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Hollow microspheres and aqueous phase behavior of pH-responsive poly(methyl methacrylate-*co*-methacrylic acid) copolymers with a blocky comonomer distribution

Kiattikhun Manokruang, Evangelos Manias *

Polymer Nanostructures Lab – Center for the Study of Polymer Systems (CSPS), and Department of Materials Science and Engineering, 325-D Steidle Building, Penn State University, University Park, PA 16802, USA

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ABSTRACT

Copolymers of methyl methacrylate (MMA) and methacrylic acid (MAA) were prepared by partial hydrolysis of PMMA in bad solvent. These copolymers evidently have a blocky comonomer distribution –strands of predominantly MMA or MAA across the polymer– and show sharp transitions in aqueous solution upon pH change. Additionally, their hollow microcapsules show an exceptional and prolonged stability at acidic conditions (pH 2) and pH-triggered release at physiological conditions.

1. Introduction

Polymers containing ionizable groups, such as carboxylic acid and amines, are the focus of intensive research, since their pH-dictated ionization can be utilized for the development of systems with reversible pH-controlled water solubility [1–7]. These polymers are typically water-soluble when charged, and become water-insoluble when neutral. Beyond the basic research focused on their phase behavior, these materials also find application, *e.g.*, in the form of hollow microspheres, in fields such as controlled release of drugs [7], protection of sensitive species in acidic environments [8], and microreactors [9].

Acrylic and methacrylic acid copolymers, including commercial materials [10,11], are particularly relevant for biomedical use because, beyond their biocompatible characteristics, they are neutral at low pH and become negatively charged at higher pH ($pKa^{COOH} \approx 4$). However their proliferation in drug delivery applications has been hampered by challenges in designing polymers with sharp transition tailored at a predefined pH, while maintaining high stability when in their water-insoluble state [10,11]. Both these requirements necessitate a well-defined copolymer microstructure that contains long sequences of single type monomers –chargeable-only or hydrophobic-only strands– currently only common in block-copolymers [1–3]. Their much easier preparation by free radical copolymerization [5,10,11] suffers, in most cases, from poor control over the comonomer distribution (*cf.* statistical monomer insertion) and over the copolymer composition (*cf.* compositional drift during the reaction results in a very wide distribution of copolymer compositions). Both these factors broaden the copolymer transition [12] and severely impact the ability to control the structure of the collapsed state and the pH-onset of the phase separation [13].

Here we report on an approach to prepare copolymers of methyl-methacrylate (MMA) and methacrylic-acid (MAA), by partial hydrolysis of PMMA in a bad solvent. These copolymers evidently have a blocky microstructure, with strands composed predominantly of MMA or MAA, and show sharp transitions in aqueous solutions upon pH variation, consistent with a hydrophobically-stabilized collapsed state. We further demonstrated their use in preparing hollow microcapsules, which showed an exceptional and prolonged stability in acidic environments (pH 2), and readily release at physiological conditions (pH 7).

2. Experimental

2.1. Copolymer preparation and characterization

All reagents were purchased from Sigma-Aldrich in *purum* grade and used with no further purification; all water is deionized (>10 MΩ cm). The copolymer preparation was carried out by alkaline hydrolysis [14] of a commercial poly(methyl methacrylate) (PMMA, Mw = 350,000 g/mol, Mw/Mn ≈ 4.5, Sigma-Aldrich), which yields well-controlled conversion of methyl methacrylate (MMA) to methacrylic acid (MAA). In brief, for each copolymer, 10 g of PMMA were hydrolyzed in a mixture of 1 eq NaOH and 1 eq deionized water in 80 g of isopropyl alcohol at 85 °C (a rather bad solvent). The degree of hydrolysis, *cf.* the copolymer acid content, was controlled by varying the reaction time (12 to 72 h). The hydrolyzed product was

* Corresponding author. Tel.: +1 814 863 2980; fax: +1 814 865 2917.
E-mail address: manias@psu.edu (E. Manias).

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dissolved in a large excess of water, and the copolymer was precipitated by HCl, filtered, and dried at 50 °C (vacuum, 24–48 h). The MAA content of each copolymer was determined by potentiometric titration of a 0.2 wt.% solution in 0.05 M standardized NaOH, by recording the pH while slowly adding 0.05M HCl. In addition, the copolymer composition was independently confirmed by ^{1}H NMR. Turbidity (cloud point) measurements followed the pH of a copolymer solution (0.03 wt.% to 2 wt.%, in 0.125 M aqueous NaOH) as acetic acid was added, while recording the transmitted intensity of a laser ($\lambda = 650$ nm, 2 mW) with a digital photodetector. As the pH decreases, the solution remains optically clear (transmittance $\equiv I_{\text{transmitted}}/I_{\text{incident}} = 1$) and upon phase-separation (demixing) it changes abruptly to almost 0; the cloud point (binodal pH) is set *ad hoc* at 0.8 transmittance.

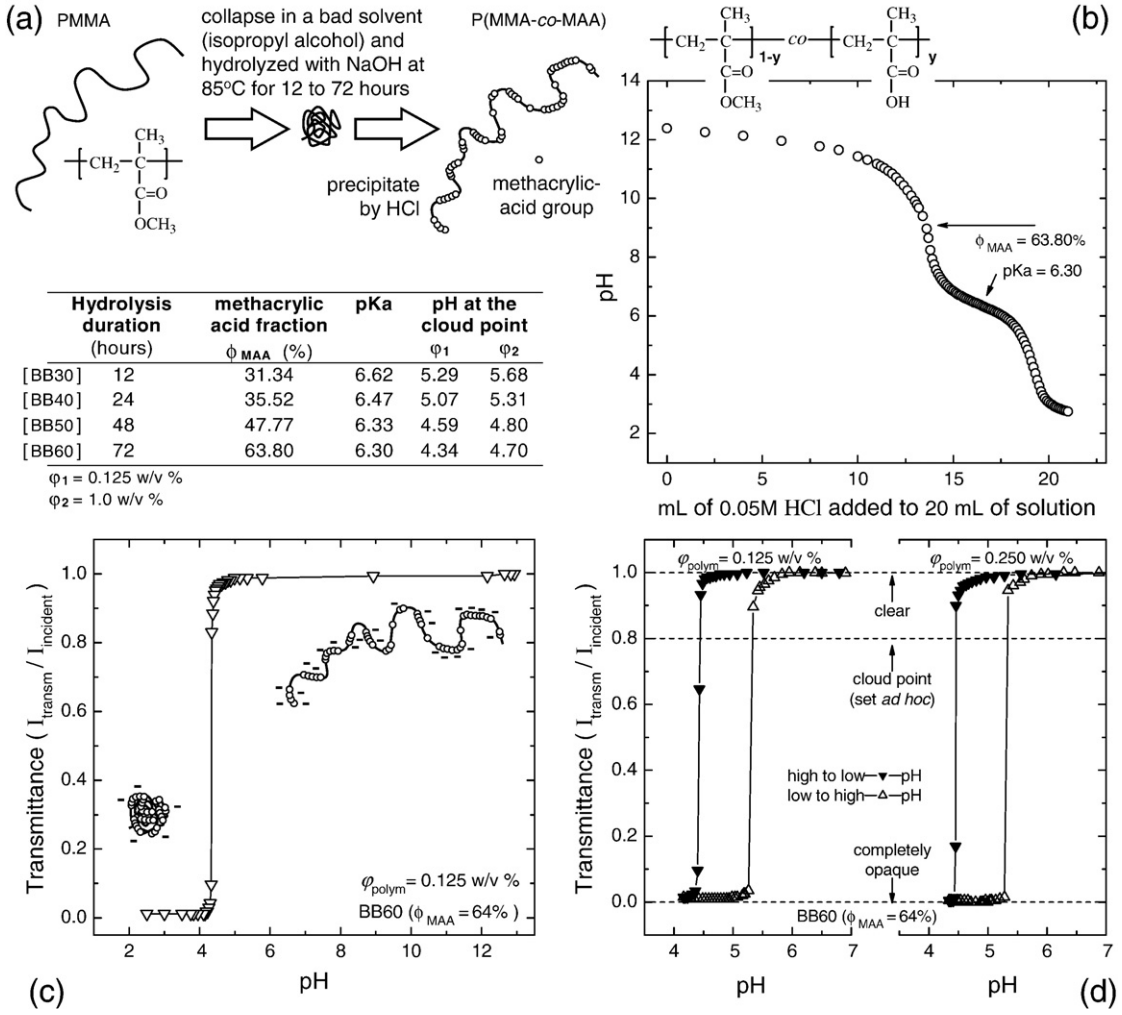
2.2. Microcapsule preparation and characterization

An oil-in-oil encapsulation method [15,16] was used to form copolymer microcapsules containing an organic solution of a water-soluble dye (0.1 wt.% methylene-blue, MB, in 95/5 methanol/benzyl-alcohol). Specifically, a predefined mass of copolymer (0.5, 0.7 and 1.0 g) was dissolved in 10 g of the MB solution and was slowly emulsified in 53 g of a second oil phase (paraffin oil containing 1 wt.% emulsifier, sorbitan trioleate) under constant stirring (*ca.* 150 rpm), at room temperature, until all methanol evaporated (*ca.* 24 h). The microcapsules were retrieved, washed with n-hexane, and dried under vacuum at room temperature. The microcapsules were characterized in saline suspension by optical microscopy, and dry by environmental scanning electron microscopy (ESEM, FEI Quanta 200), also after freezing with liquid Nitrogen and fracturing. Release studies were performed from 30 mg of microcapsules dispersed in 100 mL of two release media with different pH (phosphate buffered saline solution, PBS, pH 7.4; and aqueous HCl solution at pH 2.3). Small amounts of the release medium were withdrawn as a function of time, and the concentration of released encapsulant was measured by UV–vis absorbance (following the intensity of the 664 nm peak of MB).

3. Results

3.1. Aqueous phase behavior and pH-response of the copolymers

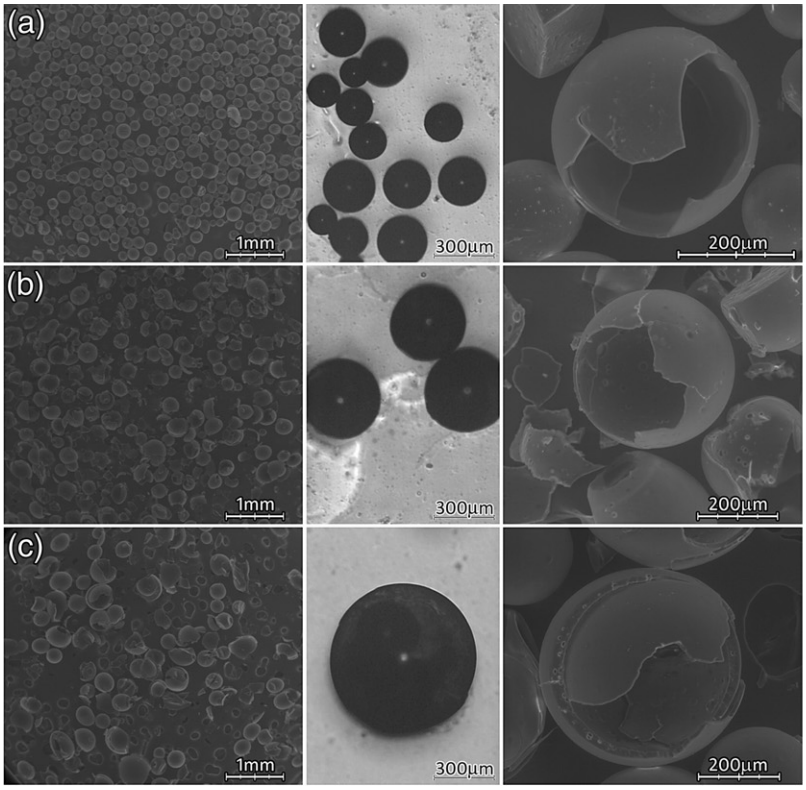
The partial hydrolysis of PMMA resulted in methyl methacrylate/methacrylic acid copolymers, poly(MMA-*co*-MAA), with the same molecular weight and varied methacrylic acid content (ϕ_{MAA}-controlled by the duration of hydrolysis; Fig. 1). Since the hydrolysis was carried out in a bad solvent the copolymer microstructure is expected to be segmented [12], *i.e.*, rather blocky with long strands composed predominantly or exclusively by chargeable (MAA) or by hydrophobic (MMA) groups. The results for four copolymers are shown in Fig. 1. The MAA-fraction in the copolymer was determined by titration (Fig. 1b) from the first equivalence point around pH 9, and was varied between 31.34 ± 0.01 mol.% and 63.80 ± 0.01 mol.%, for 12 to 72 h of hydrolysis (ϕ_{MAA} was also confirmed by ^{1}H NMR, *e.g.*, at 61 ± 1 mol.% MAA for BB60, in

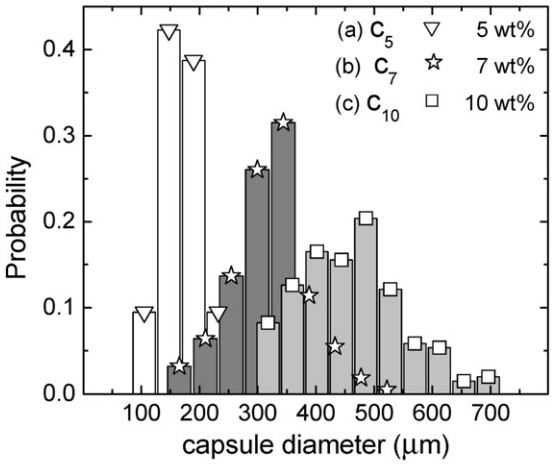


	Hydrolysis duration (hours)	methacrylic acid fraction ϕ_{MAA} (%)	pKa	pH at the cloud point φ_1	φ_2
[BB30]	12	31.34	6.62	5.29	5.68
[BB40]	24	35.52	6.47	5.07	5.31
[BB50]	48	47.77	6.33	4.59	4.80
[BB60]	72	63.80	6.30	4.34	4.70

φ_1 = 0.125 w/v %
φ_2 = 1.0 w/v %

Fig. 1. Aqueous phase behavior and characteristics of the copolymers. (a) A schematic of the PMMA hydrolysis and details of the P(MMA-*co*-MAA) copolymers as a function of hydrolysis duration. (b) Potentiometric titration of 20 mL of 0.2 wt.% BB60 solution; the MAA content can be determined from first equivalence point (at about pH 9) and increases with more prolonged hydrolysis. (c) pH-induced phase separation of an aqueous solution of the BB60 (64%-MAA) copolymer at 25 °C, including a schematic of the corresponding copolymer charge and conformations. (d) Hysteresis of the phase separation transition upon reducing (higher-to-lower) or increasing the pH.





c_{emuls} (wt%)	Average radius (μm)	Thickness of microcapsule wall (μm)
(a) 5	94 ± 16	9
(b) 7	170 ± 33	20
(c) 10	239 ± 44	45

Fig. 2. Microcapsules prepared via an oil-in-oil method from (a) 5, (b) 7, and (c) 10 wt.% of BB50 copolymer solutions. Optical micrographs of the capsules in saline (center) and scanning electron micrographs of the capsules after freezing and fracturing. The diameter distributions were measured by optical microscopy (at least 250 capsules per system) and the microcapsule wall diameter was calculated from the compositions and confirmed by SEM measurements.

quantitative agreement with the titration). The pKa of each copolymer is determined at the midpoint of the buffer capacity plateau (at *ca.* pH 6) and decreases with MAA content, as expected (moving towards the pKa of the polymethacrylic acid homopolymer as ϕ_{MAA} tends to 1); the width of this plateau increases with ϕ_{MAA}, also as expected [3]. The phase behavior of the copolymers' aqueous solutions was determined by turbidity measurements, shown in Fig. 1c, as the pH was lowered by gradual addition of acetic acid. The MAA groups are ionizable, *i.e.*, they are negatively charged at high pH, promoting polymer solubility in water, and become uncharged at lower pH, eventually rendering the copolymer water-insoluble. Despite its very sharp character, this transition shows a strong hysteresis (when lowering *vs.* increasing the pH, Fig. 1d), which indicates that the transition is conformational in nature, rather than a first-order thermodynamic phase transition [17,18,12,13]. This hysteresis reflects a high stability for the collapsed/phase-separated structures, akin to those of micelles [19], which probably originates from the hydrophobic attraction [20] of long MMA strands –which can promote MMA aggregation leading to a polymer collapse and precipitation [21,17,19]. Finally, the pH-onset of the copolymers' transition shows a systematic dependence on ϕ_{MAA} and an almost-linear dependence on the polymer concentration in solution, which further indicate that these transitions are due to a microphase separation mediated by interchain and intrachain hydrophobic-attraction [17,19]. All the above phase behavior trends, confirm *a posteriori* the supposition of a blocky comonomer distribution in the copolymers.

3.2. Microcapsules and their pH-controlled behavior

Controlling the onset of pH-response by the copolymer composition, allows for the design of systems with specific pH solubility profiles, *i.e.*, systems that are stable at a predefined pH-range and undergo a transition at a desired pH-value. For example, here we demonstrate the preparation of microcapsules that are stable at high acidic conditions and readily release the encapsulated matter at physiological conditions: Specifically, as per the typical requirements [7] for colon-targeted drug delivery of orally-administered medicines (that dictate protection of the active species in the stomach environment –*ca.* pH 2, and subsequent release in the colon/intestine –*ca.* pH 7.4, within 3 to 5 h) we designed capsules based on the BB50 copolymer. In Fig. 2, we show copolymer microcapsules made by oil-in-oil emulsion method; their size was controlled by the copolymer concentration in the solution (*i.e.*, capsule diameter of 190, 340 and 480 μm for $c_5 = 5$, $c_7 = 7$ and $c_{10} = 10$ wt.% BB50 copolymer, respectively, at constant stirring rate, Fig. 2).

The pH-controlled release from the capsules was quantitatively observed by UV–vis absorption (Fig. 3). All capsules had an exceptional stability in high acidic environment

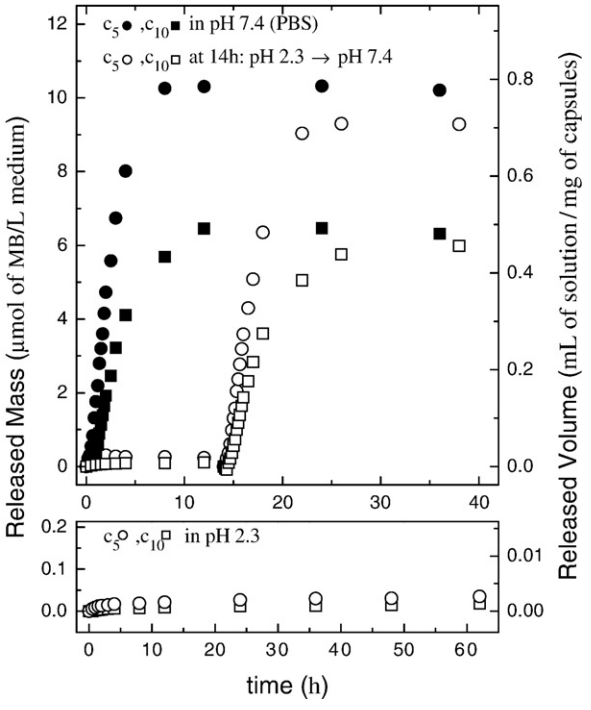


Fig. 3. The release behavior from the c_5 and c_{10} capsules as followed by UV–vis absorbance. The capsules are stable at pH 2.3 showing no release after 62 h (bottom, open symbols), whereas they readily dissociate and release at pH 7.4 (top, filled symbols). In addition, their pH-stimulated release is exemplified by holding the capsules at pH 2.3 for 14 h and subsequently increasing the pH to 7.4 (top, open symbols); in this case, the capsules are stable at acidic conditions and release at physiological pH identically to when introduced directly to PBS.

(Fig. 3, bottom panel), showing no appreciable release [22] for up to 62 h at pH 2.3, and were stable for up to 2 months at these conditions (data not shown). This is consistent with the pH collapse shown in Fig. 1, and reflects the high stability of the micelle-type structure of the collapsed copolymers in solution. In addition, the capsules readily dissociate and release at a physiological pH (phosphate buffered saline solution, PBS, pH 7.4, Fig. 3 top, filled symbols), with a release rate that is consistent with the capsule wall-thickness [23], and achieve 80% release in 3.8 and 7.5 h, for c_5 and c_{10}, respectively. The ultimate release amount was, in all cases, equal to the volume of encapsulated material, denoting a complete dissociation of the capsules.

Finally, we also show pH-triggered release from capsules held for 14 hours at pH 2.3, followed by an increase in pH to PBS values (Fig. 3 top, open symbols). In this case, the microcapsules were stable [22] at pH 2.3 (first 14 h) and readily release upon increasing the media's pH to 7.4 (the release takes place in a manner identical to when introduced directly to PBS). This behavior clearly denotes that holding the capsules at high acidic pH does not cause a marked change in their structure or stability, in concert with what is expected of copolymers with a blocky comonomer distribution.

4. Conclusions

pH-responsive copolymers were prepared from controlled partial hydrolysis of hydrophobic PMMA homopolymer in a bad solvent. The copolymers demonstrated sharp pH-induced phase-separations in water, whose transition-onset depended on the copolymer composition. Hollow copolymer microcapsules were also made, employing an oil-in-oil emulsion approach and loaded by a water-soluble dye in an organic solvent. The capsules showed exceptional stability at low pH, and a pH-triggered release at physiological conditions (pH 7.4 in PBS). Both the copolymer phase behavior, as well as the pH-induced release from the capsules, indicate a highly stable collapsed phase, attributed to the hydrophobic attraction of extended sequences of hydrophobic monomers along the copolymer chain.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.matlet.2009.01.046.

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