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Synthesis of multi-responsive polymeric nanocarriers for controlled release of bioactive agents†

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Polymeric nanocarriers based on the amphiphilic block copolymer poly(2-dimethylamino)ethyl methacrylate-*b*-poly(2-nireobenzyl acrylate) exhibited multi-responsive to UV, temperature, CO_2 and pH, could be used for controlled release of bioactive agents.

In the few past decades, polymer nanocarriers that respond to environmental stimuli such as light, temperature, pH, and redox potential have been prevalently designed for drug delivery.[1–7] For example, poly(*N*-isopropylacrylamide) based temperature-sensitive micelles have been most commonly studied and used as a representative model to demonstrate how stimuli-sensitive properties can be modulated and utilized for drug delivery.[8,9] pH stimuli-sensitive polymer micelles based on poly(methacrylic acid)[10,11] and UV-sensitive micelles prepared from poly(2-nitrobenzyl methacrylate) have also been widely reported.[12,13] But a majority of them deal with the response to a single stimulus. In nature, the change in behavior of a macromolecule (proteins and nucleic acids) is often a result of its response to a combination of environmental changes, rather than a single factor.[14] Therefore, formulation of materials which can respond to multiple stimuli in a predictable manner would be of great interest.

Poly(2-dimethylamino)ethyl methacrylate (PDMAEMA) is a candidate material for multi-stimuli sensitive micelles as drug carriers.[15,16] In our previous research, we have found that PDMAEMA showed intriguing changes in structures and properties in response to pH and temperature changes.[17] Besides, it has been proven that the tertiary amine groups of PDMAEMA can react with CO_2 in water, which induces a change of its solubility.[18–20] PDMAEMA modified hydrogels as antidote delivery vehicles that respond to changes in CO_2 concentration have been fabricated by Satav *et al.*[21] Hydrogels prepared from poly(DMAEMA-*co*-hydroxyethyl methacrylate) have been employed as glucose responsive insulin delivery vehicles, as well as CO_2 sensors.[22] In contrast, de-protonation of tertiary amine groups takes place along with the removal of CO_2.[23] As compared to protonation/de-protonation conducted by acidoid/alkaline substances (such as HCl/NaOH), CO_2 induced reaction avoids generating salts.

Photo-responsive polymers containing nitrobenzene groups have been widely used to prepare polymeric nanocarriers.[12] Also, remote motivated spatial and temporal release of loaded molecules from theses nanocarriers can be achieved. In particular, polymers bearing 2-nitrobenzyl groups can under go photolysis *via* either one-photon UV or two-photon near-infrared absorption,[24] indicating their potential application in controlled drug release. Photo-sensitive brushes for release of dye molecules have been prepared from block copolymer polystyrene-*b*-poly(4,5-dimethoxy-2-nitrobenzyl methacrylate) by Kumar *et al.*[25] Yan *et al.*[26] has prepared light-dissociable amphiphilic block copolymer micelles from amphiphilic copolymer utilizing poly(ethylene oxide) as hydrophilic block and poly(2-nitrobenzyl methacrylate) as hydrophobic block. In this communication, novel nanocarriers made from amphiphilic block copolymer poly(2-dimethylamino)ethyl methacrylate-*b*-poly(2-nitrobenzyl acrylate) ($PDMAEMA_m$-*b*-$PNBA_n$) that are responsive to UV, temperature, CO_2 and pH have been developed.

As shown in Scheme S1,† 2-nitrobenzyl acrylate monomer was firstly prepared by reacting acryloyl chloride with 2-nitrobenzyl alcohol. And PDMAEMA is prepared *via* reversible additional-fragmentation chain transfer (RAFT) polymerization, using synthesized *S*-1-dodecyl-*S*′-(α,α′-dimethyl-α′′-acetic acid)

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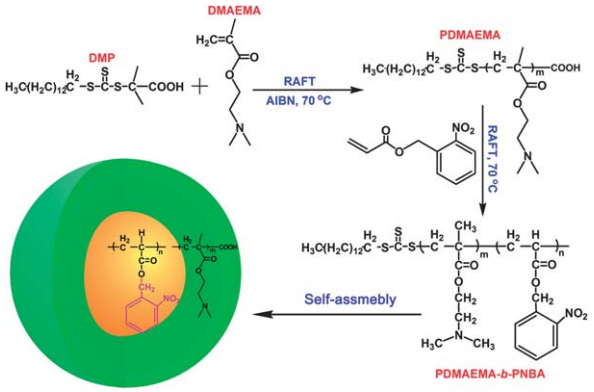
† Electronic supplementary information (ESI) available: Details of synthesis of monomer, copolymer, polymeric micelles, drug loading and release, characterization, digital image of micelles dispersed in water before and after UV irradiation, FT-IR spectra of multi-stimuli sensitive micelles before and after UV irradiation, DLS analysis for reversibility of micelles under multi-stimuli. See DOI: 10.1039/c3py00746d

trithiocarbonate (DMP) as a RAFT agent. Their structures are confirmed by ^{1}H NMR spectra (Fig. 1A and B), and the number average molecular weight (M_n) of PDMAEMA is 1.28×10^4 g mol^{-1} determined by gel permeation chromatographic (GPC) measurement. Then, PDMAEMA is used as a macro-RAFT agent to copolymerize with 2-nitrobenzyl acrylate to form block copolymer PDMAEMA$_m$-*b*-PNBA$_n$ ($M_n = 1.72 \times 10^4$ g mol^{-1}) determined by GPC, where the values of m and n are 80 and 21, respectively. Dissolving the resultant PDMAEMA$_{80}$-*b*-PNBA$_{21}$ in tetrahydrofuran (THF) and dialysis against DI-water with constant shaking at room temperature for 4 hours, multi-responsive micelles with hydrophilic PDMAEMA chains as the shell and hydrophobic PNBA chains as the core are finally obtained (Scheme 1).

Self-assembly of amphiphilic block copolymer in aqueous media is of fundamental interest for application in biotechnology and medicine because most drug molecules are hydrophobic and they can be loaded into self-assembled polymeric carriers. As shown in Fig. 1D, self-assembled micelles from PDMAEMA$_{80}$-*b*-PNBA$_{21}$ are observed with diameter ranging from 60 nm to 120 nm. The relatively small size of micelles are beneficial to their applications in biology, since nanocarriers with a size under 200 nm are more likely to be taken up by cells.[27,28]

The stimuli responsive properties of micelles were investigated by DLS and TEM measurements (Fig. 2). Under UV irradiation at 365 nm for 30 min, the color of micelles dispersion has been turned to yellow (Fig. S1†). It is caused by photocleavage of the chromophore of *o*-nitrobenzyl groups. Hydrophobic 2-nitrosobenzaldehye molecules are detached from PNBA, and hydrophobic PNBA chains are converted into hydrophilic poly(acrylic acid).[29] The nanostructure of micelles based on the hydrophilic–hydrophobic balance is wrecked and micelles are dissembled. However, the photocleaved copolymers still can form some aggregates instead of unimers because the formed carboxylic acid groups after UV irradiation can be



Scheme 1 Preparation of multi-stimuli sensitive micelles.

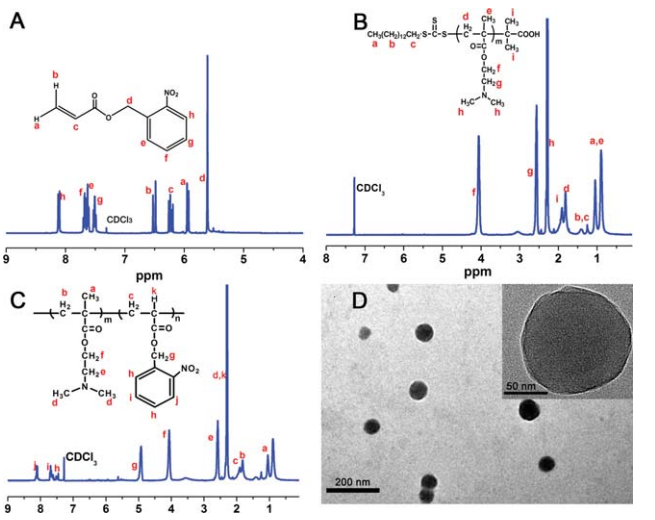


Fig. 1 ^{1}H NMR spectra of 2-nitrobenzyl acrylate (A), PDMAEMA (B), PDMAEMA$_{80}$-*b*-PNBA$_{21}$ (C), and TEM image of multi-stimuli sensitive micelles (D).

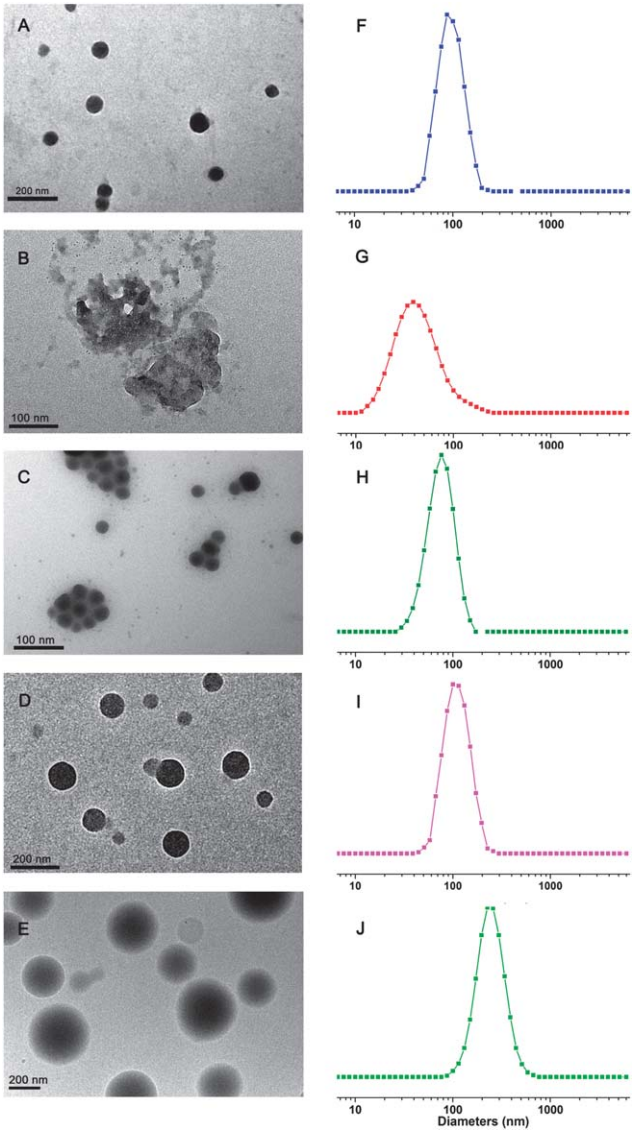


Fig. 2 TEM images and DLS analysis of as-prepared micelles (A and F), after UV irradiation for 30 min (365 nm, 75 mW cm^{-2}) (B and G), at 60 °C (C and H), after bubbling with CO_2 for 10 min (D and I), and at pH 3.0 (E and J).

reacted with amine groups of PDMAEMA to form cross-links, preventing the further dissociation of the aggregates (Fig. 2B, G and S2†).[30] Thus, the diameter detected by DLS decrease while PDI increase inversely.

However, the size of micelles is decreased when increasing the temperature of micelles' solution to 60 °C. Micelles with diameter around 30–40 nm can be found concurrently some slight aggregation by TEM image (Fig. 2C and H). The reason is that micelles shrunk as the hydrophilicity of PDMAEMA segments decreased when the solution temperature above its LCST (44 °C). Much larger size is measured by DLS due to the aggregation and swollen of micelles in water.

Larger micelles are detected by bubbling CO_2 into the solution, which is caused by protonation of tertiary amine groups,[31–33] as shown in Fig. 2D and I. Better solubility of PDMAEMA in water is achieved since the polarity is increased due to the protonation. PDMAEMA chains are more extended in solution and the density of polymer network gets lower. In addition, de-protonation of PDMAEMA can be easily achieved by removal of CO_2 using gas like N_2.

Much larger micelles can be observed when the pH value of the micelles solution was adjusted to 3.0 (Fig. 2E and F), which is mainly caused by protonation, too. PDMAEMA chains are stretched by electrical repulsion after protonation in an acid environment. Besides, the looser network of micelles is contributing to their swelling in aqueous solution, thus enlarging the size of micelles. It is worth noting that the responses to multi stimulus mentioned above are reversible, expect for UV irradiation, due to the collapse of micelles by photocleavage (Fig. S3†).

The hydrophobic drug indomethacin was used as a model drug to investigate release behaviors of the as-prepared multi-responsive micelles as carriers. The control experiment was conducted in PBS at pH 7.4 and 37 °C. Then, the environmental factors, such as light, temperature, pH, and CO_2, were adjusted to investigate the stimuli controlled release of micelles. As shown in Fig. 3A, the drug release rate is faster under UV irradiation than that under visible light, which is caused by photocleavage of PNBA chains. PNBA converted into hydrophilic poly(acryl acid) since 2-nitrosobenzaldehye molecules are detached from PNBA. The hydrophilic–hydrophobic balance of micelles is disturbed, thus micelles disassembled, leading to release of the loaded drugs. Temperature dependence of release of indomethacin from the nanocarriers is displayed in Fig. 3B. Drug released rate can be accelerated with increasing the temperature. Interestingly, the release of indomethacin from micelles can be significantly accelerated when the temperature is increased to 50 °C. Because the hydrophilicity of PDMAEMA segments is decreased as temperature increased above its LCST (44 °C). Drugs are squeezed out from nanocarriers when nanocarriers get shrunk at temperatures above the LCST.

As shown in Fig. 3C and D, the speed and efficiency of drug release are simultaneously enhanced with CO_2 bubbling or in an acidic environment. The reason is the protonation of tertiary amine groups. The polarity of PDMAEMA units decreased due to the formation of carbonic acid upon CO_2 bubbling in water which caused the better solubility of PDMAEMA in water. So, the shell of micelles becomes looser as the PDMAEMA segments are stretched. The protonation caused by CO_2 is reversible with assistance of N_2 (Fig. S4†). Scheme 2 shows the mechanism for the temporally controlled polymer-based drug release systems. We envision that this multi-responsive polymeric release system will lead to a new generation of on–off drug delivery vehicles and stimulate the development of unique and clinically applicable therapies.

In conclusion, novel multi-responsive $PDMAEMA_{80}$-*b*-$PNBA_{21}$ amphiphilic block copolymer was synthesized *via* a RAFT route. They could self-assemble into nanoparticles in aqueous solution and could be used to encapsulate hydrophobic guest molecules. The as-prepared polymeric micelles showed good response to UV, temperature, pH and CO_2 bubbles. Stimuli controlled drug release behaviors were investigated *in vitro*. The results showed that this series of polymers could be used to fabricate nanoparticle platforms for repeated and effective on–off drug release, in which temperature and pH were utilized as a switch to regulate the release of drug, while UV- and CO_2 bubble-responsive behaviors were used to accelerate the drug release which only worked in the 'on' state.

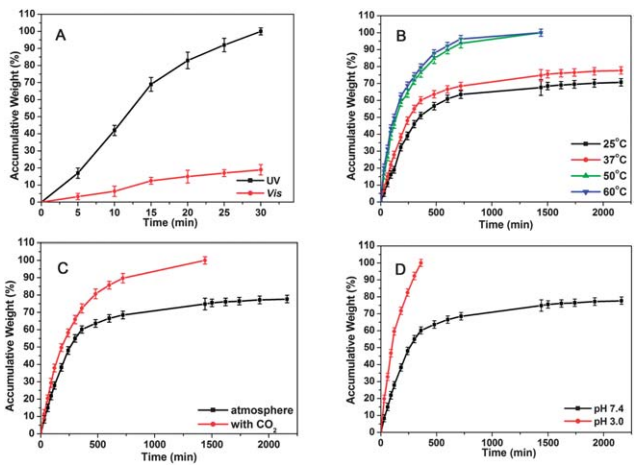
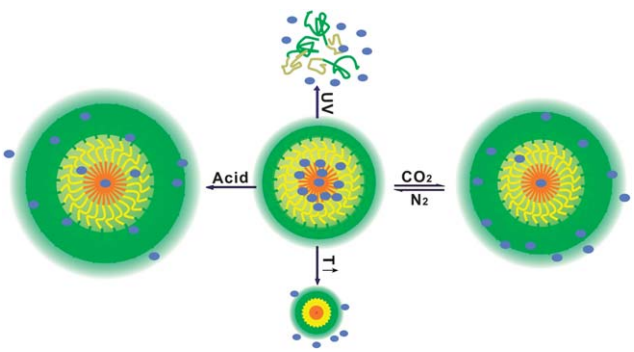


Fig. 3 The release of indomethacin under UV and visible-light irradiation (A), at different temperature (B), with CO_2 bubbling (C), at pH 3.0 and pH 7.4 (D). Data are presented as average standard deviation ($n = 3$).



Scheme 2 Release of drug from nanocarriers under different stimulus.

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