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SHORT COMMUNICATION

Photo-induced synthesis glucose-responsive carriers for controlled release of insulin in vitro

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Abstract The glucose-responsive amphiphilic poly(PEGMA-*co*-AAPBA) copolymer micelles as drug carriers have been developed using 3-phenylpyruvic acid (3-PPA) as photoinitiator. Under the light irradiation, 3-PPA could be self-polymerized into the trimer structure to execute the emulsifier and initiator functions that confirmed by gas chromatography-mass spectrum (GC-MS) analysis. The structure and morphology of resultant poly(PEGMA-*co*-AAPBA) micelles were characterized by ^{1}H NMR and TEM. Insulin, as the model drug of diabetes, was loaded into micelles during the photopolymerization process. The as-prepared polymeric micelles exhibited the excellent glucose sensitivity. The loaded insulin could be released from micelles triggered by regulation of temperature and glucose concentration in the environment. The new drug carriers provided a potential application for the therapy of diabetes based on glucose-sensitive controlled drug delivery systems.

Keywords Photopolymerization · Glucose sensitive · Insulin · Drug release

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Introduction

Photopolymerization is one of significant synthesis methods, which has attracted increasing attention due to its facile controllable reaction by the light source. It holds numerous applications in coatings, adhesives, inks, printing plates, optical waveguides, microelectronics, biomaterials, etc. [1–5]. In the photochemical systems, photoinitiator plays a vital role in initiating the polymerization by generating active radicals under UV light irradiation through cleavage and hydrogen abstraction reactions [6]. Benzophenone and its derivatives are widely used as photoinitiator [7–10]. For example, Chung et al. [11] reported that a series of urethane acrylate (UA) polymers had been prepared initiation by polyurethane (PU)-grafted benzophenone via photopolymerization method. Shi et al. [12] synthesized a novel polymeric photoinitiator based on benzoxazine by introducing 4-hydroxy benzophenone, diglycolamine, and paraformaldehyde into the macromolecular chain. However, most photoinitiators are water insoluble or oil soluble. This inevitably uses organic solvents in the photopolymerization process. Development of water-soluble photoinitiators provides a good strategy to resolve the serious environmental pollution caused by organic solvents. Aliphatic ketones containing carbonyl and carboxyl groups can be used as water-soluble photoinitiators and emulsifier [13–15]. For example, Griffith and coworkers [16] have reported a double-tailed surfactant product via spontaneously self-assemble into stable vesicles using 2-oxooctanoic acid (2-OOA) under irradiation, resulting in the production of the radical intermediate 2-OOA. Then, two 2-OOA radicals recombine to form the OOA-OOA dimmer, a double-tailed surfactant followed by self-assembly. Recently, our groups have reported that the multi-sensitive micelles were prepared via a photo-initiated polymerization and self-assembly route using 2-ketobutyric acid (2-KBA) as effective water-soluble

photoinitiator and emulsifier [17]. It provides a new platform for development of drug delivery vehicles with reducing possible utilization of organic solvents, initiators and surfactants.

Herein, we report using 3-phenylpyruvic acid (3-PPA) as a new aromatic ketones photoinitiator and emulsifier to synthesize amphiphilic copolymers. Poly(ethylene glycol) methyl ether methacrylate was designed as hydrophilic monomer and 3-acrylamide boronic acid as hydrophobic monomer. Amphiphilic poly(PEGMA-*co*-AAPBA) copolymers were synthesized via photopolymerization route. Due to the good biocompatibility of PEG chain and glucose sensitive of phenylboronic acid groups, the poly(PEGMA-*co*-AAPBA) copolymers can be self-assembled to form micelles as drug carriers to encapsulate insulin, a hydrophobic drug. The loaded insulin could be released from micelles triggered by regulation of glucose concentration and temperature in the environment.

Experimental

Materials

3-phenylpyruvic acid (3-PPA, 98 %), poly(ethylene glycol) methyl ether methacrylate (PEGMA, $\overline{M} = 950$), N,N-Dimethylformamide (DMF, AR) were purchased from Aladdin Reagent Co. Ltd (Shanghai, China), and other reagents used as received. 3-acrylamide boronic acid (AAPBA) were synthesized according to the literature procedure [18].

Photopolymerization of poly(PEGMA-*co*-AAPBA)

The poly(PEGMA-*co*-AAPBA) copolymers were synthesized by photo-initiation polymerization. The general procedure was shown as follows: 10 mg of 3-phenylpyruvic acid was dissolved in 0.1 mL of DMF, and then added into 10 mL deionized water with ultrasonicated until becoming a transparent solution. 0.15 g of PEGMA monomer and 0.15 g of AAPBA dissolved in 0.5 mL of DMF added dropwise into the above transparent aqueous solution with ultrasonicated until becoming a homogeneous solution, respectively. The homogeneous solution was transferred to a flask. Then the flask was immersed in liquid nitrogen followed by three cycles of freeze-pump-thaw procedures. The homogeneous solution was reacted under UV irradiation for 6 h. The obtained product was dialyzed against deionized water for 3 days using a dialysis bag (MWCO=3500 Da) to remove the DMF. The final product was freeze-dried for 24 h.

Photopolymerization of insulin-loaded poly(PEGMA-*co*-AAPBA)

The insulin-loaded poly(PEGMA-*co*-AAPBA) copolymers were prepared via one-pot photopolymerization method under UV irradiation in the same way as the procedure for synthesis of poly(PEGMA-*co*-AAPBA) copolymers. Briefly, 2 mg of insulin dissolved in certain water and ethanol mixture. Then, the insulin solution was added into the above homogeneous photopolymerization system. The oxygen in the solution was removed by three cycles of freeze-pump-thaw procedures. The polymerization was conducted under UV irradiation for 6 h. The obtained product was dialyzed against deionized water for 3 days using a dialysis bag (MWCO=7000 Da) and freeze-dried for 24 h.

Characterization

The ^{1}H NMR spectra were recorded on a Bruker AV 400 MHz spectrometer using $CDCl_3$ as solvent and tetramethylsilane as internal standard. Transmission electron microscopy (TEM) measurements were recorded on JEM-2100 with an accelerating voltage of 200 kV. Dynamic light scattering (DLS) measurements were performed in aqueous solution using a Malvern Zetasizer Nano Series instrument. Gas chromatography-mass spectrum (GC-MS) analysis was performed on gas chromatography-mass spectrometer (MS 5973I, GC 6890N; Agilent Technologies, USA). The detection temperature was controlled at 50–200 °C. UV-vis spectrophotometry was recorded on Hitachi U3900H instrument. The influence of the glucose on the hydrodynamic radius (R_h) of poly(PEGMA-*co*-AAPBA) copolymer was studied by dynamic light scattering (DLS) at different glucose concentrations ranged from 0 to 10 mg/mL at 37 °C. Circular dichroic (CD) spectral study was undertaken in the region of 190–250 nm (range characteristic of secondary structure of insulin) using 1 cm path length rectangular quartz cuvette to compare and assess structure stability of native insulin and released insulin.

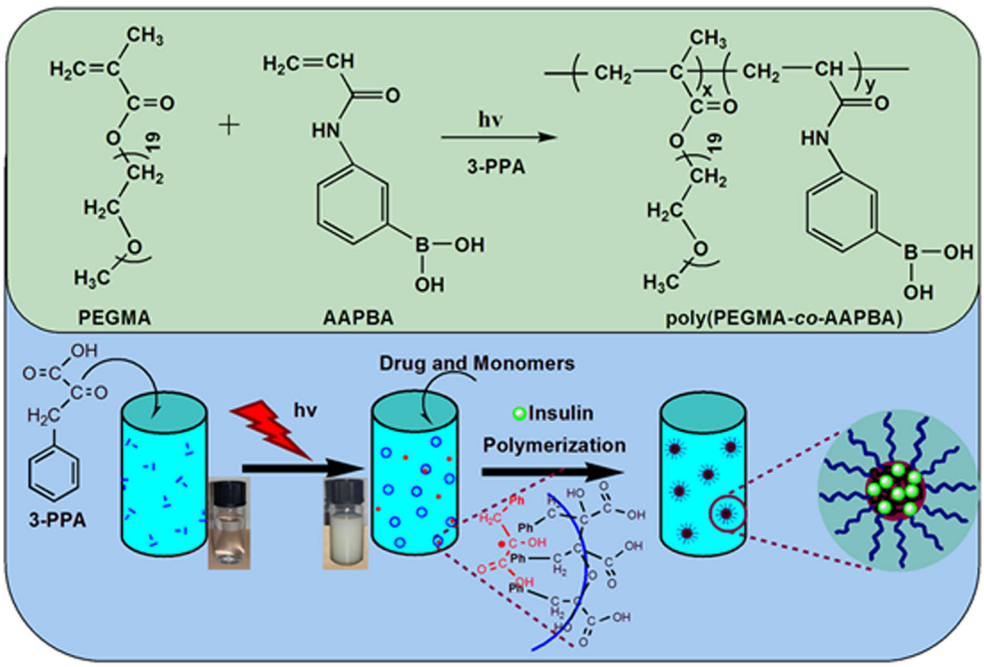
In vitro insulin release from the insulin-loaded micelles was evaluated with different glucose concentrations (0, 1, 3, and 5 mg/mL) or temperature (25, 37, and 40 °C) in PBS at pH 7.4. Typically, 10 mg insulin-loaded micelles solid was first dispersed in 5 mL of PBS and subsequently introduced into a dialysis bag (MWCO 7000 Da). The release experiment was initiated by placing the end-sealed dialysis bag into 150 mL of PBS at 37 °C with continuous shaking at 60 rpm. At a predetermined time point, the same amount of release medium was taken out and replaced by the same volume of fresh PBS. The drug concentration was detected by UV-vis spectrophotometry absorbance at 235 nm.

Results and discussion

The synthetic route of poly(PEGMA-*co*-AAPBA) copolymer was shown in scheme 1. 3-PPA is kind of aromatic acid. It can absorb light through its carbonyl chromophore. An absorption peak concentrated at 283 nm can be observed in UV-vis spectrum. After UV irradiation, the color of 3-PPA aqueous solution becomes milky color, which suggests the formation of micelles due to the 3-PPA photolysis. The photolysis of 3-PPA under UV irradiation was confirmed by GC-MS analysis. As shown in Fig. 1a, 3-PPA molecule is divided into different ion peaks (*m*/*z* 42, 50, 77, and 105) in the dark. However, the dimer $(PPA)_2$ (*m*/*z* 329.2) and trimer $(PPA)_3$ (*m*/*z* 469.3) molecular structures are formed under the assistance of UV irradiation, as shown in Fig. 1b. Figure 1c illustrates the photochemical and polymerization mechanism resulting in the production of the dimer or trimer molecule, as well as radicals. 3-PPA first absorbs light through its carbonyl chromophore, resulting in the production of radicals, analogous to the well-known photochemistry of pyruvic acid [17, 19, 20]. 3-PPA is reversibly hydrated in aqueous solution. In aqueous solution, some 3-PPA exists in its keto form. The keto form contains a UV chromophore, which can be excited in the near-UV state to induce photolysis. 3-PPA molecule can react with a ground-state 3-PPA molecule to efficiently form the radical intermediate 3-PPA. Some radicals can be reacted each other to form dimer molecules. The dimer molecules can be further reacted with the radical intermediate 3-PPA·to form trimer molecules. The peak of *m*/*z* 329.2 is attributed to the molecular weight of the dimer $(PPA)_2$. But the measured molecular weight of trimer $(PPA)_3$ has slight deviations compared with the theoretical value due to the low amount of $(PPA)_3$ and easy to cleavage. In addition, the ion trap (ionization source) of mass spectrometers also causes the deviations [21]. The dimer and trimer molecules contain hydrophobic aromatic ketones and hydrophilic carboxyl groups. They can be acted as the surfactant or emulsifier to form micelles in solution. The free radicals are dissociated in solution to initiate the polymerization of monomers.

The poly(PEGMA-*co*-AAPBA) copolymers were synthesized via photo-initiated polymerization. The resultant poly(PEGMA-*co*-AAPBA) copolymers were firstly confirmed by ^{1}H NMR analysis. As shown in Fig. 2, the peaks at 7.00, 7.52, and 7.72 ppm were attributed to the phenyl protons, and the peaks at about 8.0 ppm were assigned to secondary amine proton. The peaks at 3.64 and 4.25 ppm were attributed to the protons from PEG segment. Combined with the integral value of phenyl protons at 7.00~7.72 ppm and PEG segment protons ($-CH_2CH_2O-$) at 3.64 and 4.25, the ratio of the degree polymerization *x* and *y* can be calculated around 1:3.97. These characteristic peaks indicate that poly(PEGMA-*co*-AAPBA) copolymers were successfully synthesized via photopolymerization method.

Due to the amphiphilic property of copolymers, they can be further self-assembled into micelles in the aqueous solution. The micelles with hydrophilic PEGMA segment as the shell and hydrophobic PAAPBA segment as the core are finally obtained, as shown in Fig. 3a. The micelles with the spherical structure can be observed. The diameter of insulin-loaded micelles (~100 nm) is larger than that of copolymer micelles (~50 nm) which analyzed by transmission electron microscope (TEM) measurement. The average hydrodynamic diameter of poly(PEGMA-*co*-AAPBA) copolymer is around 190 nm obtained from the



Scheme 1 Synthetic route of poly(PEGMA-*co*-AAPBA) copolymer using 3-PPA as photoinitiator

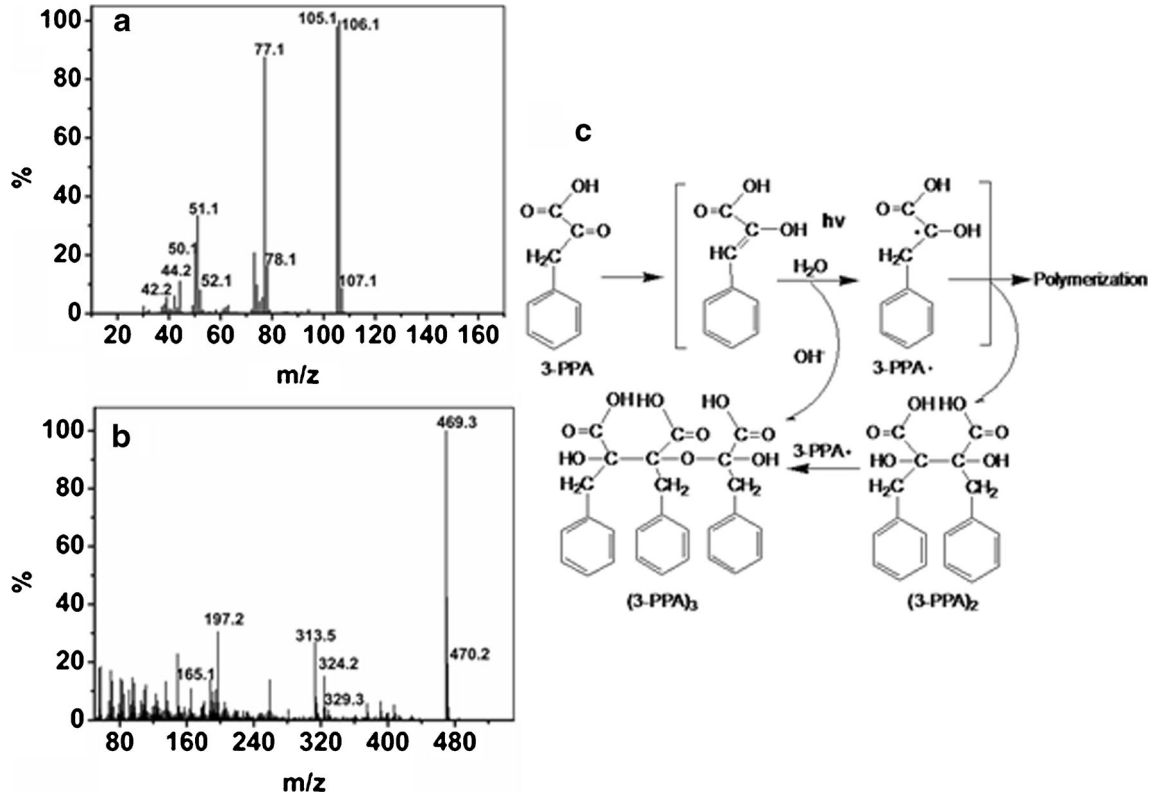


Fig. 1 GC-MS spectra of 3-PPA **a** in dark and **b** under UV light irradiation. **c** Photochemical and polymerization mechanism for the production of the dimer or trimer molecule from 3-PPA with assistance of light irradiation

dynamic light scattering (DLS) analysis compared with that of insulin-loaded poly(PEGMA-*co*-AAPBA) micelles at around 230 nm (Fig. 3b). The average diameter determined by DLS is larger than that determined by TEM. This discrepancy is widely considered to be induced by the process of sample preparation and the difference of investigation method between DLS and TEM [22–25].

To investigate the glucose sensitivity of the as-prepared polymeric micelles, the hydrodynamic radius of the copolymer micelles under different glucose concentrations were measured by dynamic light scattering (DLS). As shown in Fig. 4a, the average hydrodynamic radius is increased from 186.5 to 433.2 nm with glucose concentration range from 0 to 10 mg/mL. Phenylboronic acid (PBA) has widely selective glucose sensitivity in aqueous milieu due to the specific interaction between boronic acids and cis-diol compounds by forming a five- or six-membered boronic cyclic ester [26–29]. The concentration of charged phenylborates is improved with increasing the concentration of glucose; and the hydrophilicity of the copolymers with pendant phenylborate moieties is improved as well. The hydrophilic/hydrophobic balance shifts to a more hydrophobic nature, resulting in an increase of the particle size. It presented a good glucose response, which could be a promising candidate for glucose-response drug carriers.

The hydrophobic drug, insulin, was chosen as a model drug to investigate release behaviors of the as-prepared copolymer micelles as carriers. The control experiment was conducted in PBS at pH 7.4 and 37 °C. Then, the environmental factors, such as temperature and glucose concentration, were adjusted to investigate the stimuli-

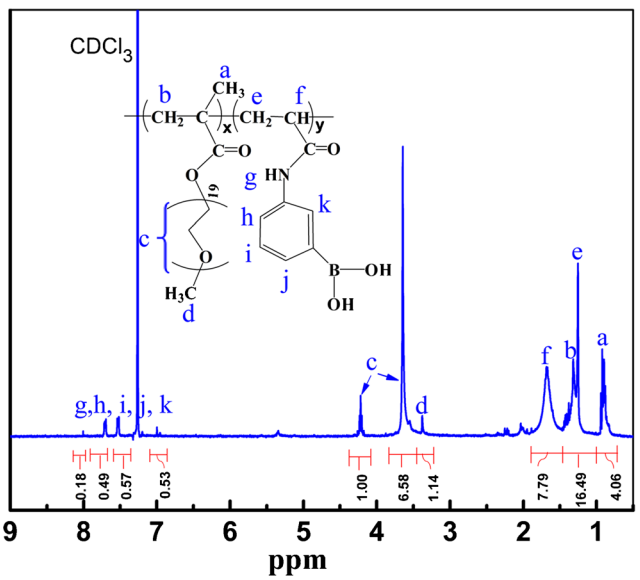


Fig. 2 ^{1}H NMR spectrum of poly(PEGMA-*co*-AAPBA) copolymer using $CDCl_3$ as solvent

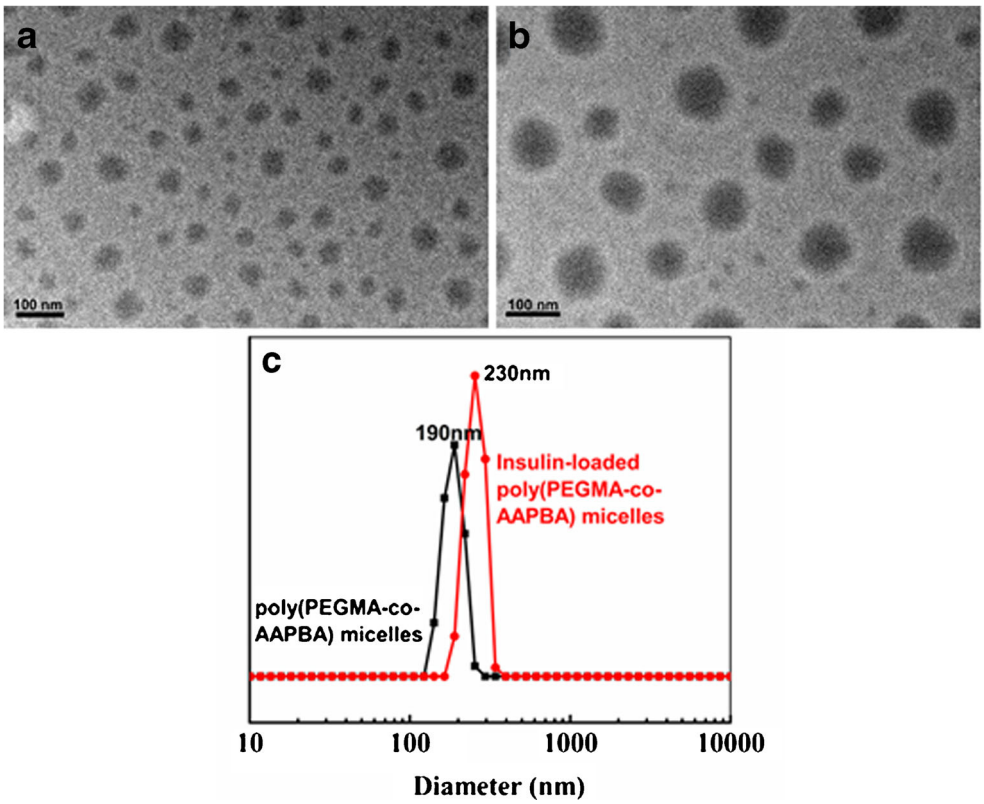


Fig. 3 TEM images of poly(PEGMA-*co*-AAPBA) micelles **a** without and **b** with loaded insulin. **c** DLS analysis of micelles without and with loaded insulin

controlled release of drug. As shown in Fig. 4b, drug-released rate can be accelerated with increasing the temperature due to the swelling of the micelles and higher diffusion rate. The effect of glucose concentration on released rate is shown in Fig. 4c. Because of the affinity interactions between phenylboronic acid and the hydroxyl

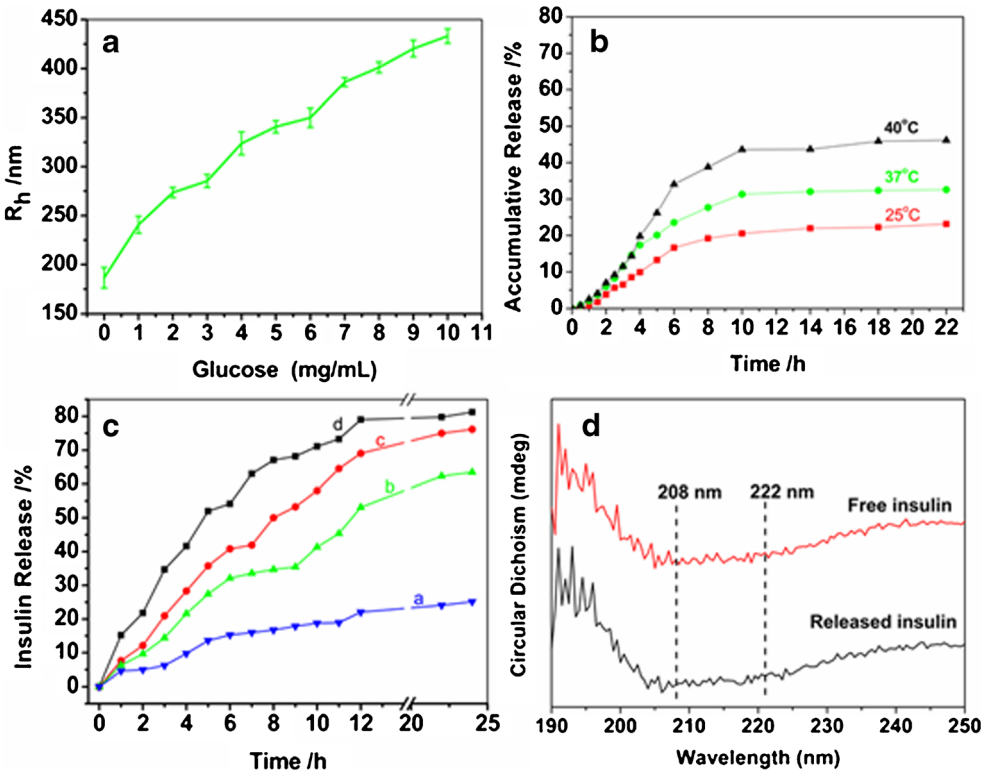


Fig. 4 **a** Dependence of the hydrodynamic radii (R_h) of poly(PEGMA-*co*-AAPBA) micelles on glucose concentration. **b** The release of insulin under different temperature and **c** glucose concentration (*a* 0, *b* 1, *c* 2, and *d* 5 mg/mL). **d** Circular dichroism (CD) spectra of free and released insulin

groups of glucose, the released rate can be accelerated with increasing the glucose concentration. Without glucose in the PBS solution, only 25.1 % insulin has been released after 24 h due to the drug diffusion. However, the amount of released insulin is increased to around 63.4 % after 24 h with the concentration of glucose at 1 mg/mL. Further increasing the concentration of glucose to 5 mg/mL in the PBS solution, about 81.2 % of loaded insulin can be released. The higher concentration of glucose in medium generates more hydrophilic glucose-PBA complex, leading to swell of micelles and release of loaded drug as well.

The activity of the released insulin was assayed for the structure stability analysis of released insulin using CD spectra. The micelles containing insulin was prepared and allowed to release insulin for 24 h at 37 °C. CD spectrum of the released insulin was compared with native insulin dissolved in 0.05 N HCl. Native insulin showed two negative bands at 208 and 222 nm in the far-UV region (Fig. 4d), which matched with the previous observations [18, 30, 31]. The CD band at 208 nm primarily arises from the α-helix structure, while that at 222 nm corresponds to β-pleated sheet structure. The ratio between both bands ($[\Phi]_{208}/[\Phi]_{222}$) can be used to generate a qualitative measure of the overall conformational structure of insulin. As shown from the CD spectra, released insulin displayed similar spectrum as that of the native insulin. The $[\Phi]_{208}/[\Phi]_{222}$ ratio (1.34) for released insulin was close to that of native insulin (1.12), indicating that secondary structure of the insulin had not been distorted after the release.

Conclusion

The polymeric micelles were prepared by the photopolymerization of poly(ethylene glycol) methyl ether methacrylate and 3-acrylamide boronic acid using 3-phenylpyruvic acid (3-PPA) as photoinitiator. Under UV light irradiation, 3-PPA can be excited to form radical intermediate 3-PPA·. Some radicals can be reacted to each other to form dimer or trimer molecules that can be acted as the surfactant or emulsifier to form micelles in solution. The other free radicals are dissociated in solution to initiate the polymerization of monomers. The model drug (insulin) can be loaded into the copolymer micelles during the photopolymerization process. The loaded insulin can be released with different rate under different stimuli factor. The released insulin remained its secondary structure similar to the native insulin.

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