an excess of chloroform. The resulting precipitates were filtered, washed by acetone three times, and dried *in vacuo.* White solids of MAH–β-CD were obtained at a yield of 69%.

Preparation of the AAPBA-*co*-(MAH–β-CD) copolymer

AAPBA and MAH–β-CD at a ratio of 3 : 1 (w/w) were dissolved in methanol, in which AIBN (2% of the total weight of the monomer) was added. We started the free-radical polymerization by heating the reaction mixture to 70°C under nitrogen bubbling and left it to react for 7 h. After that, the reaction mixture was poured into a large excess of ether; the resulting precipitates of AAPBA-*co*-(MAH–β-CD) were filtered and dried *in vacuo.* In the ^{1}H-NMR spectra of the copolymer, the integral area of the AAPBA protons was 40.6% of the total area of the protons, from which a molar ratio of AAPBA to MAH–β-CD in the copolymer of 8 : 1 was inferred.

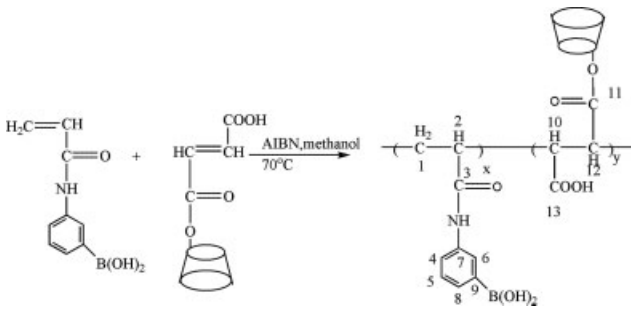

Figure 1 Synthesis route of the AAPBA–(MAH–β-CD) copolymer.

The synthesis route of the AAPBA-*co*-(MAH–β-CD) copolymer is shown in Figure 1.

Self-assembly of the AAPBA-*co*-(MAH–β-CD) microspheres

A certain amount of polymer was dissolved in methanol (5 mL). Water (50 mL) was added dropwise into the polymer solution, and the reaction occurred for 3 h at room temperature; then, the polymer was turned into latex, and the hard latex was collected by centrifugation. After it was rinsed with deionized water, the microspheres were lyophilized for over 48 h and stored *in vacuo.*

Drug load of the AAPBA-*co*-(MAH–β-CD) microspheres

Gliclazide was used as the model medicine for studying the drug release of the copolymer microspheres. A mixture of gliclazide and copolymer (ratio = 1 : 10 w/w) was dissolved in 5 mL of methanol; then, 50 mL of H_2O was added. The same procedures were performed according to the self-assembling procedures of the copolymer.

Glucose-sensitive properties of the microspheres

To gain insight into the glucose sensitivity of the microspheres, a set of various conditions experiments was carried out with the solutions containing different concentrations of glucose. An appropriate amount of dried microspheres loaded with the drug was transferred in a dialysis bag, and the bag was put in a pH 9.0 phosphoric buffer containing 10% alcohol. The absorptions of gliclazide were recorded by monitor (λ = 225 nm) on an ultraviolet–visible spectrometer at 37 ± 0.5°C. When the releasing rate approached zero, the dialysis bag was transferred into a solution of phosphoric buffer containing glucose (3 g/L) to dialyze for 30 min. After that, the

dialysis bag was put back in the phosphoric buffer without glucose for 30 min. This procedure was repeated several times to get the regulated-release curves.

RESULTS AND DISCUSSION

Characterization of AAPBA-*co*-(MAH–β-CD)

NMR spectra

In the ^{1}H-NMR spectra of the copolymer, the peaks at δ = 7.76–7.09 ppm were assigned to the protons on benzene rings. The peak at δ = 7.90 ppm was the contribution of the amine proton (NH), the protons of the polymer straight chains were around δ = 4.66 ppm, and the protons of β-CD appeared at 1.83 and 2.445 ppm. The ^{13}C-NMR spectrum of AAPBA-*co*-(MAH–β-CD) and its assignments[26,27] are shown in Figure 2. The peak around 18 ppm was the contribution of the number 10 carbon, and the numbers 11 and 9 carbons appeared at about 138 ppm; the chemical shift of carbon (number 13) in the maleic acid carbonyl group was at 176 ppm.

IR spectra

The stronger characterized vibration of C_{phen}—B bond was at 1336 cm^{-1},[28] the C=O unsymmetrical band was at 1656 cm^{-1}, and the broad peak at 3350 cm^{-1} was the contribution of OH groups of β-CD.

Effect of the MAH–β-CD content on the yield of the polymer

The yield of the copolymer decreased with increasing MAH–β-CD content in the polymer because the competing polymerization rate of MAH was lower than that of acryloyl derivatives; thus, MAH–β-CD was not easily linked on the chains of the copolymer. The yields of the copolymers with various ratios (w/w) of AAPBA to MAH–β-CD are summarized in Table I.

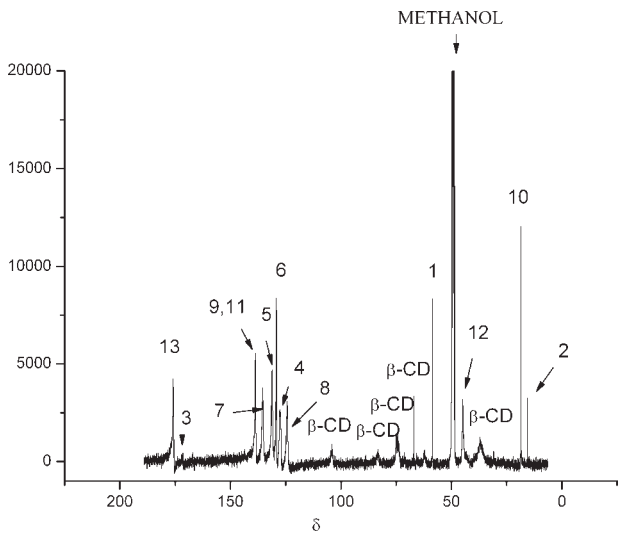

Figure 2 ^{13}C-NMR spectrum of the copolymer.

TABLE I
Yields of the Polymer with Various Ratios of AAPBA to MAH–β-CD

Polymer	AAPBA/MAH–β-CD (w/w)	Yield (%)
AM1	3 : 1	32
AM2	5 : 1	43
AM3	10 : 1	56
AM4	15 : 1	61

Self-assembling mechanism of the microspheres

The copolymer was insoluble in many solvents, including tetrahydrofuran, dimethyl sulfoxide, DMF, $CHCl_3$, CH_2Cl_2, 1,4-oxetone, acetone, and water, but it dissolved easily in alcohol's solvents. Among them, methanol was the best solvent. When the copolymer was dissolved in methanol, it exhibited self-assembling behavior by the addition of water into the system. The copolymer did not dissolve in nonalcoholic solvents, even at the boiling temperature. When a small amount of methanol was dropped into the boiling solution, the polymer dissolved rapidly, and the solution immediately became transparent. The excellent solubility properties of the copolymer in methanol are shown in Table II.

In self-assembling experiments, we also found that the soluble cases of the copolymers with the same amounts varied in methanol with the same volume. The higher the content of AAPBA in the copolymer was, the more easily it was dissolved. The polymer with a ratio of 15 : 1 (w/w) was dissolved in methanol under slight stirring at room temperature, whereas the copolymer with a ratio of 3 : 1 (w/w) did not dissolve in methanol unless it was stirred violently and heated. The reason was just that the boric acid groups in the copolymer reacted with methanol to generate stable complexes.[29–31]

The copolymer was insoluble in other solvents because AAPBA groups in one chain of the polymer bonding linked with the OH groups on the β-CD

TABLE II
Solubility of the Polymer in Various Solvents

Solvent	Solubility	Solubility with methanol added to the solvent
Dimethyl sulfoxide	Insoluble	Soluble
Tetrahydrofuran	Insoluble	Soluble
Acetone	Insoluble	Soluble
DMF	Insoluble	Soluble
Methanol	Soluble after heating	—

skeleton in the neighboring chain to generate a networked crosslinking polymer that was difficult to dissolve in nonalcoholic solvents. The reaction ability of methanol with AAPBA was stronger than that of β-CD with AAPBA; moreover, the stereoinhibition of methanol was weaker than that of β-CD. When the copolymer was put into methanol, the bonds of β-CD with AAPBA were broken, and straight-chain polymers containing the AAPBA–methanol complex were generated, which made the copolymer dissolve easily in methanol.

The copolymer containing AAPBA groups and β-CD groups exhibited a self-assembling behavior in the H_2O–MeOH cosolvent. When H_2O was added in the system, the β-CD moieties of the polymer rolled up because of the hydrophilicity of β-CD. In the initial stage of the self-assembling process, there was only a small amount of water in the system, and the AAPBA groups in the copolymer reacted with the methanol molecules to lead the rolls of the polymer to dissociate. With increasing H_2O amount, the methanol drops were wrapped up inside the microspheres under stirring, and β-CD moieties were on the surface of the microspheres. The drug dissolved in MeOH was also wrapped inside the microspheres; this is shown in Figure 3.

A scanning electron microscopy photograph of poly[AAPBA-*co*-(MAH–β-CD)] microspheres is shown in Figure 4. The microspheres were basically uniform, and the average diameter was in the range 100–300 nm.

We synthesized a series of copolymers with various ratios of AAPBA to MAH–β-CD, in which the crosslinking agents were added. The crosslinking copolymers basically did not self-assemble, and no microspheres were found.

In summary, the self-assembling behavior of the polymer resulted not only from the interaction between AAPBA groups and the alcohol's solvents but also from the hydrophilic properties of β-CD groups.

Drug-release function of the microspheres

We studied the drug release of the microspheres by monitoring the maximum absorption of gliclazide (λ = 225 nm) at pH = 9.0 and 37°C. The release curves of the macrospheres loaded with the drug are shown in Figure 5.

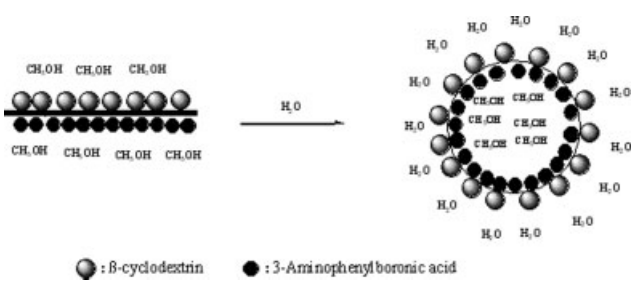

Figure 3 Sketch map of the self-assembly of the copolymer.

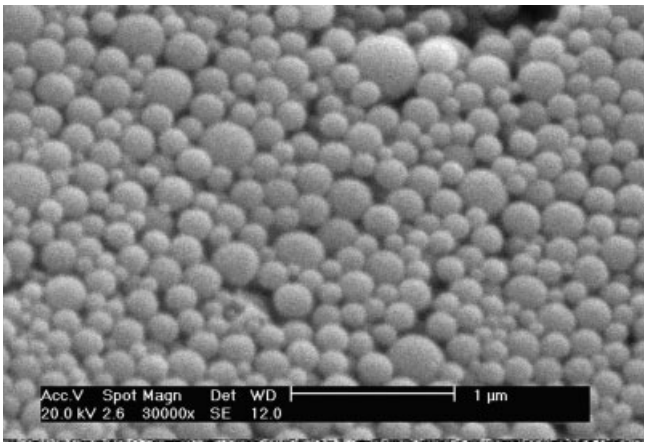

Figure 4 Scanning electron microscopy photograph of AAPBA–(MAH–β-CD) microspheres.

We observed four features in Figure 5: (1) there were an obvious sudden release stage (before 30 min) and a sustained release stage (after 1 h) for every sample, (2) the sudden release concentration and sustained release time of the microspheres with a ratio of MAH–β-CD to AAPBA of 1 : 3 (w/w) were much higher and longer than the others, (3) the sudden release concentration and sustained release time decreased with decreasing MAH–β-CD content in the copolymer microspheres, and (4) after certain amounts of the drug were released, the microspheres stopped releasing the drug because the drug encapsulated inside microspheres could not be released without stimulation of glucose. These results not only proved the component of the microspheres but also indicated that the drug encapsulated inside the microspheres could not be released without stimulation of glucose.

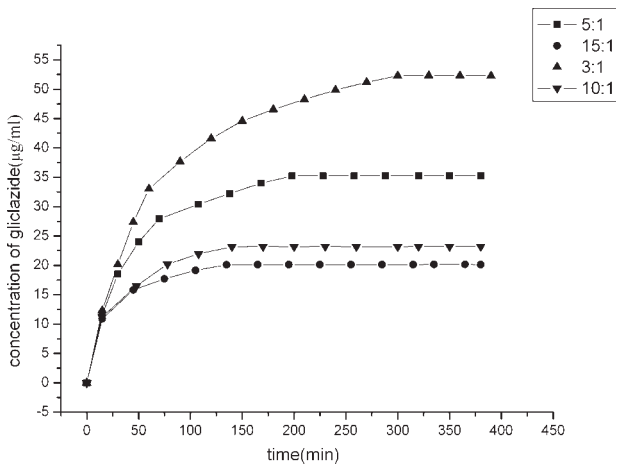

Figure 5 Plots of controlled release in phosphate-buffered solution without glucose.

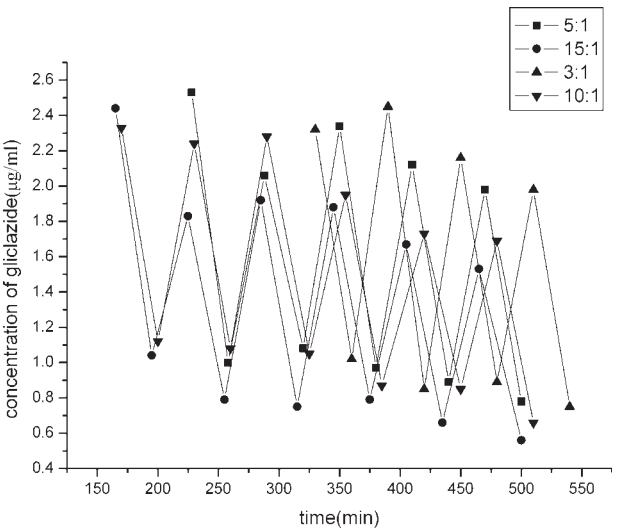

Figure 6 Plots of self-regulated release of AAPBA–(MAH–β-CD) gliclazide. Microspheres in the solution contained 3 g/dm^3 glucose.

Glucose-sensitive properties of the microspheres

The microspheres loaded with the drug at various ratio (w/w) of monomers were put in mediums containing 3 g/dm^3 of glucose and in a medium without glucose; the absorptions via time (hours) plots are shown in Figure 6.

The results show that the microspheres loaded with the drug responded rapidly to the glucose in 30 min and released rapidly in the solution containing 3 g/dm^3 glucose, which is consistent with the blood glucose concentration for the diabetes. The drug release stopped automatically without glucose in the medium. These results show that the materials loaded with the drug possessed very good stimulus–response sensitivity to glucose. The experiments were carried out several times, and the on–off regulated release of gliclazide was successfully repeated in a synchronizing manner four to six times with a change in the concentration of external glucose (Fig. 6), and the responding ability continued for 6 h, which indicated that the microspheres loaded with the drug could multideliver the drug and meet clinical requirements.

CONCLUSIONS

New amphiphilic copolymers microspheres were prepared with various ratios (w/w) of monomers. The self-assembling behavior of the microspheres loaded with gliclazide were studied in CH_3OH–H_2O cosolvent. The results of the *in vitro* release experiments of the microspheres loaded with the drug show that after the drug release on the surface of the microspheres was finished, the drug encapsulated inside the microspheres could not be released into the medium. The results of the *in vitro* oscillating release tests indicate that the microspheres responded to glucose rapidly in 30 min, and the microspheres exhibited self-regulated on–off release behavior four to six times between the solution with 3 g/L glucose and the medium without glucose in 6 h; this meets the clinical requirements of multidrug delivery.

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