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# Fabrication of nanoaggregates of poly(ethylene oxide)-*b*-polymethacrylate by complex formation with chitosan or methylglycolchitosan

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**Abstract**

Nanoaggregate formation of poly(ethylene oxide)-*b*-polymethacrylate (PEO-b PMA) is induced by chitosan (Ch) or methylglycolchitosan (MGC). The nanoaggregates are basically obtained by electrical charge neutralization of the anionic PMA block of the PEO-b-PMA polymer with cationic Ch or MGC, which results in insolubilization of the PMA block to form the core of the aggregates. Formation of the nanoaggregates was confirmed by dynamic light scattering, fluorescence spectroscopy, atomic force microscopy and zeta-potential measurements. The properties of the nanoaggregates depend upon the concentration of the polymer as well as the concentration of Ch or MGC. The significance of these aggregates is their ability to incorporate ionic species, leading them to potential applications as drug carriers and nanoreactors.


*Keywords:* Poly(ethylene oxide)-*b*-polymethacrylate; Chitosan; Methylglycolchitosan; Nanoaggregates; Counterions

## 1. Introduction

In the past decades, extensive attention has been paid to preparation of micelles from block copolymers. The polymeric micelles have wide applications in drug delivery systems [1,2] and separation techniques [3]. On dissolving the amphiphilic block copolymer into water, its self-assembly occurs to form the polymeric micelles having a hydrophobic core and a hydrophilic corona [4,5]. Although the micelles of amphiphilic block copolymers have various advantages compared to the micelles of low molecular-weight surfactants, there is a drawback that they cannot incorporate ionic species into the core because the core is hydrophobic in nature [6].

Recently, steadily increasing attention has been paid to the *double hydrophilic copolymers* (DHBCs) because they have a unique self-assembling property which leads them to form polymeric micelles in the presence of some suitable counterions [7–9]. The DHBC cannot form the micelle by itself on dissolving it into water. However, when one block of it is insolubilized with a suitable counterion such as a surfactant, metal ion or polyion, it forms a micelle having a core of the insolubilized block. The electrostatic interaction (or coordinate bond in some cases) between the added counterion and one block of the DHBC plays a primary role in the micellization process. For example, poly(ethylene oxide)-*b*-polymethacrylate (PEO-b-PMA) can form micelles when the cationic PMA block is insolubilized with a counterion such as poly-L-lysine (PLS) [9]. This nanoaggregate had proved to be stable, monodispersed and able to incorporate ionic drugs into the core due to its ionic nature. Other examples of DHBC assemblies have been reported by Kataoka et al. [10–13], Kabanov et al. [14,15], and Bronstein et al. [16,17]. Nanoaggregate formation of DHBCs has been extensively reviewed by Cölfen [7].

Based on our earlier studies [8,9,18,19] and those of others [20–25], we tried to fabricate the nanoaggregates using PEO-b-PMA and the chitosan family such as chitosan (Ch) and methylglycolchitosan (MGC). These chitosans are polycations and thus may bind to the anionic PMA block of

*Abbreviations:* (PEO-b-PMA), poly(ethylene oxide)-*b*-polymethacrylate (PEO-b-PMA); Ch, chitosan; MGC, methylglycolchitosan; DN, apparent degree of neutralization; cac, critical aggregation concentration

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PEO-b-PMA by electrostatic interaction, resulting in insolubilization of the PMA block. Ch exhibits antimicrobial and wound-healing properties [26]. Several investigations so far have been attempted regarding its function in health parameters. These studies include chitosan's hypocholesterolemic properties [27], as well as its effect on arthrosis [28], ulcers [29], osteoporosis [30], etc. Thus, formation of nanoaggregates of Ch and its family with PEO-b-PMA may break fresh ground for drug delivery systems as well as basic research on nano-science. As we expected, we have obtained the nanoaggregate of PEO-b-PMA and Ch or MGC. The nanoaggregate formation was confirmed by dynamic light scattering (DLS) measurements, fluorescence spectroscopy, atomic force microscopy (AFM) and zeta-potential measurements.

## 2. Experimental section

### 2.1. Materials

PEO-b-PMA was supplied from Polymer Source, Inc., and used as received. The molecular weight of each block is $\bar{M}n$(PEO) = 7500 and $\bar{M}n$ (PMA) = 15,500. Ch and MGC were purchased from Wako and used without further purification. The molecular weight of Ch ranges from 3000 to 30,000, and that of MGC is more than 150,000. The *deacetylation degree* (DA) of Ch and MGC is 40–50% and 100%, respectively. Pyrene (Py) was purified by vacuum sublimation.

### 2.2. Preparation of nanoaggregates

A known amount of PEO-b-PMA stock solution (2 g/L) was titrated with a solution of Ch or MGC. The amount of added Ch or MGC is expressed by an *apparent degree of neutralization* (DN), which is defined as:

$$\mathrm{DN}(\%) = \frac{\mathrm{DA}\times(\text{amount of counterions in base molar units})\times 100}{\text{amount of COOH groups in mole units}} \quad (1)$$

We assume that DA = 0.4 for Ch and DA = 1.0 for MGC.

All the solutions were prepared using water purified with the Millipore MiliQ system. The solutions of counterion/PEO-b-PMA were transparent over the whole range of DN and stable for several weeks. In the case of the Ch/PEO-b-PMA nanoaggregates solution, the desired pH is obtained by adding hydrochloric acid or sodium hydroxide.

The pH of the solutions of MGC/PEO-b-PMA was 7.2, where about 70% of the carboxylic groups in PMA and almost all the amine groups in MGC are ionized [31].

In the Ch/PEO-b-PMA systems, the pH was adjusted to 4.65 to realize a condition where both the carboxylic groups in PMA and the amino groups in Ch have an ionic form. However, only 10% of the carboxylic groups have an ionic form at this pH [31]. In spite of this fact, we did not choose another pH value because most of the carboxylic groups have a nonionic form at a pH lower than 4.65, while almost all of the amino groups have a nonionic form at a pH higher than 4.65. For these reasons, DN does not have its original meaning. However, we use DN just to show the amount of $NH_2$ groups in the added chitosans relative to that of COOH groups in the PMA block.

### 2.3. DLS measurements

DLS measurements were carried out with an Otsuka ELS-800 apparatus at a fixed 90° scattering angle. Correlation functions were analyzed by a histogram method and used to determine the diffusion coefficient ($D$) of the samples. The hydrodynamic radius ($R_h$) was calculated from $D$ using the Stokes–Einstein equation:

$$R_h = \frac{k_B T}{6\pi\eta D}, \quad (2)$$

where $k_B$ the is Boltzmann constant, $T$ the absolute temperature, and $\eta$ the solvent viscosity.

### 2.4. Zeta-potential measurements

The measurements of electrophoretic mobility (EPM) were performed at 25 °C with an Otsuka ELS-8000 apparatus. The zeta-potential of the particles was calculated from the EPM using the Smoluchowski equation:

$$\mu_E = \frac{\zeta\varepsilon}{\eta}, \quad (3)$$

where $\mu_E$ is EPM, $\zeta$ the zeta-potential, $\varepsilon$ the permittivity of the solvent, and $\eta$ the viscosity.

### 2.5. Fluorescence spectroscopy

Steady-state fluorescence spectra of the samples were recorded with a Hitachi F-6500 fluorescence spectrophotometer (right angle geometry, 1 cm × 1 cm quartz cell). First a known volume of Py stock solution ($6 \times 10^{-5}$ M in methanol) was transferred into a 10 mL volumetric flask. The solvent was evaporated by gentle heating under a nitrogen gas stream. A known volume of PEO-b-PMA stock solution was then added to the volumetric flask containing Py, followed by the addition of counterion solutions. Finally, hydrochloric acid or sodium hydroxide was added to the volumetric flask to obtain the desired pH. The concentration of Py was 0.6 μM which is close to the saturation concentration of Py in water at 22 °C. Py is excited at 334 nm. The band widths were 3 and 1 nm for excitation and emission, respectively.

### 2.6. Atomic force microscopy

AFM images were obtained in the dynamic force mode (corresponding to the tapping mode) with an SPA 300 unit together with an SPI 3700 control station (Seiko Instruments