Electronic Supplementary Information

Development of novel self-assembled poly(3-acrylamidophenylboronic acid)/ poly(2-lactobionamidoethyl methacrylate) hydrid nanoparticles for improving nasal adsorption of insulin

Cui Cheng, Xinge Zhang*, Yanxia Wang, Chao Zheng, Zhentan Lu, Chaoxing Li*

The Key Laboratory of Functional Polymer Materials of Ministry Education.

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

* Corresponding author:

The Key Laboratory of Functional Polymer Materials of Ministry Education. Institute of Polymer Chemistry, Nankai University, 94 Weijin Road, Tianjin 300071, China

Tel: +86-22-23501645; Fax: +86-22-23505598

E-mail address: zhangxinge@nankai.edu.cn; lcx@nankai.edu.cn

S1. Materials.

3-Aminophenylboronic acid monohydrate was purchased from Nanjing Kangmanlin Chemical Industry Co. Ltd. (Nanjing, China) without further purification before use. 1-Dodecanethiol and tricaprylylmethylammonium chloride was purchased from Alfa Aesar (Tianjin, China). Fluorescein isothiocyanate (FITC) was purchased from Beyotime Institute of Biotechnology (Nantong, China).

S2. Characterizations.

The FT-IR spectra were recorded respectively on a Fourier Tranform Infrared Spectrometer (FTS-6000, Bio-Rad Co.) with a KBr tablet containing the powders of above samples at a resolution of 8 cm^{-1}. ^{1}H-NMR spectra were recorded at room temperature on a Varian UNITY-plus NMR spectrometer at 400 MHz. The thermogravimetric analysises of dry pAPBA/pLAMA NPs and the solid mixture of pAPBA and pLAMA were conducted in nitrogen, at a heating rate of 5 °C/min between 25 and 800 °C, using a thermogravimetric analyzer (TGA; TG 209, NETZSCH).

S3. Synthesis of 2-lactobionamidoethyl methacrylate (LAMA).

Lactobionolactone (10.0 g, 29.4 mmol) was dissolved in methanol at 40 °C. After cooling to room temperature, 2-aminoethyl methacrylate hydrochloride (10.0 g, 60.4 mmol), triethylamine (10.0 mL) and hydroquinone (0.25 g, 2.3 mmol) were added to the lactobionolactone solution. Then the mixture was stirred for 5 h, concentrated under vacuum and precipitated into isopropanol. The formed white solid was filtered, washed with isopropanol, dried under vacuum. Purified LAMA was obtained in 65%

yield. δ_H (400 MHz; D_2O): 6.1 (1H, s, -CH_2=C), 5.7 (1H, s, -CH_2=C), 4.5-3.2 (16H, m, -CH_2O, -CH_2N, -CHO), 1.9 (3H, s, -CH_3).

S4. Synthesis of raft agent 4-cyanopentanoic acid dithiobenzoate (CPADB).

Distilled ethyl acetate (80.0 mL), dry ACVA (5.84 g, 21.0 mmol) and Di(thiobenzoyl) disulfide (DTBD) (4.25 g, 14.0 mmol) were added to a 250 mL round-bottomed flask and then the reaction solution was heated reflux for 18 h at 85 °C. After this, the ethyl acetate was removed in vacuum. The crude product was isolated by column chromatography (silicagel 60 Å, 100-200 mesh) using ethyl acetate:hexane (2:3) as eluent. Red fractions were collected and dried by anhydrous sodium sulfate overnight. After removing the solvent mixture under vacuum, the red oily residue was placed in a freezer at -20 °C to crystallize. Finally, the target product was recrystallized from benzene. δ_H (400 MHz; $CDCl_3$): 7.9-7.4 (m, 5H, Ar-H), 2.8-2.5 (m, 4H, -CH_2), 1.9 (s, 3H, -CH_3).

S5. Preparation of 3-acrylamidophenylboronic acid (APBA).

3-aminophenylboronic acid monohydrate (5.0 g, 32.2 mmol) was dissolved in sodium hydroxide solution (40 mL, 129.0 mmol), then freshly distilled acryloyl chloride (3.3 mL, 40.0 mmol) was added dropwise to the 3-aminophenylboronic acid solution in an ice bath, while stirring over a period of 30 min. The mixture was stirred for a further 2 h at room temperature. Then the beige precipitate was obtained by moderating the mixture to pH= 8 using 0.1 M dilute HCl. The precipitate was filtered and washed five times with cold water, and then recrystallized from water (80 °C). The resulting crystals were filtered, crushed, and left to dry in a desiccator. δ_H (400

MHz; DMSO-d_6): 10.1 (2H, s, -$B(OH)_2$), 7.9, 7.8, 7.5, 7.3 (4H, m, Ar-H), 6.5, 6.3, 5.7 (3H, m , CH_2=CH-).

S6. Synthesis of 2-dodecylsulfanylthiocarbonylsulfanyl-2-methylpropionic acid (DMP).

1-Dodecanethiol (8.1 g, 0.04 mol), acetone (1.9 g, 0.3 mmol), and tricaprylylmethylammonium chloride (0.6 g, 1.6 mmol) were added to a jacketed reactor cooled to 10 °C. Under a nitrogen atmosphere, 50% NaOH solution (3.3 g, 0.04 mol) was added to the mixed solution over 20 min. The reaction was stirred for an additional 15 min before carbon disulfide (3.0 g, 0.04 mol) in acetone (4.1 g, 0.07 mol) was added over 20 min. Ten minutes later, chloroform (7.1 g, 0.06 mol) was added slowly, followed by dropwise addition of 50% sodium hydroxide solution (16 g, 0.2 mol) over 30 min. The reaction was stirred overnight and then 60 mL of water was added, followed by 10 mL of concentrated HCl (caution! gas, odor) to acidify the aqueous solution. Nitrogen was purged through the reactor with vigrous stirring to help evaporate off acetone. The solid was collected with a Buchner funnel and then stirred in 100 mL of 2-propanol. The undissolved solid was filtered off and the 2-propanol solution was concentrated to dryness, and the resulting solid was recrystallized from hexanes. δ_H (400 MHz; $CDCl_3$): 0.89 (3H, t, -CH_3), 1.26-1.69 (20H, m, -CH_2-), 1.73 (6H, s, -CH_3), 3.29 (2H, t, -CH_2-), 10.2 (1H, s, -COOH).

S7. Synthesis of fluorescein isothiocyanate (FITC) labeled insulin.

2 mL FITC solution in DMSO (1 mg/mL) was slowly added into a 10 mL insulin solution (1mg/mL, carbonate-bicarbonate buffer solution (pH 9.5). After stirring in

the dark at 4 °C for 12 h, the coupling reaction was stopped by adding 2 mL NH_4Cl (50 mM). The mixture was stirred in the dark for another 2 h at 4 °C. Unbound FITC was separated by dialysis in the dark. The FITC-labeled insulin was frozen and lyophilized, stored at 4 °C in the dark.

S8.Thermal analysis

The traces of thermogravimetric (TG) and derivative thermogravimetric (DTG) for pAPBA/pLAMA NPs and the solid mixture of pAPBA and pLAMA are shown in Fig. S3. The pAPBA/pLAMA NPs displayed two-stage weight loss patterns. It is obviously different from the degradation of the solid mixture which has four-stage weight loss patterns. However, the weight loss of first stage from 28 to 198 °C of the NPs is similar to that of the solid mixture. It both has an 11 wt% loss, which is attributed to desorption of loosely bound water.[37]

For the solid mixture, there is a 10% weight loss in the second stage from 198 to 282 °C, corresponding to the loss of the pendant lactose residue; a 14% weight loss in the third stage from 282 to 358 °C is assigned to the decomposition of the pLAMA backbone, that is because pLAMA has lower thermal stability;[38] and a 37% weight loss in the fourth stage from 358 to 488 °C is attributed to the degradation of pAPBA.

For the NPs, it has a 54% weight loss from 198 to 488 °C in the second stage. As shown in Fig. S3a, because the complexation between the hydroxyl groups in pLAMA and boronic acid groups in pAPBA increased the thermal stability of the NPs, the degradation rate of the NPs in the range of 198-749 °C is slower than that of the solid mixture.

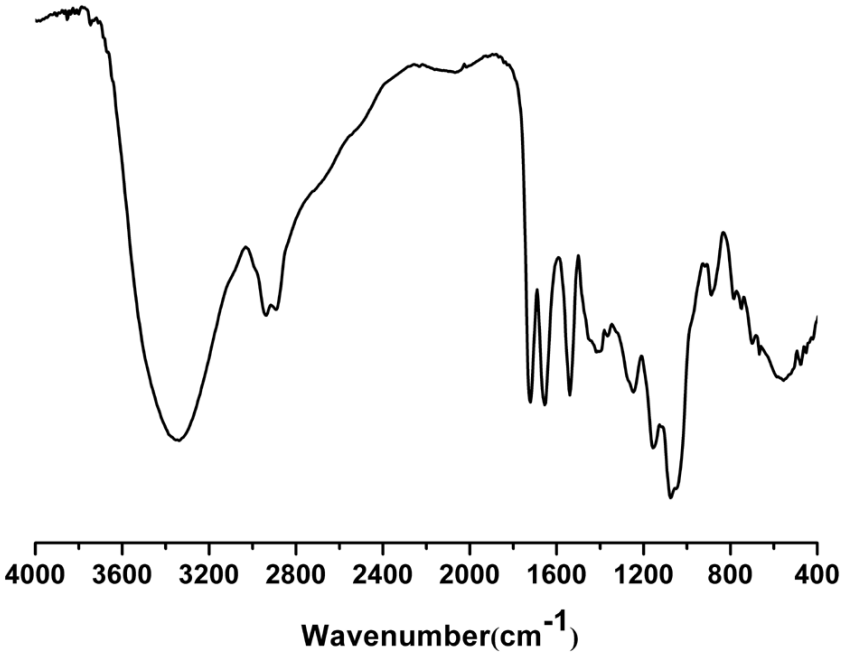

Fig. S1. The FT-IR spectrum of pLAMA.

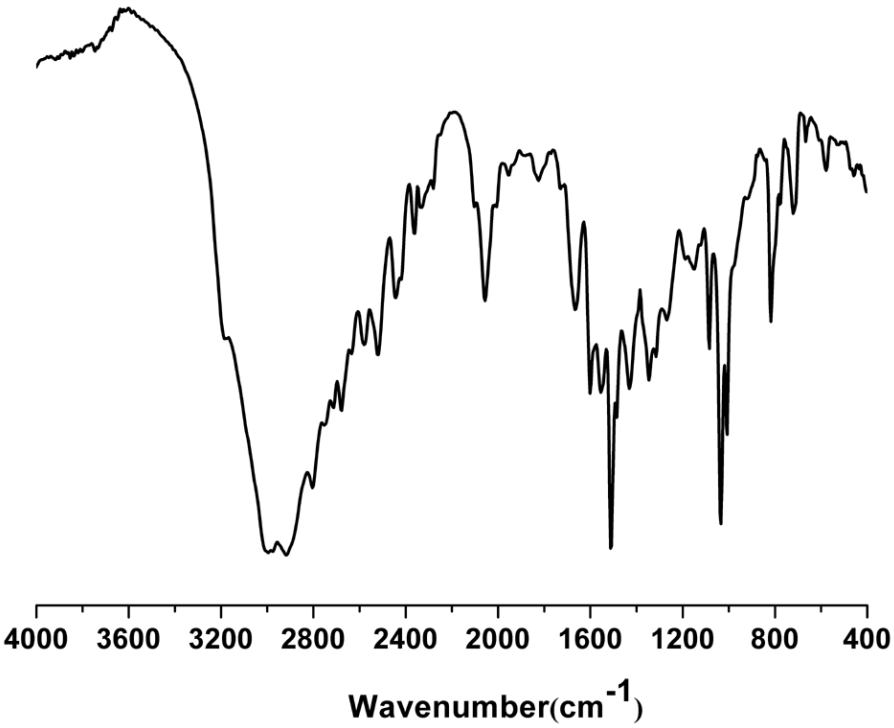

Fig. S2. The FT-IR spectrum of pAPBA.

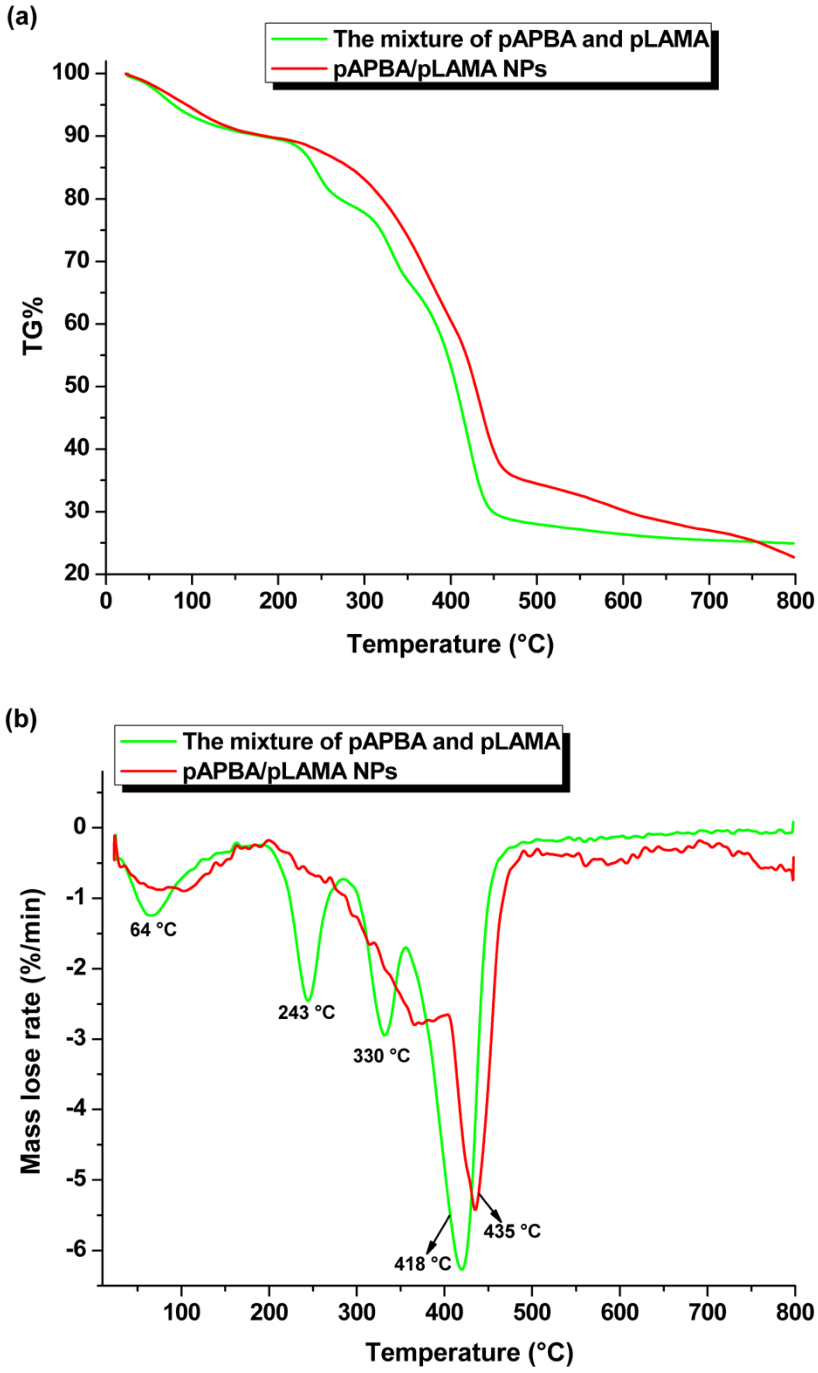

Fig. S3. Thermal analysis of the pAPBA/pLAMA NPs and the solid mixture of pAPBA and pLAMA: (a) TG; (b) DTG.