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Short communication

# Photoinitiated synthesis of multi-sensitive micelles for drug release

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ABSTRACT

The multi-sensitive micelles were prepared through a photoinitiated polymerization and self-assembly route using 2-ketobutyric acid (2-KBA) as effective water-soluble photoinitiator and emulsifier. The drug release behaviors were investigated under different glucose concentration, pH and temperature conditions. This work provides a new platform for development drug delivery vehicles with reducing possible utilization of organic solvents and initiators and surfactants.



## 1. Introduction

Over the past decade, significant attention has been paid to environmental-sensitive polymeric micelles for medical application [1,2]. As a facile one-batch process, emulsion or miniemulsion polymerization is the most common method to synthesize monodisperse polymeric micelles [3–7]. At the beginning of polymerization, all the reaction ingredients, including the monomer, initiator, stabilizer (or surfactant) and reaction medium (continuous and dispersed phase), are mixed together [8]. Depending on the polarity of the continuous medium and the dispersed phase, both oil-in-water (o/w, direct) and water-in-oil (w/o, inverse) microemulsions can be prepared. Despite the many advantages of microemulsion polymerization, the high concentration of surfactant relative to monomer and the control of molecular weight limit the industrial viability of microemulsion polymerization [9].

Photoinitiated polymerization provides an effective tool to tailor the physical and mechanical properties as well as the control of reaction by the light source. Furthermore, photoinitiated polymerization can be done under mild reaction conditions, thus reducing possible denaturation of biological active agents. Therefore, the development of a highly responsive photocontrolled initiation procedure, which affords control over the chain growth process, is both a major opportunity as well as challenge for the future of polymerizations [10–13]. Aliphatic ketones is a kind of significant photoinitiator which plays a vital role in initiating the polymerization by generating active radicals under light irradiation through cleavage and hydrogen abstraction reactions [14]. Griffith and coworkers [15] showed the photochemical synthesis of a double-tailed surfactant from a single-tailed one followed by spontaneous self-assembly into stable vesicles. The single-tailed

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molecule, 2-oxooctanoic acid (2-OOA, an 8-carbon oxo-acid), was utilized as the surfactant. 2-OOA first absorbed light through its carbonyl chromophore, resulting in the production of the radical intermediate 2-OOA·. Then, two 2-OOA radicals recombine to form the OOA–OOA dimer, a double-tailed surfactant followed by self-assembly without external perturbation.

Inspired by the above example, the analogs of 2-OOA may be used as the stabilizer (or surfactant) in the microemulsion polymerization solution. Furthermore, the intermediate radicals can be used as the initiator for the microemulsion polymerization. The conventional free radical microemulsion polymerization is well documented in the literature [16–18]. To the best of our knowledge, there are only a few examples of the combination of photoinitiated and microemulsion polymerization known to exist today. Inhere, 2-ketobutyric acid (2-KBA) was chosen as photoinitiator and emulsifier due to it contains aliphatic ketones and carboxyls groups. Multi-sensitive copolymeric micelles based on poly(N, N-dimethylaminoethyl methacrylate-*co*-3-Acrylamide phenylboronic acid) (P(DMAEMA-*co*-AAPBA)) were prepared by a photoinitiated polymerization and self-assembly route. An anti-inflammatory agent and non-negligible side effects drug, indomethacin (IND), was chosen as a model drug to simulate the drug release under the different glucose concentration and temperature conditions.

## 2. Results and discussion

The synthetic route of P(DMAEMA-*co*-AAPBA) copolymer was shown in Fig. 1. 2-KBA is firstly dissolved in deionized water. The DMF solution of DMAEMA and AAPBA monomers is added. The obtained mixture is stirred at room temperature for about 30 min with $N_2$ bubble to form a homogeneous solution. Then, the solution was irradiated to polymerize under a halide lamp (400 W) as UV–vis light source. Prior to exposure to light, the mixture solution is clear. However, after irradiation for 7 h, the solution becomes opalescent indicating the presence of particulates in solution (see Fig. S1b in Supplementary Material). The product is obtained by dialysis against deionized water for 3d to remove DMF using a dialysis bag (MWCO = 3500 Da) and freeze-dried for 24 h.

The structure of P(DMAEMA-*co*-AAPBA) copolymers is certified by $^1$H NMR spectrum (see Fig. S2 in Supplementary Material). The peaks at 7.0–7.7 ppm and 8.0 ppm are attributed to the phenyl protons and secondary amine proton, respectively. These characteric peaks indicate successful synthesis of P(DMAEMA-*co*-AAPBA). Fourier transform infrared (FT-IR) spectroscopy is further conducted to confirm the successful synthesis of P(DMAEMA-*co*-AAPBA) (see Fig. S3 in Supplementary Material). The number average molecular weight ($M_n$) of P(DMAEMA-*co*-AAPBA) is $1.39 \times 10^4$ g mol$^{-1}$ determined by gel permeation chromatographic (GPC) measurement. The degree of polymerization $x$ and $y$ are calculated at 46 and 35, respectively.

For further confirmation the role of 2-KBA, a certain amount of isopropyl alcohol (IPA, a quencher of radicals, ~1 wt%) is added into the solution prior to exposure to light. After irradiation, the color of solution still keep in clear and transparent which indicates no reactions taken place due to active radicals from 2-KBA photolysis captured by inhibitor IPA (see Fig. S1c in Supplementary Material). However, only 2-KBA dissolved in the water, the light milky color of solution can be founded after irradiation,

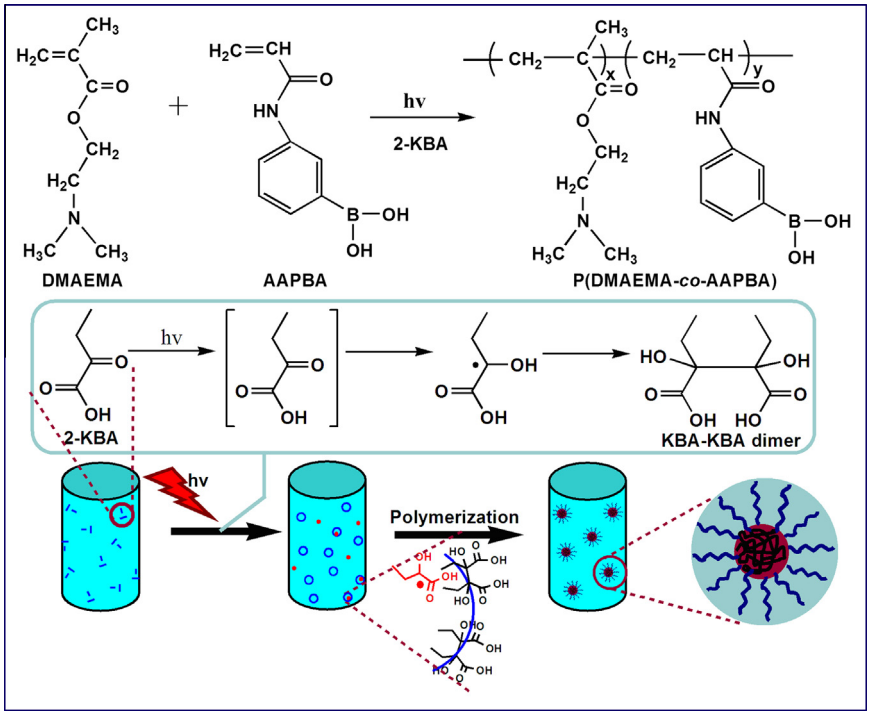


**Fig. 1.** Synthetic route of P(DMAEMA-co-AAPBA) copolymer with 2-KBA as photo-initiator.

which suggests the formation of micelles due to the 2-KBA photolysis (see Fig. S1a in Supplementary Material) [15]. Fig. 1 illustrates the photochemical and polymerization mechanism resulting in the production of the dimer molecule as well as radicals. 2-KBA first absorbs light through its carbonyl chromophore, resulting in the production of radicals, analogous to the well-known photochemistry of pyruvic acid [15,19]. 2-KBA is reversibly hydrated in aqueous solution. In aqueous solution, some 2-KBA exists in its keto form, with the majority existing as its gem-diol. The keto form contains a UV chromophore, which can be excited in the near-UV state to induce photolysis. 2-KBA molecule can react with a ground-state 2-KBA molecule to efficiently form the radical intermediate 2-KBA·. Some radicals can be reacted each other to form dimer molecules. The dimer molecules contain hydrophobic aliphatic ketones and hydrophilic carboxyl groups. They can be acted as the surfactant to form micelles in solution. Other free radicals are dissociated in solution to initiate the polymerization of monomers.

Due to the amphiphilic property of copolymers, they are further self-assemblied into micelles with core–shell structure. The micelles with hydrophilic PDMAEMA segment as the shell and hydrophobic PAAPBA segment as the core are finally obtained, as shown in Fig. 2A. The diameter of micelles is around 150 nm that analyzed by transmission electron microscope (TEM) measurement. Dynamic light scattering (DLS) analysis shows that the average hydrodynamic diameter of P(DMAEMA-*co*-AAPBA) copolymer is around 190 nm (Fig. 2B). The average micelle 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 [20]. The IND was loaded into P(DMAEMA-*co*-AAPBA) micelles according to the similar procedure for preparation of copolymers. The average hydrodynamic diameter of IND-loaded copolymer had expanded to around 210 nm.

To investigate the glucose-sensitivity of obtained micelle, the hydrodynamic radius of micelles against different glucose concentration were analysis by DLS. As shown in Fig. 3A, with increasing the concentration of glucose, the average hydrodynamic radius of micelles are increased from the 188.0 nm to 326.8 nm with glucose concentration range from 0 to 7 mg/mL. Phenylboronic acid has been widely utilized for the design of chemosensors in the detection of saccharides over the past decades [21–23]. The covalent interactions between phenylbbronic acid and the hydroxyl groups of saccharides (1,2- or 1,3-diols) lead to the formation of five- or six-membered rings and allow bronic acid to be a sensitive detector for saccharides. The glucose molecules are diffused into the inner of micelles to react with phenylboronic acid of PAAPBA, and the volume of micelles is expanded accordingly. After the phenylboronic acid of PAAPBA saturated with enough glucose molecules, the size of micelles tends to balance.

PDMAEMA is a weak polyelectrolyte with p$K_a \approx 6$ [24]. Thus, the Lower Critical Solution Temperature (LCST) of PDMAEMA is strongly dependent pH. This phase transition should lead to a collapse of the outer PDMAEMA block of our micelles. Using dynamic light scattering (DLS) measurements, the phase transition of P(DMAEMA-*co*-AAPBA) was investigated in three different buffer solutions. As shown in Fig. 3B, at pH = 6, the apparent hydrodynamic radius, $R_h$, is ≈178 nm and does not significantly change with increasing temperature. This value is close to the expected maximum (fully stretched) size of the micelles. At pH = 7 the $R_h$ values are somewhat smaller (≈152 nm) since PDMAEMA is less protonated and the polymer chains are more contracted. They slightly decrease with increasing temperature, which might be due to a slight contraction when approaching the cloud point. However, at pH = 8 where the apparent hydrodynamic radius at room temperature is much higher ($R_h \approx 210$ nm), which in principle might be unexpected since PDMAEMA should be even less protonated. The possible reason for this phenomenon is the intermolecular aggregates at room temperature due to hydrophobic interaction of the insufficiently protonated PDMAEMA chains during the dissolution process. A strong decrease of $R_h$ is observed in the temperature range from 35 to 60 °C due to the collapse of the PDMAEMA chains above LCST.

The above results imply the micelles exhibit a good glucose, pH and temperature-sensitive response, and they may be one of promising candidates for drug delivery. The hydrophobic drug, indomethacin (IND), was used as

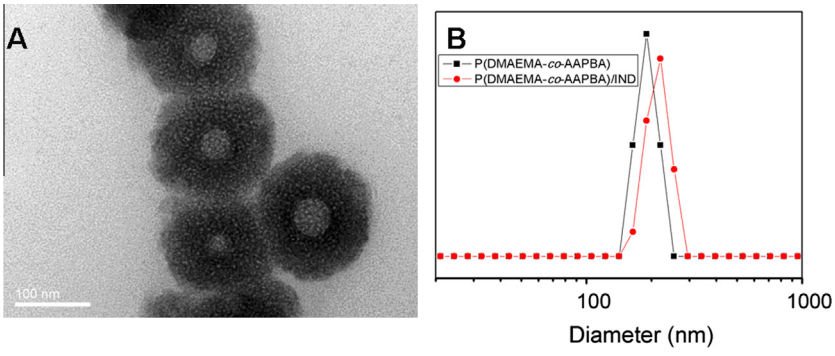


**Fig. 2.** TEM image of P(DMAEMA-*co*-AAPBA) micelles (A), and DLS distribution curves of P(DMAEMA-*co*-AAPBA) and P(DMAEMA-*co*-AAPBA)/IND in the solution.

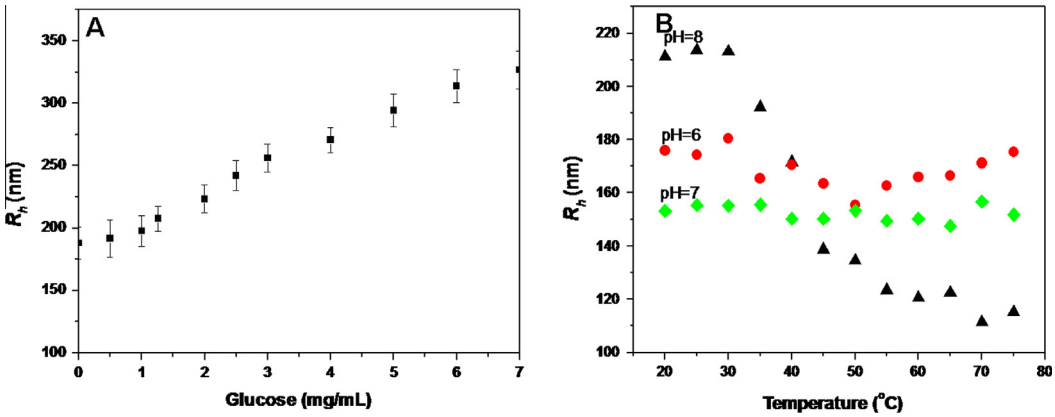


**Fig. 3.** Dependence of the hydrodynamic radii on glucose concentration (A) and temperature (B).

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 temperature, pH, and glucose concentration, were adjusted to investigate the stimuli controlled release of drug. As shown in Fig. 4A, drug released rate can be accelerated with increasing the temperature. The release of IND from micelles can be significantly accelerated when the temperature is increased to 45 °C. Due to the hydrophilicity of PDMAEMA segments decreased as temperature increased above its LCST (~44 °C), drugs are squeezed out from micelles when they get shrunk at temperatures above the LCST. The effect of glucose concentration on released rate is shown in Fig. 4B. With increasing the glucose concentration, the released rate can be accelerated. Because of the affinity

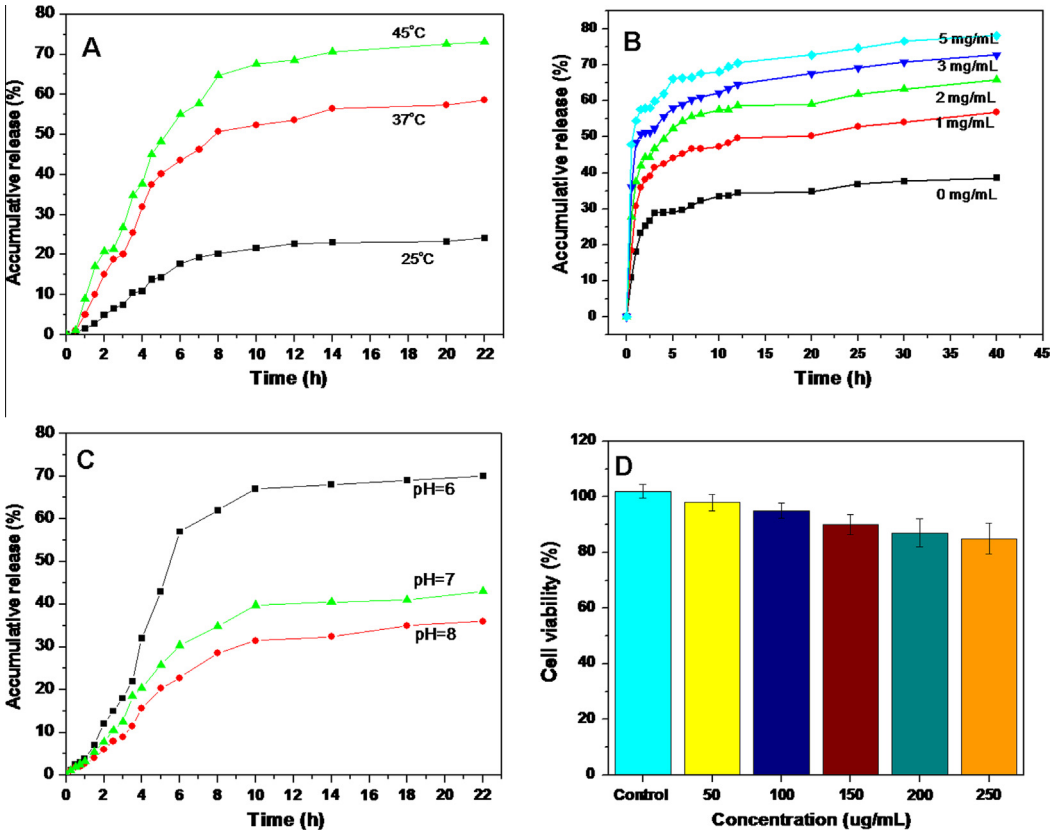


**Fig. 4.** The release of indomethacin under different temperature (A), glucose concentration (B) and pH (C) conditions. Viability of Hela cells against as-prepared micelles for 24 h by MTT assays (D). Data are presented as average standard deviation ($n$ = 5).

interactions between phenylbbronic acid and the hydroxyl groups of glucose lead to the swelling of micelles' core and allow loaded drug to be released. The speed and efficiency of drug release are also can be enhanced in an acidic environment. As shown in Fig. 4C, the reason is the protonation of tertiary amine groups. So, the shell of micelles becomes looser as the PDMAEMA segments are stretched [16].

The relative cytotoxicity of as-prepared micelles was estimated by MTT viability assay against Hela cells [25]. Fig. 4D shows the cell viability after 24 h of incubation with micelles at the different concentrations. When the concentration of micelles reaches 200 μg $mL^{-1}$, the viability of cells is still kept above 85%, indicating low toxicity of the micelles. It partly owes to limited organic solvents and initiators (or surfactants) were involved during preparation procedure.

## 3. Conclusion

In summary, the multi-sensitive micelles with core and shell structure were prepared through a photoinitiated polymerization route using 2-ketobutyric acid (2-KBA) as effective water-soluble photoinitiator and emulsifier. The as-prepared micelles exhibited excellent sensitivity to temperature, glucose concentration and pH, leading to significant differences on release rate under the different conditions. This work provides a new platform for development drug delivery vehicles with reducing possible utilization of organic solvents and initiators (or surfactants).

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eurpolymj.2014.08.020.

## References

[1] Jiang G, Xia W, Chen W. Des. Monomers Polym. 2008;11:105–22.
[2] Cheng R, Meng F, Deng C, Klok H-A, Zhong Z. Biomaterials 2013;34:3647–57.
[3] Wang Y, Jiang G, Sun X, Ding M, Hu H, Chen W. Polym. Chem. 2010;1:1638–43.
[4] Wang Y, Jiang G, Zhang M, Wang L, Wang R, Sun X. Soft Matter 2011;7:5348–52.
[5] Jiang G, Wang Y, Zhang R, Wang R, Wang X, Zhang M, et al. ACS Macro Lett. 2012;1:489–93.
[6] Ethirajan A, D'Olieslaeger L, Vandenbergh J, Lutsen L, D'Olieslaeger M, Vanderzande D, et al. Macromol. Chem. Phys. 2013;214:1859–964.
[7] Ethirajan A, Baeten L, Conradi M, Ranieri K, Conings B, Boyen H-G, et al. Polym. Chem. 2013;4:4010–6.
[8] Tan J, Rao X, Jiang D, Yang J, Zeng Z. Polymer 2014;55:2380–8.
[9] Ciftci M, Tasdelen MA, Li W, Matyjaszewski K, Yagci Y. Macromolecules 2013;46:9537–43.
[10] Xu J, Jung K, Atme A, Shanmugam S, Boyer C. J. Am. Chem. Soc. 2014;136:5508–19.
[11] Fors BP, Hawker CJ. Angew. Chem. Int. Ed. 2014;51:8850–3.
[12] Tasdelen MA, Uygun M, Yagci Y. Macromol. Chem. Phys. 2014;211:2271–5.
[13] Anastasaki A, Nikolaou V, Simula A, Godfrey J, Li M, Nurumbetov G, et al. Macromolecules 2014;47:3852–9.
[14] Guo R, Gao Y, Wu M, Wang H. Polymer 2013;54:4940–7.
[15] Griffith EC, Rapf RJ, Shoemaker RK, Carpenter BK, Vaida V. J. Am. Chem. Soc. 2014;136:3784–7.
[16] Wang X, Jiang G, Li X, Tang B, Wei Z, Mai C. Polym. Chem. 2013;4:4574–7.
[17] Wang X, Jiang G, Wang Y, Wang R, Sun X, Hu R, et al. J. Macromol. Sci. Part A: Pure Appl. Chem. 2013;50:644–52.
[18] Wang Y, Fang Y, Jiang G, Lv Q, Sun X, Ding M, et al. J. Macromol. Sci. Part A: Pure Appl. Chem. 2011;48:890–5.
[19] Griffith EC, Carpenter BK, Shoemaker RK, Vaida V. Proc. Natl. Acad. Sci. USA 2013;110:11714–9.
[20] Jiang G, Sun X, Ma Y, Cao J, Wang Y, Wang R, et al. Soft Mater. 2013;11:288–93.
[21] Nishiyabu R, Kubo Y, James TD, Fossey JS. Chem. Commun. 2011;47:1106–9.
[22] Ma R, Shi L. Polym. Chem. 2014;5:1503–18.
[23] Sanjoh M, Miyahara Y, Kataoka K, Matsumoto A. Anal. Sci. 2014;30:111–7.
[24] Arslan H, Pfaff A, Lu Y, Stepanek P, Müller AHE. Macromol. Biosci. 2014;14:81–91.
[25] Wang X, Jiang G, Wei Z, Li X, Tang B. Eur. Polym. J. 2013;49:3165–70.