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Letter to the Editor

Questionable micelle formation of the double hydrophilic block copolymer PEG-pHPMA

Dear editor,

Polymer micelles are self-assembled core–shell nanostructures formed from amphiphilic polymer structures (e.g. block, gradient, graft copolymers) in a solvent that is selective for one part of the polymer. In water, double hydrophilic block copolymers can form micelles when one part has an ionic character and is complexed with counterion (macro) molecules, forming so called polyion complex micelles (Harada and Kataoka, 1995; Magana et al., 2020). However, neutral block copolymers cannot form micelles if the two parts of the polymer are both highly soluble. Poly(ethylene glycol) (PEG) and poly(2-*N*-hydroxypropyl methacrylamide) (pHPMA) are two typical examples of polymers that are highly soluble in water, both having very similar Flory-Huggins χ-parameters in water, which represent the Gibbs free energy change upon polymer dissolution (i.e. 0.484 for pHPMA (Bohdanecký et al., 1974) and 0.426 for PEG (Merrill et al., 1993)). Taking this into account, it is very surprising that the group of Prof. Biswas and Ghosh reported in three recent papers the formation of micelles/nanoparticles in water from mPEG-*b*-pHPMA block copolymers (Fig. 1) (Bobde et al., 2021a, 2021b; Ch et al., 2021). This remarkable observation prompted us to have a close look at these papers. In this letter, we share our view on the reported observations, and we support claims with own data.

One of the key characteristics of micelles is the existence of a critical micelle concentration (cmc). The fluorescent probe method is the most applied means to determine cmc's, for which pyrene is by far the most popular probe (Piñeiro et al., 2015). A red shift in the excitation spectrum of pyrene (with λ_{em} at 390 nm) is observed when going from a polar to a more apolar environment, which is typically indicated by an increase in the I_{338}/I_{333} excitation intensity ratio. Based on this phenomenon, it is generally assumed that a steep increase of I_{338}/I_{333} with increasing polymer concentration is representing the cmc. Fig. 2(a) shows own representative data for mPEG-*b*-pHPMA block copolymers in which the pHPMA block is completely modified with benzoyl (Bz) or benzoyl plus monolactate (Lac_1) groups to endow the polymer with amphiphilic properties (Shi et al., 2013, 2015). Clear I_{338}/I_{333} inflection points are visible, showing that mPEG-*b*-p(HPMAm-Bz) micelles have a lower cmc (1.3 μg/mL) than mPEG-*b*-p(HPMAm-Bz/HPMAm-Lac_1) micelles (50 μg/mL). This can be explained by the fact that the lactate groups present in the latter polymers make the core of the micelles less hydrophobic. However, in the papers of Biswas and Ghosh clear inflection points are not visible, for example when the cmc of $mPEG_{2K}$-*b*-$pHPMA_{1.1K}$ was determined (mP-*b*-H in Fig. 2(b)), (Bobde et al., 2021b) which makes it impossible to determine an accurate cmc in such case.

In the paper of Bobde et al. (2021b), mPEG-*b*-pHPMA was functionalized with all-trans retinoic acid (ATRA). The authors observed an increase in molecular weight by GPC after modification, and from that increase they calculated an ATRA to polymer molar ratio of 1.6:1. However, the authors made the mistake that GPC is not an absolute measurement of the molecular weight and such calculations are therefore not justified. Even more strikingly, in the same paper it is reported that the product contained 15 μg ATRA (=0.05 μmol) per mg of polymer based on more reliable UV spectrophotometric analysis. Combined with the reported M_n of the polymer, one can calculate a molar ratio ATRA to polymer of 0.2: 1, which is very different from the value 'determined' by GPC analysis. This implies that on average, only 1 out of 5 polymer molecules were functionalized with an ATRA unit. In conclusion, the polymer product contained a very low degree of functionalization, and the majority of the polymers constituting the supposed micelles were still the non-functionalized double hydrophilic mPEG-*b*-pHPMA that should readily dissolve in water. Biswas and Ghosh argue that mPEG-*b*-pHPMA forms micelles because "the polymethacrylate moiety of HPMA block imparts hydrophobicity to the mPEG-*b*-HPMA" (Bobde et al., 2021a). If this is true then pHPMA should also be insoluble in water, which of course is not the case.

DLS data that were presented in Biswas and Ghosh' papers nevertheless suggest the unexpected formation of micelles mainly consisting of mPEG-*b*-pHPMA, although the PDI values were high (>0.3, up to >0.6 for drug-loaded micelles in Bobde et al., 2021a) suggesting (extremely) high polydispersity. Remarkably, in Bobde et al. (2021a), they report particle sizes of 25–69 nm for mPEG-*b*-pHPMA micelles (non-loaded and doxorubicin-loaded) with pHPMA chain lengths of 21–155 units, while in Ch et al. (2021) particles of 161–503 nm were reported for moxifloxacin-loaded micelles using polymers of similar chain lengths. The most reliable way to confirm micelle formation would be to directly observe the particles by high-resolution electron

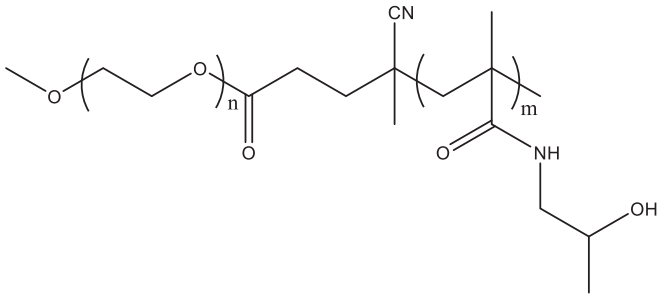

Fig. 1. Structural formula of mPEG-*b*-pHPMA.

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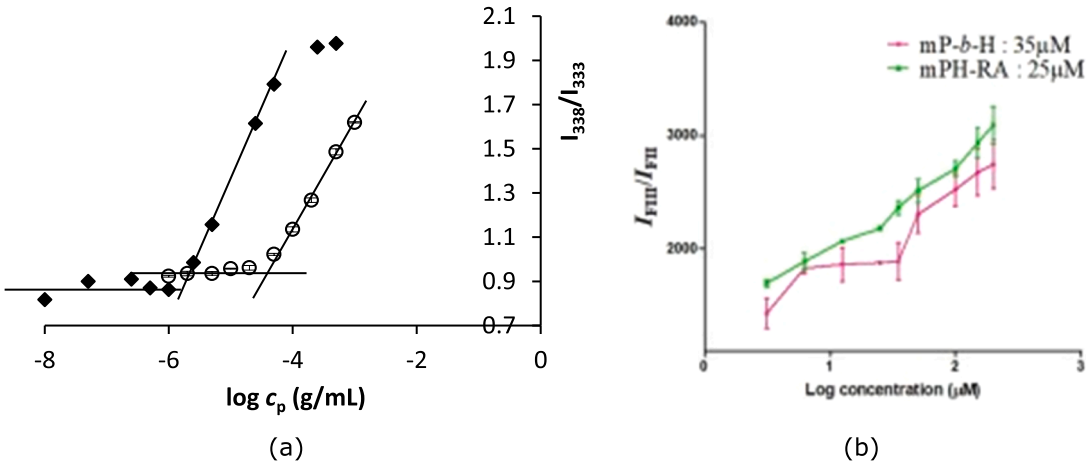

Fig. 2. (a) Fluorescence excitation intensity ratio of pyrene (I_{338}/I_{333}) as a function of the concentration of mPEG$_{5K}$-*b*-p(HPMAm-Bz/HPMAm-Lac$_1$)$_{10K}$ (with 75:25 Bz/Lac$_1$ ratio, open dots) and mPEG$_{5K}$-*b*-p(HPMAm-Bz)$_{9K}$ block copolymers (closed diamonds). (b) Fluorescence data of pyrene as a function of the concentration of mPEG-*b*-pHPMA (mP-*b*-H) and the corresponding retinoic acid modified block copolymer (mPH-RA), reprinted from Bobde et al. (2021b), with permission.

microscopy. This is challenging, considering the soft nature and small size of the micelles. Nevertheless, the authors presented SEM pictures with unprecedentedly high resolution. The grain-like and mostly non-spherical appearance of the particles suggest solid and probably even crystalline particles and it is very difficult to believe that these were actually micelles.

Regarding drug loading in polymeric micelles, one has to realize that drug loading efficiency and stability can only be substantial when the drug is sufficiently hydrophobic to allow partitioning in the micellar core. To take an example of probably erroneous data interpretation, we again refer to one of Biswas and Ghosh' papers. Upon hydration of a solid film of mPEG-*b*-pHPMA (10 mg) and moxifloxacin (1 mg) with 3 mL of water, they report an "encapsulation efficiency" of 47.5% (i.e. a final concentration of 0.158 mg/mL). However, this drug has an aqueous solubility of 0.168 mg/mL (Drugbank online); therefore, the authors most likely just measured the dissolved fraction of the drug.

Another remarkable observation in both papers was the authors' explanation of the pH-dependent release of loaded doxorubicin. To cite from the *IJP* paper: "The given polymeric micellar system is composed of pHPMA blocks that ionize at lower pH leading to electrostatic repulsion resulting in the swelling of the polymer and disassembly of polymeric micelles" (Bobde et al., 2021b). Here, they refer to their own work published in *Coll. Surf. B,* (Bobde et al., 2021a) in which they again make the similar statement and again refer to own work (*Eur. Polym. J.*) (Bobde et al., 2020). However, it is obvious from the structural formula presented in Fig. 1 that the mPEG-*b*-pHPMA blockcopolymer employed in these studies is not ionizable, at least not at (close to) physiological pH. In theory, the amide group in the polymer can be hydrolyzed to yield polymethacrylic acid, but such process will be extremely slow at physiological pH.

Coming back to the basic question whether mPEG-*b*-pHPMA block copolymers can form micelles or not: In own previous work (Soga et al., 2004, 2005), we prepared thermosensitive micelles of mPEG-*b*-poly (HPMA-mono/dilactate), formed upon rapidly heating ice-cold polymer solutions to above the cloud point of the polymer. Upon hydrolysis of the lactate side-groups, mPEG-*b*-pHPMA is formed, which was accompanied by complete loss of the scattering intensity in DLS and release of the encapsulated drug, thus unambiguously indicating dissociation of the micelles. Analogously, the group of Sumerlin reported the complete thermal dissociation of azo-crosslinked star-PEG-pHPMA in water into soluble mPEG-*b*-pHPMA fragments (Dai et al., 2017). We furthermore recently reported micelle formation from pHPMA-*b*-p(HPMA-Bz) block copolymers, in which pHPMA forms the hydrophilic shell (Wang et al., 2020; Talelli et al., 2010). In line with this, pHPMA has been frequently used as a scaffold to make soluble polymer-drug conjugates (Chytil et al., 2018; Yang and Kopeček, 2016; Lammers, 2010).

To summarize, our own data and the probably erroneous interpretation of their data by the group of Biswas and Ghosh demonstrated that double hydrophilic mPEG-*b*-pHPMA block copolymers are unable to form micelles.

This "Letter to the editor/Rebuttal/Response to Rebuttal/Editorial comment" is part of a series of comments on the article "Polymeric micelles of a copolymer composed of all-trans retinoic acid, methoxy-poly(ethylene glycol), and b-poly(N-(2 hydroxypropyl) methacrylamide) as a doxorubicin-delivery platform and for combination chemotherapy in breast cancer" (https://doi.org/10.1016/j.ijpharm.2021.120866):

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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