Concentration dependent response to pH modification and salt addition of polymeric dispersions of C_{60} fullerenes

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ABSTRACT

Diblockopolymers containing the pH-responsive monomer 2-(diethylaminoethyl) methacrylate (DEAEMA) were synthesized via RAFT polymerization. cryo-TEM imaging revealed the formation of micelles that were used for dispersion of C60 fullerenes in native pH, and their responsiveness to pH modification and salt addition was monitored at different concentrations. Concentration-dependent responsiveness was observed: Just above the CMC in native pH, pH reduction results in precipitation (release) of pre-dispersed C60. At high polymer concentrations the C60 dispersions are non-responsive. Dispersions of Carbon Black, a colloidal particle of much larger diameter, characterized as a reference system, showed a different nanomorphology and were not responsive to pH modifications, due to a different dispersion mechanism. We concluded that good size matching between the dispersed particles is necessary for the formation of micelle-entrapped dispersions, and that the responsivity of the C60 dispersion to pH reduction is limited to a concentration range between the CMC in native conditions and the CMC in acidic conditions.

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1. Introduction

C_{60} fullerenes are all-carbon molecules with exceptional electron accepting properties and strong singlet oxygen sensitising ability as well as non-linear optical activity [1–3]. Biomedical applications of C_{60} fullerenes have been extensively explored over the recent years, suggesting antiviral and antibacterial activity [4,5], amyloid formation inhibition [6] and efficient antioxidant and neuroprotective activity [7], to name a few (for a comprehensive review see Ref. [8]).

A major obstacle for their utilization in biomedical applications is their hydrophobic nature. Solubilization of C_{60} in aqueous solutions relies on chemical modification [9], preparation of cage-like complexes and dispersion using surfactants and amphiphilic polymers [8]. A few biocompatible surfactants were successfully employed for dispersion of fullerenes aggregates with a typical diameter in the range of 60–70 nm [10] and a broad size-

distribution [11]. As aggregation reduces the production of reactive oxygen species (ROS) and modifies the size-dependent activity of fullerene clusters [12] reduction of the aggregate size to nanometric dimensions is desirable. Furthermore the ability to control the upload of fullerenes and their release via pH modification and salt addition are valuable for different applications that rely on tunable stability.

Here we report the utilization of the block-copolymers poly (O950)-b-(DEAEMA-co-BMA) comprising the monomer 2-(diethylaminoethyl) methacrylate (DEAEMA), the hydrophobic comonomer, butyl methacrylate (BMA), and a neutral hydrophilic monomer polyethylene glycol monomethylether methacrylate (PEGMA) (designated O950) for dispersions of C_{60} fullerenes [13]. Similar copolymers were shown before to form pH-responsive emulsions due to the presence of the DEAEMA residue that is a weak polybase with a pKa of the conjugated acid of about 7.3 [13]. UV-Vis measurements clearly indicate the presence of dispersed C_{60} in the micellar polymeric solutions and enable quantitative measurement of the concentration of the dispersed C_{60}. Dispersions of Carbon Black (CB) where the primary particles are characterized by a diameter of 30–50 nm were used as a reference system. Our findings indicate that size-matching between the

nano-structure and the molecular assemblies determine the nano-structure of the dispersions. In particular, while C_{60} seem to be entrapped within the polymeric micelles, CB aggregates are dispersed by the polymers. The stability of C_{60} dispersions and their responsiveness to pH modification and salt-addition depend on the concentration of the polymers: In low polymer concentrations, close to the CMC in native pH, the uptake of C_{60} is relatively low (about 10 μg/ml). Lowering the pH (from about 7 to around 1.5–2) or addition of salt de-stabilizes the dispersions, leading to release of pre-dispersed C_{60}. At high polymer concentration (about 4 times the CMC or more) the uptake of C_{60} is higher (and levels at about 60 μg/ml) and the dispersion is stable and non-responsive to pH modification and salt addition. Cryo-TEM imaging of the dispersions reveal the presence of polymeric micelles while non-dispersed C_{60} aggregates are not observed.

2. Experimental

2.1. Materials

Four di-block copolymers (sketch 1) were synthesized via reversible addition-fragmentation chain transfer (RAFT) polymerization (Table 1). The details of the synthesis were described before [13] see also the SI.

poly(PEGMA)-b-(DEAEMA$_{co}$BMA)
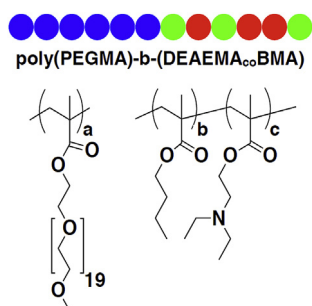
Buffers: Phosphate buffered saline (PBS) and 4-(2 Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) pK_a (25 °C) = 7.5 (Sigma-Aldrich) were used following pH adjustment by addition of NaOH or HCl solution.

C_{60} fullerenes: C_{60} (99.95% purity cat. No. 600–9950) were purchased from SES research Houston, Texas. The powders were dissolved in toluene and re-dried before use [10].

Carbon black: A dry powder of carbon black (CB), Vulcan P grade, was purchased from Cabot Corp. and used as received. Typical values of specific area are 700–1000 m^2/g, and typical diameters of the primary particles are 30–50 nm.

Polymer solutions and dispersions: were prepared by dissolving the polymers in water (di-ionized water, DIW) or buffer solutions at room temperature (22–24 °C). To form dispersions the polymer solutions were added to a C_{60} or CB (1–2 mg/ml) powder and stirred vigorously by a magnetic stirrer for 2–3 h.

Surface tension measurements were carried out using Du Nouy ring method [14] (KSV Sigma70 tensiometer), at room

temperature (22 °C) using 20 ml of polymer solution or C_{60} dispersion.

Uv-Vis absorption: the concentration of dispersed C_{60} was determined by UV-Vis absorption measurements in the range of 200–700 nm using Lambda35 P.E. spectrophotometer and standard quartz cuvettes. The dispersions were centrifuged (10 min at 2200 rpm) prior to the measurement. The intensity of the absorbance peak at around λ = 350 nm and the reported value of the extinction coefficient, 0.0263 (Lmg^{-1}cm^{-1}) [10] were used for calculating the dispersed concentration of C_{60} following the Beer-Lambert-Bouguer law.

Cryo-TEM. The technique is based on rapid vitrification of liquid samples (formation of a glassy solid) induced by plunging the liquid dispersion into a suitable cryogen (here liquid ethane) [15]. Such physical fixation of the liquid state preserves the native structures and allows examination of the polymeric assemblies in the high vacuum of the electron microscope at cryogenic temperature. Thinning the sample by blotting off the excess liquid prior to the plunging in a controlled environment (temperature and humidity) limits solvent sublimation. The rapid cooling and vitrification of the solvent molecules prevents the formation of either cubic- or hexagonal ice. A 3 μL drop was applied to a TEM grid (300 mesh Cu Lacey substrate, Ted Pella, Ltd.) following a short pre-treatment of the grid via glow discharge. The excess liquid was blotted and the specimen was vitrified by rapid plunging into liquid ethane pre-cooled by liquid nitrogen using a vitrification robot system (Vitrobot mark IV, FEI). The vitrified samples were examined at −177 °C using FEI Tecnai 12 G^2 TWIN TEM operated at 120 kV and equipped with a Gatan model 626 cold stage. The images were recorded by a 4 K × 4 K FEI Eagle CCD camera in low dose mode. TIA (Tecnai Imaging & Analysis) software was used to record the images and to measure the diameters of the assembled polymers. The average diameter was calculated from diameter values obtained from measuring 50–200 micelles of each polymer in each solution.

Positive staining. Ammonium molybdate (AM) solution was prepared by dissolving 30 mg of AM salt (Ammonium molybdate tetrahydrate, Sigma-Oldrich, PubChem ID 24896471) in 1 ml of deionized water to form a 3 wt% solution. The staining solution was added in a 1:1 vol ratio to the sample followed by mixing on vortex for few seconds prior to preparation of the cryo-TEM specimens.

3. Results and discussion

C_{60} fullerenes were dispersed in aqueous solutions of four di-block copolymers: poly(O950)-b-(DEAEMA-co-BMA) having different molecular weights and ratio between the hydrophilic and hydrophobic residues. The effect of polymer concentration, pH modification and addition of salt was investigated in dispersions of the di-block copolymer designated A.

The native polymers: The concentration −dependent surface tension of the four native polymers in buffer solutions and water shows similar behavior. In Fig. 1a we present polymer A and B in water and PBS solutions at pH = 7.4, 7 and 5.5. The transitions seen in the curves are generally attributed to a collapse of the polymer molecules adsorbed at the water-air interface, with the plateau value indicating the formation of micelles (CMC) [16,17]. In a previous study [17] we found out that the CMC of these polymers depends on the pH of the solution and on the buffer composition. The calculated CMC values for the different solutions are presented in Table 2.

The assemblies formed by the block-copolymers in the solutions above the CMC were imaged using cryo-TEM (Fig. 1b and c). To increase the inherently low contrast of the polymer assemblies investigated here the cryo-TEM images were obtained with the objective lens under focused by 2–4 μm to induce a phase shift of

Table 1
Composition, molecular weight and polydispersity index of the diblock copolymers. The macro-CTA DP (Degree of Polymerization) is fixed to 16 (see also Table S1).

Polymer notation	DEAEMA DP	BMA DP	Mn (g/mol)	PDI	Block ratio
A	26	20	21,060	1.2	0.4
B	53.4	37.2	32,900	1.15	1.1
C	79.2	55.5	41,300	1.14	1.6
D	154.8	106.8	68,600	1.13	3.4