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Preparation and drug release property of CO_2 stimulus-sensitive poly(*N*, *N*-dimethylaminoethyl methacrylate)-*b*-polystyrene nanoparticles

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ABSTRACT

The CO_2 stimulus-sensitive nanoparticles based on poly(*N*, *N*-dimethylaminoethyl methacrylate)-*b*-poly styrene (PDMAEMA-*b*-PS) were prepared via surfactant-free miniemulsion reversible addition–fragmentation chain transfer (RAFT) polymerization. The as-prepared nanoparticles exhibited core–shell structure with about 120 nm in diameter. Their dispersion/aggregation in water can be adjusted by alternatively bubbling of CO_2 and N_2. Drug release from these nanoparticles can be accelerated (or delayed) by bubbling (or removing) of CO_2.

1. Introduction

Stimuli-responsive polymeric nanoparticles are materials of considerable interest that have been explored as carriers for controlled drug release [1–7]. A myriad of drug delivery systems based on stimuli-responsive polymeric nanoparticles have been investigated in the past decade [8–11]. For instance, poly(*N*-isopropylacrylamide) has been widely used as the base polymer for thermosensitive nanoparticle systems due to its physiologically relevant lower critical solution temperature (LCST) between 31 and 33 °C. Drugs can be quickly loaded by passive diffusion at temperatures below the LCST and slowly released after nanoparticles collapsed above the LCST [12,13]. pH-sensitive nanoparticles made from acrylic-based polymers such as poly(methacrylic acid) exhibit pH-dependent swelling, and drug can be controlled release by adjusting the acid–base property of environment [14]. Apart from this, light-responsive nanoparticles and magnetic field-responsive nanoparticles have also been widely studied as drug carriers [15–17]. However, there are few reports about CO_2-responsive nanoparticles for drug delivery [18,19].

It has been reported that overdose of analgesic causes hypoventilation, an inadequate ventilation to perform gas exchanges in lungs leading to increased CO_2 concentration in the blood [20,21]. By considering the cytotoxicity of drugs, it takes a risk using antidote to prevent respiratory depression [22]. CO_2 stimuli-sensitive drug carrier is desired to control the release of those drugs. Fortunately, *N*, *N*-dimethylaminoethyl methacrylate (DMAEMA) with dimethylamino groups has been proved to be sensitive to CO_2 [23,24]. And it has been widely used as a part of copolymer [25,26]. Hydrogels prepared from poly (DMAEMA-*co*-hydroxyethyl methacrylate) have been employed as glucose responsive insulin delivery vehicles, as well as CO_2 sensors [27]. Zhao et al. have reported sensitivity of nanoparticles prepared with DMAEMA [28]. And poly (DMAEMA)-modified hydrogels as antidote delivery

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vehicles that respond to changes in CO_2 concentration have been fabricated by Satav et al. [29]. A lot of work remains to be done to study drug release property of CO_2 stimulus-sensitive nanoparticles, since nanoparticles are one of the most popular methods to deliver drugs [30,31].

In this report, CO_2 stimulus-sensitive polymeric nanoparticles with PDMAEMA containing dimethylamino groups on the shell have been designed as a drug delivery system. PDMAEMA was firstly synthesized via reversible addition–fragmentation chain transfer (RAFT) polymerization using S-1-dodecyl-S′-(α,α′-dimethyl-α″-acetic acid) trithiocarbonate (DMP) as an initiator. Then, PDMAEMA was further employed as stabilizer, as well as initiator, for miniemulsion RAFT polymerization to copolymerize with hydrophobic monomer styrene to form core–shell structure (Scheme 1).

2. Experiment

2.1. Materails

S-1-dodecyl-S′-(α,α′-dimethyl-α″-acetic acid) trithiocarbonate (DMP) was synthesized according to previously published procedures [32]. Azobisisobutyronitrile (AIBN, 98%) was purchased from East China Chemical Co., Ltd. (Shanghai, China) and purified by recrystallization twice from ethanol and dried in vacuum prior to use. Styrene (St) purchased from Aladdin Reagent Co., Ltd. (Shanghai, China) was washed with 5 wt% NaOH solution and deionized water firstly to remove the inhibitors and then vacuum distilled prior to use. Cyclohexane, *N*, *N*-dimethylformamide (DMF), 2-(dimethylamino) ethyl methacrylate (DMAEMA), Indomethacin (IND) and other regular reagents and solvents were supplied by Aladdin Reagent Co., Ltd. and used as received. MilliQ water was used in all experiments. CO_2 (99.998%) and N_2 (99.998%) were purchased from East China Chemical Co., Ltd. (Shanghai, China) and used as received.

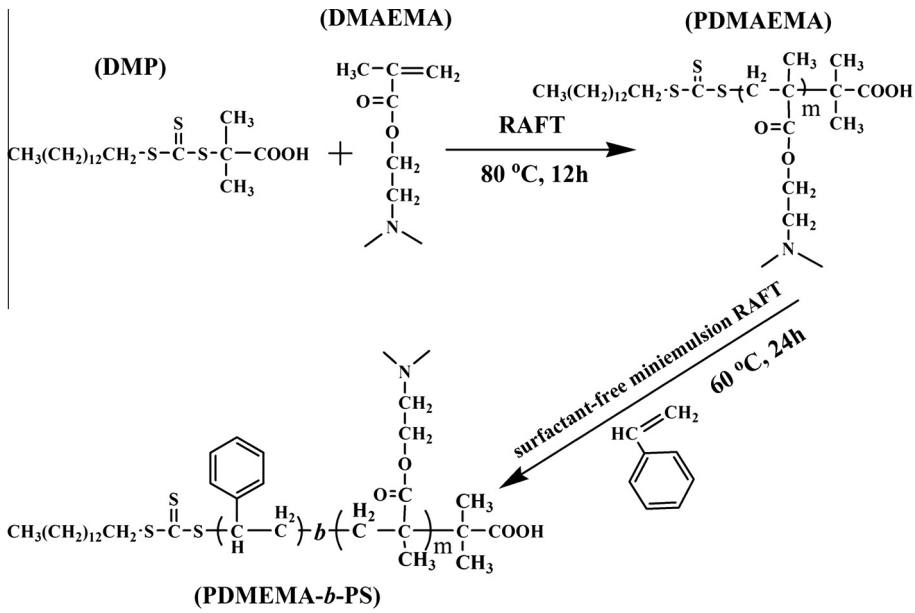
Human cervical carcinoma (HeLa) cells were cultured in dulbecco's modified eagle's medium (DMEM) supplemented with 10% FBS, 1% Penicillin/Streptomycin and 1% L-Glutamine at 37 °C in a fully humidified air atmosphere containing 5% CO_2.

2.2. Synthesis of PDMAEMA

PDMAEMA was prepared via RAFT polymerization (Scheme 1). Briefly, DMP (0.183 g, 0.5 mmol), AIBN (0.016 g, 0.1 mmol) and DMAEMA (5.39 mL, 0.03 mol) were added into a flask with a stirrer. After three times of being vacuumed and pumped with N_2, the flask was kept stirring at 80 °C for 12 h. The resultant product with $M_n = 1.68 \times 10^4$ g mol^{-1}, PDI = 1.15 (GPC) was obtained at >95% yield by lyophilization. ^{1}H NMR ($CDCl_3$, ppm): δ 0.8–0.9 (3 H, –CH_3); 1.8–1.9 (2 H, –CH_2–); 2.20–2.30 (6 H, –N(CH_3)$_2$); 2.50–2.60 (2 H, –CH_2–N); 4.00–4.20 (2 H, –CH_2–CH_2–).

2.3. Synthesis of PDMAEMA-b-PS Nanoparticles

Nanoparticles based on block polymer PDMAEMA-b-PS were synthesized via surfactant-free miniemulsion RAFT polymerization (Scheme 1). It can be described as follows. A solution containing of St (0.165 mL, 1.25 mmol), AIBN (0.0003 g, 0.002 mmol) and cyclohexane (0.055 mL, 0.5 mmol) was mixed with 10 mL aqueous solution of DMP-PDMAEMA (0.168 g, 0.01 mmol). The mixture was stirred for 10 min before added into a flask. Followed with two minutes of ultrasonic, the mixture was kept stirring at 60 °C for 24 h after three times of vacuumed and pumped



Scheme 1. Synthesis of PDMAEMA-*b*-PS via surfactant-free miniemulsion RAFT polymerization.

with N_2. PDMAEMA-*b*-PS with Mn = 3.02 × 10^4 g mol^{-1}, PDI = 1.30 was obtained by lyophilization after 3 days of dialysis against water.

2.4. Drug loading and release

Drug loaded nanoparticles was prepared in the same way as preparation of nanoparticles, except that IND (0.0178 g, 0.05 mmol) was dissolved in dispersed phase prior to emulsifiaction. The release experiment was conducted in phosphate buffer solution (PBS) at pH 7.4, 37 °C. CO_2 was purged into the buffer at different time to investigate control release of drugs conducted by CO_2. Briefly, IND loaded nanoparticles (50 mg) were dissolved in 5 mL PBS before the solution was put into a dialysis bag (MWCO 3.5 kDa) and immersed in a beaker containing 200 mL PBS. The flow rate of CO_2 was controlled to be ~50 mL/min. At predetermined time intervals, 5 mL of liquid was sampled from the outer solution, and then replaced by the same volume of release medium. The drug concentration was detected by measuring UV–*vis* absorbance at 320 nm.

2.5. Characterization

The ^{1}H NMR spectra were recorded on an AVANCE AV 400 MHz Digital FT-NMR spectrometer operating at 400 MHz using deuterated heavy water (D_2O) and chloroform-d ($CDCl_3$) as solvents. Gel permeation chromatographic (GPC) analysis was carried out using a Waters 1525 pumping system (USA) at the flow rate of 1.0 mL min^{-1} with an Ultrahydrogel 500 column (Waters). The eluent was THF. Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet 5700 spectrophotometer using an ATR cell or KBr pellets for samples. The sizes and morphologies of the resultant samples were characterized by JSM-2100 transmission electron microscopy (TEM) at an accelerating voltage of 200 kV, whereby a small drop of sample aqueous solution was deposited onto a carbon-coated copper TEM grid (230 mesh) and dried at room temperature at atmospheric pressure. The sample for TEM was prepared by diffusing 1 mL of emulsion after polymerization in 4 mL H_2O. Dynamic light scattering (DLS) measurements were performed in aqueous solution using a HORIBA Zetasizer apparatus (LB-550 V) equipped with a 5.0 mW laserdiode operating at 650 nm at room temperature. The measurements were conducted in quintuple. The results presented are the average data. UV–*vis* spectra were obtained using a Hitachi U-3010 spectrophotometer.

The relative cytotoxicity of nanoparticles was estimated by MTT assay which is a colorimetric assay for measuring the activity of cellular enzymes. It can be used to measure cellular metabolic activity via cellular oxidoreductase enzymes succinate dehydrogenase and under defined conditions, reflect the number of viable cells. Hela cells were seeded into 96-well plates at 8000 cells per well in 200 μL of complete DEMEM (Dulbecco's modified Eagle's medium) supplemented with 10% fetal bovine serum, 2% sodium bicarbonate, 1% amino acid, and 1% sodium pripate at 37 °C in 5% CO_2 atmosphere for 24 h. After the incubation, 200 μL of DMEM containing appropriate dilutions of nanoparticles were added to the cells. 24 h later, the supernatant fluid was carefully removed, and 90 μL of fresh DMEM was added along with 10 μL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/mL) solution to the cells. Then, 20 μL of MTT (5 mg mL^{-1}) assays stock solution in PBS was added to each well. After 4 h incubation, the medium containing un-reacted MTT was carefully removed. The obtained blue formazan crystals were dissolved in 200 μL $well^{-1}$ DMSO, and the absorbance was measured in a BioTek Elx800 at a wavelength of 570 nm.

3. Results and discussion

Fig. 1 shows the ^{1}H NMR spectra of PDMAEMA and PDMAEMA-*b*-PS. The peaks belong to PDMAEMA become weaken after co-polymerization of styrene, due to its ratio decreased in block copolymer. The appearance of new peaks between 6.48 and 7.25 indicates successful synthesis of block copolymer PDMAEMA-*b*-PS [33,34] The composition of the resultant block copolymers was also confirmed by FT-IR measurements (Fig. S1). As application of mini-emulsion RAFT polymerization technology, global nanoparticles with size about 50–140 nm can be obtained due to its low critical micelle concentration (CMC ≈ 0.1 mg/mL) (Fig. S2). It should be noted that surfactant-free mini-emulsion RAFT polymerization would contributed to nanoparticles' potential application in biosome, because little organic solvent was involved.

CO_2-stimulus sensitivity of PDMAEMA-*b*-PS nanoparticles was assessed in their aqueous solution by bubbling CO_2 and N_2 alternately. As shown in Fig. 2A, the average diameter of original nanoparticles measured by DLS is 228 nm, which is larger than that observed by SEM because of swelling and slight aggregation of nanoparticles in the water. After bubbling of CO_2 into nanoparticles' solution, the size of nanoparticles gets smaller and the emulsion becomes transparent. Once CO_2 is removed, the diameter of nanoparticles can be increased to around 224 nm, and the color of solution changed to be milky-like again. Nanoparticles' reversible sensitivity to CO_2 is

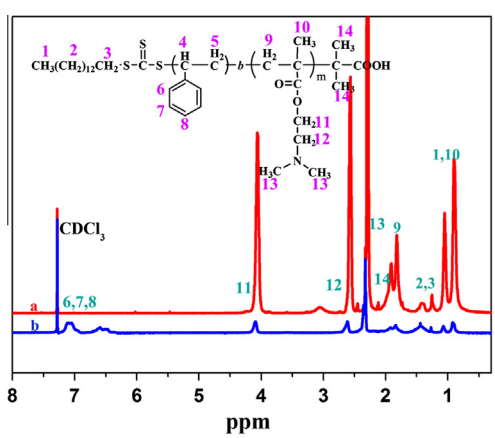


Fig. 1. ^{1}H NMR spectra of PDMAEMA (a) and PDMAEMA-*b*-PS (b).

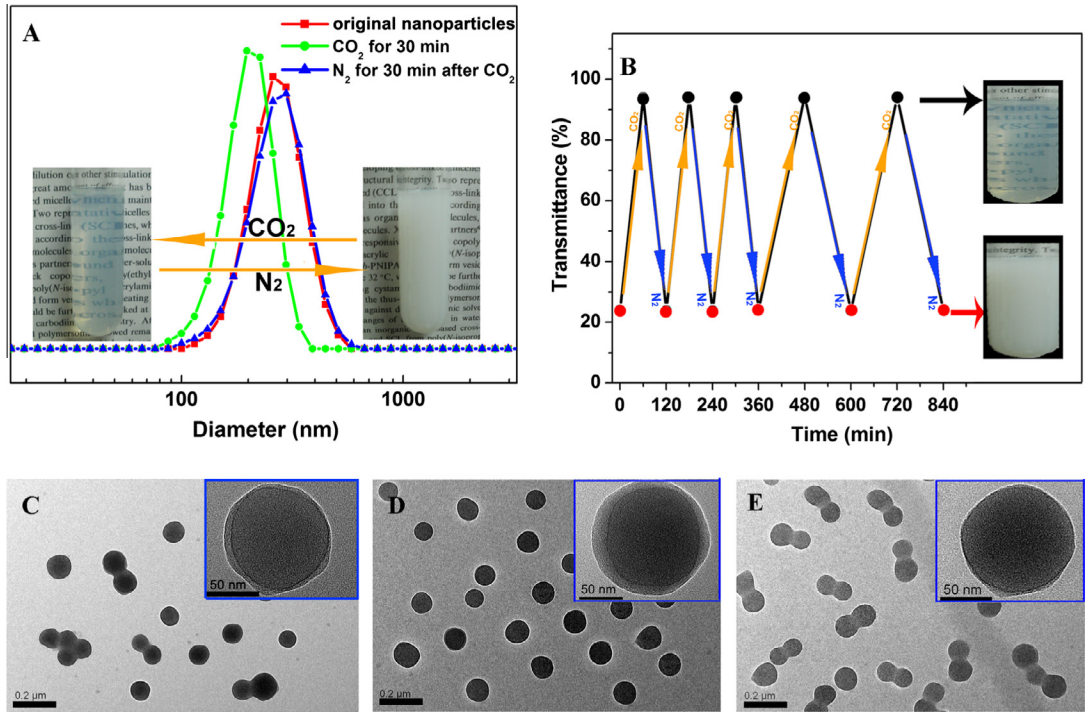
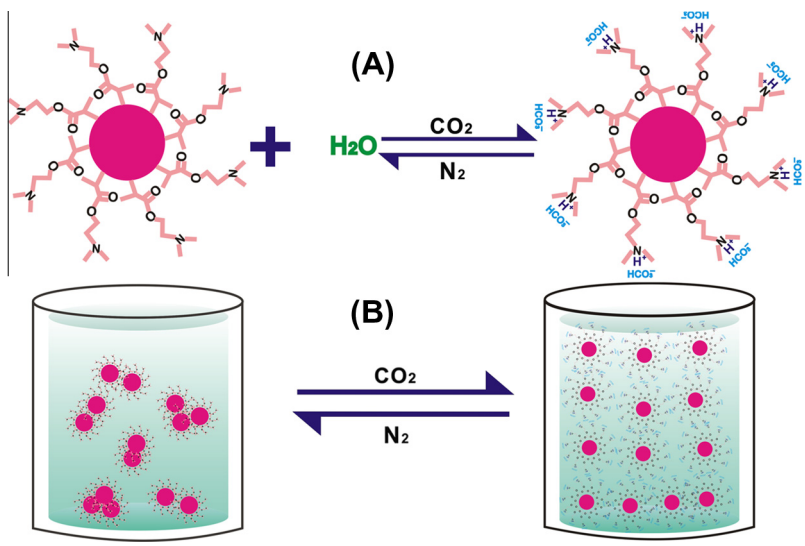


Fig. 2. DLS analysis of nanoparticles after treating with CO_2 and N_2 (A); reversible change of transmittance upon five cycles of CO_2/N_2 bubbling (B); TEM image of original nanoparticles (C), bubbling with CO_2 for 30 min (D), N_2 for 30 min after CO_2 (E).



Scheme 2. (A) Reactions of side chains of PDMAEMA on nanoparticles' surface upon CO_2 and N_2 bubbling; (B) schematic illustration of dispersion of nanoparticles in the presence/absence of CO_2.

confirmed by the change of transmittance upon cycles of CO_2/N_2 bubbling (Fig. 2B). TEM has also been applied to investigate the structure change of nanoparticles under the presence or absence of CO_2 (Fig. 2C–E). It can be founded that nanoparticles have a little aggregated after removal of CO_2. It is consistent with previous reports [35,36]. The shell of nanoparticles in the presence of CO_2 are thicker than that of without CO_2 (Fig. 2D, inset), indicating a decrease on its shell density 23.

The reason for the change of dispersion and structure of nanoparticles can be ascribed to reversible interaction of PDMAEMA and CO_2. In the presence of CO_2, tertiary amine groups of PDMAEMA are protonated due to the formation of carbonic acid in water (Scheme 2), inducing a change

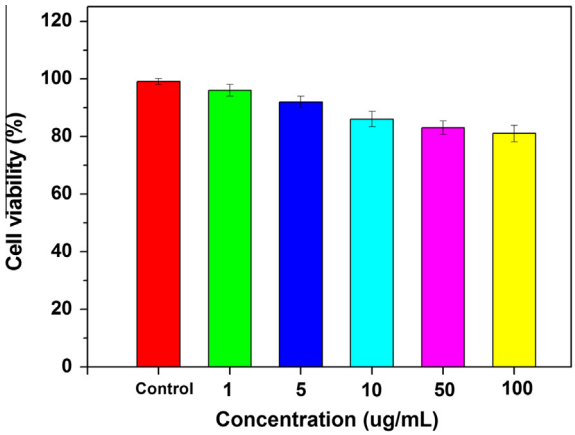


Fig. 3. Viability of Hela cells against nanoparticles for 24 h by MTT assay. Data are presented as average standard deviation ($n = 5$).

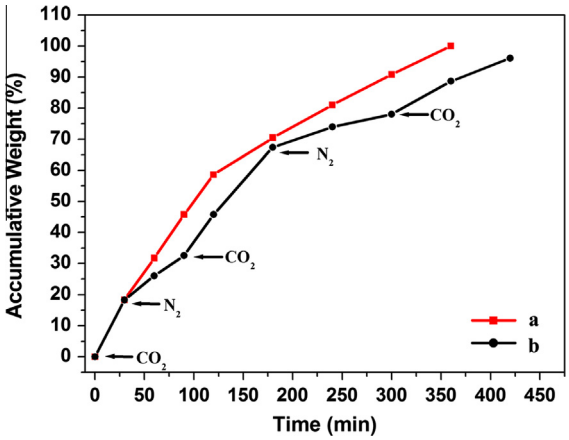


Fig. 5. Drug release from nanoparticles with CO_2 bubbling throughout the whole process (a), and with CO_2/N_2 bubbling at different time (b).

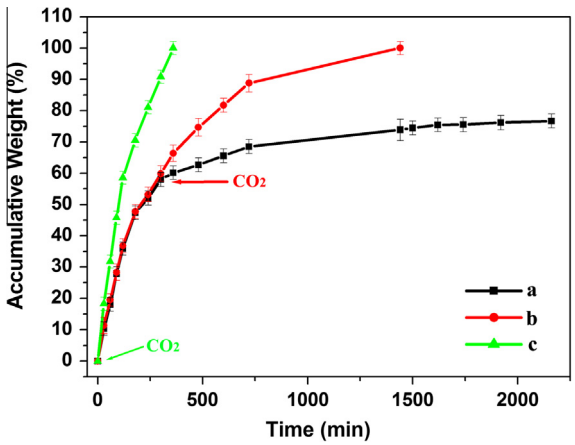


Fig. 4. Drug release from nanoparticles without CO_2 (a); with CO_2 bubbling since 360th min (b); with CO_2 bubbling throughout the whole process (c). Data are presented as average standard deviation ($n = 3$).

of their solubility [37]. The increased solubility of nanoparticles does help to improve the dispersion of the product (Fig. 2D), therefore, the excellently dispersed nanoparticles in water looks transparent. In contrast, de-protonation of PDMAEMA takes place along with the remove of CO_2, causing the aggregation of nanoparticles, which leads turbid of the solution.

The relative cytotoxicity of nanoparticles was estimated by MTT viability assay against Hela cells [37]. Fig. 3 shows the cell viability after 24 h of incubation with nanoparticles at the different concentrations. When the concentration of nanoparticles reaches 100 μg mL^{-1}, the viability of cells is still kept above 80%, indicating low toxicity of the nanoparticles. It partly owes to limited organic solvents were involved during the surfactant-free miniemulsion RAFT polymerization.

Indomethacin, a hydrophobic drug, was chosen as a model molecule to investigate the controlled release properties of the CO_2-stimulus sensitive nanoparticles *in vitro*. The loading efficiency is determined to be 13.06% by UV–*vis* spectrometry. The release of indomethacin from the PDMAEMA-*b*-PS composite nanoparticles was tested in PBS. The cumulative percentage release was plotted against time based on the maximum amount of indomethacin released from the nanoparticles. As shown in Fig. 4, about 77.24% of loaded indomethacin can be released in PBS without assistant of CO_2 (Fig. 4a). The release rate and efficiency can been synergistically improved as CO_2 bubbling. As shown in Fig. 4b, the drug release curve is overlapped with that in Fig. 4a before 300 min without of CO_2 bubbling. After adding the CO_2 bubbling, the release rate is increased and the maximum release amount about 100% can be obtained at 1500 min. While almost of all drug can be released in the short time by bubbling of CO_2 from the beginning, as shown in Fig. 4c. The main reason for these release characteristics can be described as follows. On one hand, separately disperse of nanoparticles in the presence of CO_2 benefits its interaction with buffer, results in acceleration release of drugs. On the other hand, the density of shell has been decreased as PDMAEMA being protonated, which makes it easier for drug to travel through the shell.

In the contrast, when CO_2 were removed from PBS, the drug release rate from nanoparticles can be delayed as comparing with CO_2 bubbling throughout the whole process (Fig. 5a), which is caused by de-protonation of the shell of nanoparticles. Nanoparticles become to be aggregated and shrunk as CO_2 being removed by N_2, retarding the release of drugs. The protonation/de-protonation can be reversibly adjusted by introducing/removing of CO_2, which can be used in control release of drugs from nanoparticles. It is confirmed by the indomethacin release from PDMAEMA-*b*-PS nanoparticles with assistant of CO_2/N_2 bubbling, as shown in Fig. 5b.

4. Conclusion

In summary, novel CO_2 stimulus-sensitive nanoparticles based on PDMAEMA-*b*-PS were prepared via surfactant-free miniemulsion RAFT polymerization. The as-prepare nanoparticles exhibited high gas sensitivity to

CO_2, leading to a control dispersion status in aqueous solution. This work provides a strong platform for the development of reversible regulated drug delivery vehicles. Such CO_2 controlled drug delivery vehicles offer promising opportunities to achieve the desired therapeutic effects of drugs and reduce side effects caused by overdose. It should be pointed out that this new synthetic strategy provides a new idea for preparing candidate drug carriers that "smart" to biomarkers.

Acknowledgments

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Appendix A. Supplementary material

FITR measurements and CMC of PDMAEMA-*b*-PS are presented in Supporting Information. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eurpolymj.2013.07.024.

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