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Physicochemical characterization of the Py-g-PEG copolymer at the interface

Y. Fujita[1], K. Ueno[1, 2], T. Satomi[1, 2, 3], H. Yajima[1], and H. Otsuka[1, 2, 3,*]

[1]Department of Applied Chemistry, Faculty of Science, Tokyo University of Science, 1-3 Kagurazaka, Shinjuku-ku, Tokyo 162-8601, FUJITA.Yohei@nims.go.jp, [2]Biomaterials center, National Institute for Materials Science, and [3]Division of Bioengineering and Bioinformatics, Graduate School of Information Science and Technology, Hokkaido University, *corresponding author

The novel amphiphilic graft copolymers was synthesized, which consist of poly(ethylene glycol) (PEG) as a hydrophilic segment and polypyrinyl moiety as a hydrophobic/metal affinity segment. Py-g-PEG copolymers modulated in adequate hydrophile-lipophile balance (HLB) were found to be self-assembled in water, expressing the critical micelle concentration (cmc) and highly hydrophobic micelle core. In this way, formation of micelle, cmc, hydrodynamic diameter, and morphology of the micelles were investigated. The micelles were determined to be cmc of 0.15 mg / ml and size of approximately 150 nm. Furthermore, fluorescence spectroscopy measurements with pyrene loaded in the hydrophobic core of the micelles showed that when these micelles were incubated at slightly acidic environment, they were increasingly hydrated with suggested destroyed micelle structure, in comparison with the neutral environment. The gold nanoparticles (NPs) were also prepared using the Py-g-PEG copolymers. In the study, the Py-g-PEG micelles worked as nanoreactors for Au^{3+} reduction. Au NPs were prepared via reduction by hydrazine and characterized by TEM and UV-vis. Au NPs diameter was determined to be ~8 nm with narrow size distribution. These nanoparticles are expected to have high utility in the field of biomedical applications including biodetection, diagnosis, and imaging.

Key words: amphiphilic graft copolymers, polymeric micelles, cmc, pH resposible

1. INTRODUCTION

The interaction between biomolecules and polymer surface is a fundamental aspect of many biological and industrial processes. Biomaterial surface is intensively investigated to use in many applications. The ability to control the interactions of cells and proteins is critical issue for fundamental cell biology studies, medical implants, and tissue engineering scaffolds, as well as for the development of cell integrated biochips[1, 2]. Thus, physichochemical properties and characteristics of the polymer surface are closely related to their biological interactions. Recently there are reported many studies, in which polymers achieves the interfacial stabilization by phase separation such as SAMs, micelles, vesicles[3, 4]. In particular, polymer brushed layer for controlling the interfacial reaction with biological compositions is of great techological importance for biomaterial deveropment: ,The brush-like layer forms an effective barrier to the adsorption of macromolecules[5-7] including proteins, where the steric repulsion force can be exploited for the stabilization of colloid and interface. In this study, the graft copolymer (pyridine-graft-poly(ethylene glycol); Py-g-PEG was newly synthesized, which enables the long-term interfacial stability by employing the multipoint adsorption of the pyridine unit, while the hydrophilic PEG strands formed the brushe-like layer, protruding into the interfacial water phase.

On the other hand, amphiphilic copolymers have recently attracted much attention due to their interesting behavior such as self-assembled micelles[4, 8]. The micelles have unique characteristics such as nanosize, a core–shell architecture, and a good thermodynamic stability in physiological conditions because of their low critical micelle concentration (cmc). Furthermore, the molecular structure, shape, and hydrophilicity of the polymer have great effects on the micelle properties. Thus, physicochemical property such as the solution and air / liquid interface and aggregate formation is very important even in the case in which polymer condition in material surface is examined.

In this study, the structure of Py-g-PEG copolymer was controlled, which made external stimuli to be a trigger using the pyridine unit, and the physicochemical property in these interfaces was evaluated.

2. EXPERIMENTAL SECTION

2.1 Materials

Commercial methacrylic acid (Wako), 4-pyridinemethanol (TCI), *N,N'*-dicycrohexylcarbodiimide (Wako), 4-(1-pyrrolidinyl)pyridine (Wako), SUNBRIGHT ME-020AS (a gift from NOF Corporation), 2-2'-azobis-(isobutyronitrile) (Wako), $HAuCl_4{\cdot}4H_2O$ (Wako), and hydradine monohydrate (Kanto Chemical) were used as received. The

α-methyl-ω-methacryloyl-PEG (M_n = 2080) was purified with following procedure: 50 wt. % solution in water (Aldrich) was dried over under reduced pressure, with dry $MgSO_4$. The resulting amorphous PEG was dissolved in THF followed by addition to cold 2-propanol, and the obtained precipitate was freeze-dried from benzene, yielding white powder. Reaction solvents were purchased from Wako dehydrated grade. All water used was purified by treating in reverse osmosis unit followed by a Millipore unit (18mΩ resistivity).

2.2 Synthesis

Preparation of 4-Pyridylmethyl-methacrylate. To synthesize polypyridine-graft-PEG copolymer, 4-pyridilmethyl-methacrylate as a pyridine monomer was synthesized. Methacryl acid (4.73 g, 55 mmol), 4-pyridine methanol (5.45 g, 50 mmol), and 4-(1-pyrrolidinyl)pyridine (740 mg, 5 mmol) were dissolved in dry dichloromethane (100 ml) in a glass vessel. After *N,N'*-dicyclohexylcarbosiimide (11.3 g, 55 mmol) was added to the solution, the reaction mixture was stirred for 1 h at room temperature. After the resulting insoluble urea was removed with filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, hexane / ethyl acetate) yielding colorless oil (8.1 g, 46 mmol, Y. = 91 %).

Copolymerization of Methyl-Terminated PEG Macromonomers with 4-Pyridylmethyl Methacrylates. A series of Py-g-PEG were newly synthesized with radical copolymerization. As shown in scheme 1, 4-pyridylmethyl-methacrylate (177 mg, 1.0 mmol), several amount of α-methyl-ω-methacryloyl-PEG (M_n = 2080), and AIBN (1 mol% of monomer) were dissolved in dry DMF (ca. 10 times amount of monomer mass). After the mixture was frozen and degassed 3 times, the solution was stirred for 24 h at 60°C. The reaction mixture was dropped into 2-propanol (100 ml), the solution was stirred for few minutes. The resulting precipitate was separated with centrifugation and freeze-dried from benzene to give white powder.

Preparation of α-methyl-ω-methyl(4-pyridilmethyl-carboxylate)-PEG, (single-Py-PEG). Synthetic route of pyridine-PEG (single-Py-PEG) was shown in scheme 2. After α-methyl-ω-methyl(N-hydroxysuccinimidyl-carboxylate)-PEG (SUNBRIGHT ME-020AS) (1.14 g, 0.50 mmol), 4-pyridinemethanol (109 mg, 1.0 mmol), *N,N'*-dicyclohexylcarbosiimide (113 mg, 0.55 mmol), and 4-(1-pyrrolidinyl)pyridine (15 mg, 0.10 mmol) were added to dry dichloromethane, the mixture was stirred for 24 h at room temperature. After the resulting insoluble urea was removed with filtration, the solvent was removed under reduced pressure. The residue was precipitated from 2-propanol at 4°C, the precipitate was separated with centrifugation, freeze-dried from benzene to yield white powder (697 mg).

Analysis. ^1H NMR spectra were obtained using $CDCl_3$ solution with JEOL AL-300 spectrometer at 300 MHz. Tetramethylsilane was used as an internal standard. The molecular weight and molecular weight distribution of the synthesized PEG were obtained using TOSOH HLC8220 GPC equipped with a gel permeation column (TSKgel $G4000_{HR}$ + $G3000_{HR}$). DMF containing 10 mM LiCl was used as an eluent.

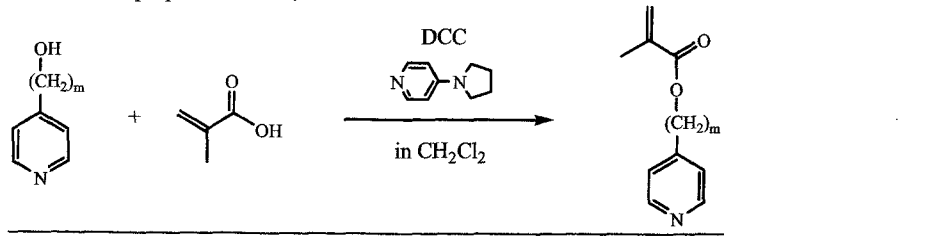
2.3 Preparation of self-organization and chacterization.

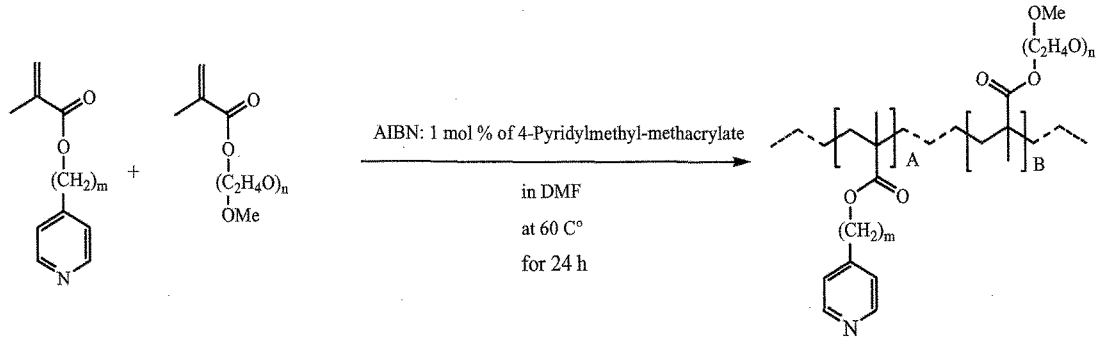
Py-g-PEG copolymer was dissolved in *N,N'*-dimethyl-acetamide (DMAc), a good solvent of both the hydro- philic and hydrophobic segments, in a 5 mg / ml concentration. The solution was into a preswollen semi- permeable membrane (MWCO = 12,000–14,000 g / mol) and dialyzed for 48 h against a 100-fold excess of distilled water. The dialysate was exchanged at times 2, 5, 8 and 24 h.

The hydrodynamic radii and the size distribution of micelles were determined by dynamic light scattering (DLS). All measurements were carried out at 25 °C on a light scattering spectrometer (DLS-7000, Otsuka Electronics), and the scattering was carried out with a vertically polarized incident beam at 488 nm supplied by an argon ion laser.

In addition, the Py-g-PEG copolymers were analyzed by transmission electron microscopy (TEM). TEM observation was performed for the samples dried on carbon-coated copper grids.

The surface tensions of aqueous solutions of the Py-g-PEG copolymers were measured with a Krüss K100 tensiometer by the Wilhelmy plate technique. Sets ofmeasurements to obtain equilibrium surface tension were taken until the change in surface tension was less than 0.01 mN m^{-1} every 1 min. The cmc and surface tension at the cmc were determined from the break point of the surface tension and logarithm of concentration curve. The adsorption amount of surfactants for heterogemini surfactants is calculated using the Gibbs adsorption isotherm equation, and other parameter was calculated. Moreover, the fluorescence measurements (Hitachi High-Technologies Co.) were performed. Pyrene solubilization has been used previously for the determination of the cmc in block copolymer solution[4]. The fluorescence of this probe is sensitive to changes in the microenvironment which permits monitoring its incorporation in micelles at concentrations exceeding the cmc. The excitation wavelength was 339 nm, and λ_{em} = 395 nm, where the concentration of pyrene was 6 x 10^{-5} M for each solution.





Scheme 1 Copolymerization of Methyl-Terminated PEG Macromonomers with 4-Pyridylmethyl Methacrylates.

the pyridine site in the intramolecule[9]. Therefore, gold nanoparticles (Au NPs) were prepared by using Py-g-PEG copolymer. Au NPs have been reported on their optical and electric properties. Au NPs were prepared by stirring aqueous solutions containing Py-g-PEG2k.5(19.7%) micelles and $HAuCl_4$ for 24 h and then reducing the encapsulated Au metal complex with hydrazine. The Au NPs obtained were analyzed by TEM and UV-visible (Agilent) absorption spectroscopy. The size distribution of the Au NPs was determined from about 200 particles.

3. RESULTS AND DISCUSSION

Polypyridine-*graft*-poly(ethylene glycol) copolymers were synthesized by radical polymerization using methyl-terminated PEG macromonomer and 4-pyridylmethyl methacrylate (Scheme 1). Py-g-PEG copolymers were characterized by ^{1}H NMR (Fig. 1). The number of each pyridine and PEG molecules per obtained graft copolymer can be controlled by changing initial ratio of PEG/Py. PEG/Py was calculated from the proton ratio of ^{1}H NMR. Table 1 summarizes the molecular characteristics of the synthesized copolymers.

It was indicated that Py-g-PEG modulated in adequate parcent hydrophobic balance (20-30 %) are self-assembled to form micelles in water. Fig. 2 shows an increasing micelle size with increasing polymer concentration. This may be due to a interpolymer aggregation at higher polymer concentration[10]. Therefore, the surface tension was measured on the orientation to air/liquid interface and cmc using the copolymer solution (Fig. 3). The figure show surface excess concentration (γ_{cmc}), and occupied area per molecule at the cmc (A_{cmc}) were the lowest value at the Py-g-PEG2k.5(19.7%). Consequently, it was indicated that Py-g-PEG2k.5(19.7%) was excellent in surface orientation and micelle forming ability.There were two break points of the solution, it seems to be appropriate, because it is similarly confirmed in Pluronic of which this tendency is the representative polymer activator[11]. In addition, there is hardly the change in the surface tension of Py-g-PEG2k.8(28.1%). This result indicates that the

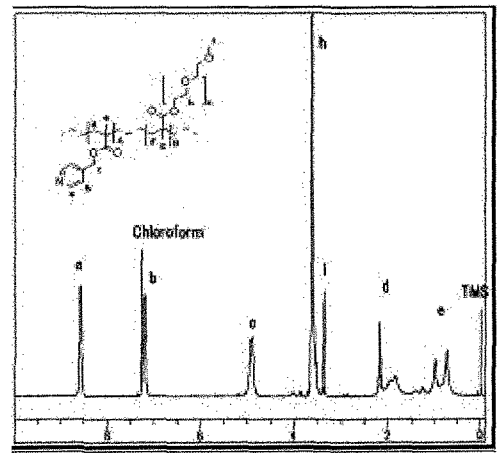


Fig. 1 ^{1}H NMR spectra were obtained using $CDCl_3$ solution at 300 MHz. Tetramethylsilane was used as an internal standard.

unimer micelle has been formed . A similar tendency was observed for other amphiphilic- diblock copolymers having carboxylic acid in the hydrophilic chain[11]. TEM observation near cmc was done, in order to know micelle size and shape in the bulk. Fig. 4 shows the Py-g-PEG micelles were spherical structure and size of approximately 150 nm.

Moreover, the change of the micellar microenvironment was confirmed from the uptake of the hydrophobic substance to the micelle core. The solubilization of pyrene was carried out. Pyrene was irradiated at the specific excitation wavelength and emits light, and the strength changes by the microenvironment in the pyrene circumference[12]. Table 2 showed cmc which was lower than the data of the surface tension by the interaction of the polymer. Especially, Py-g-PEG 2k.5(19.7%) and 8(28.1%), the hydrophobic interaction of pyridine site of the micelle core is strong, and have packed the density. In the meantime, pyridine site of the hydrophobic core of the micelles has particular p*Ka*, so it is possible to cause the protonation by pH, resulting in micelle collapse.

Table 1 Result of synthesized Py-g-PEG copolymer.

No.	Sample Name	m	n	M_n[a)]	M_w / M_n[a)]	principal chain unit number	PEG unit number
3	Py-g-PEG2k.3(8.3%)	1	43	52,000	1.36	159	12.2
8	Py-g-PEG2k.5(19.7%)	1	43	54,000	1.24	109	18.0
9	Py-g-PEG2k.8(28.1%)	1	43	34,000	1.28	57	12.6
11	Py-g-PEG2k.10(55.8%)	1	43	59,000	1.29	69	24.5

[a] M_n = number-average molar weight and M_w = weight-average molar weight determined by GPC.

Table 2 Parameters of adsorption and micellization.

Sample Name	cmc[a] ($mg\ ml^{-1}$)	γ_{cmc} ($mN\ m^{-1}$)	A_{cmc} ($nm^2\ molecule^{-1}$)	cmc[b] ($mg\ ml^{-1}$)
Py-g-PEG2k.3(8.3%)	$1.56x10^{-1}$	50.1	1.76	$1.56x10^{-2}$
Py-g-PEG2k.5(19.7%)	$1.47x10^{-1}$	44.8	1.48	$1.04x10^{-1}$
Py-g-PEG2k.8(28.1%)	$9.02x10^{-4}$	62.5	1.63	$6.5x10^{-2}$
Py-g-PEG2k.10(55.8%)	$1.24x10^{-2}$	57	2.47	$5.5x10^{-2}$

The [a]cmc measured in surface tension and [b]cmc was obtained from the fluorometry.

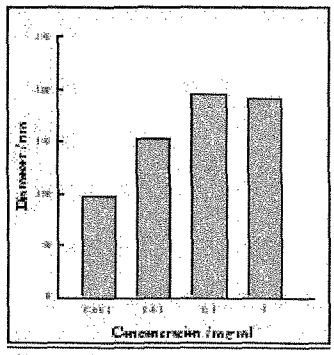


Fig. 2 The effect of the polymer concentration on the size of Py-g-PEG2k.5(19.7%) micelles.

Furthermore, it is possible to prepare Au NPs that is stabilized by the interaction between gold and pyridine in the Py-g-PEG micelles[9]. The solution of Au NPs has stably dispersed over 1 month. Au NPs were sphere, and it was the 6.7 ± 0.9 nm average diameter. And, the polymer has been collected in the circumference of Au NPs. It means NPs involves it for the micelles. The application of Py-g-PEG in the solid / liquid interface changed possible this result.

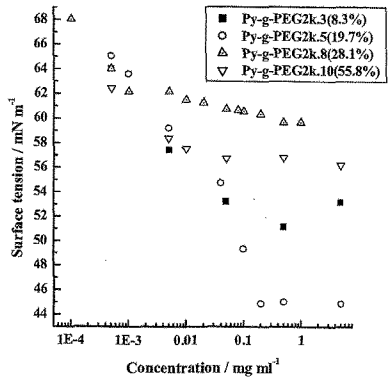


Fig. 3 Surface tension as a function of concentration for Py-g-PEG2k.x (x=3(8.3%), 5(19.7%), 10(55.8%)).

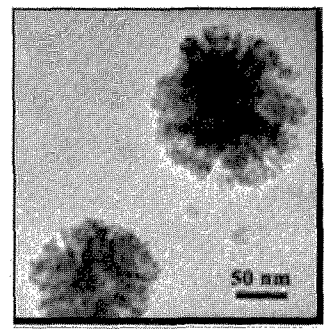


Fig. 4 TEM image of Py-g-PEG2k.5(19.7%) micelles. The polymer concentration was 0.1 mg / ml.

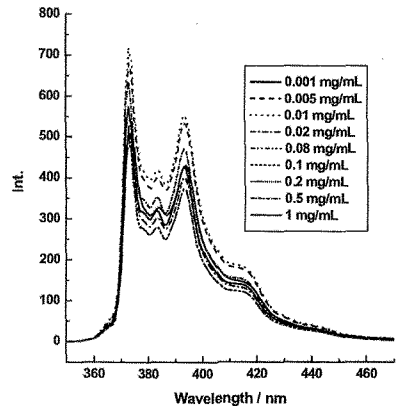


Fig. 5 Fluorescence emission spectra (λ_{ex} = 339 nm) of pyrene (6 x 10^{-5} M) in the presence of various concent rations of Py-g-PEG2k.5(19.7%).

4. CONCLUSION

The novel amphiphilic graft copolymer (Py-g-PEG) was synthesized, and the physicochemical characterization at the interface was examined. Py-g-PEGs form micelles at adequate parcent HLB (20-30%) and concentration, some physicochemical properties were measured. The micelles were determined to be cmc of 0.15 mg / ml and size of approximately 150 nm. Because the hydrophobic pyridine site was protonated under p*Ka*, the amphiphilic structure was the pH responsive dissociation. Therefore, it is possible to synthesize the polymer micelle in proportion to various pH by the change of the molecular

structure of the hydrophobic segment. The polymer synthesized in this study may have the high utility in the field of tissue engineering, drug delivery, diagnosis, as well as basic cell biology.

5. REFERENCES

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