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Synthesis of pH Sensitive Organic-Inorganic Polymer Hybrids

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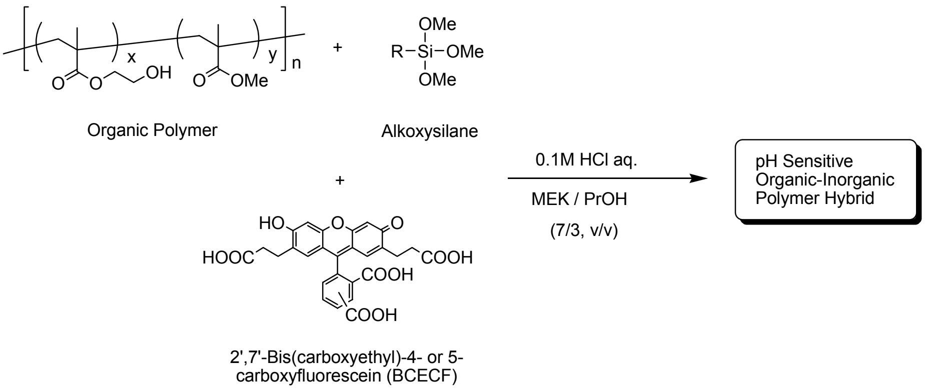
Summary

The synthesis of pH sensitive organic-inorganic polymer hybrids from poly(2-hydroxyethyl methacrylate) derivatives and alkoxysilane is described. To a reaction mixture of poly(2-hydroxyethyl methacrylate) derivatives, tetramethoxysilane, 2',7'-bis(carboxyethyl)-4- or 5-carboxyfluorescein (BCECF) as a fluorescent probe, and ten equivalents of HCl aqueous solution in methyl ethyl ketone / propanol (7/3, v/v) were added. The resulting mixture was kept stirring at room temperature for 1 h and was heated at 40 ℃ for 2 weeks. The changes of the pH were estimated as changes in the fluorescence intensity with the pH sensitive fluorescence probe BCECF trapped within the obtained polymer hybrid. Consequently, the pH value was found to be proportionate to the fluorescence intensity.

Introduction

The composite materials are expected to give the specific properties, which are not in the original material by mixing two or more components. Therefore, the composite system is one of the excellent methods to build new material cheaply starting from common materials. Preparation of organic-inorganic composite material has attracted much attention as they might show improved properties superior to those of the parent materials [1-3]. Especially, a sol-gel reaction can be easily used for the preparation of composite materials of organic and inorganic compounds. In the sol-gel process, a three-dimensional silica network is formed at relatively low temperature by hydrolysis and condensation reaction of alkoxysilanes [4]. Various kinds of organic compounds including polymers are incorporated in the reaction mixture of the sol-gel reaction to obtain the organic-inorganic hybrid materials. When the hybrid materials are functionalized, the doping of functional molecules is the simplest method. For example, the stimuli-responsive molecules such as pH sensitive molecules (BCECF [5], Quene 1 [6]), calcium ion sensitive molecules (Fluo 3 [7], Fura 2 [8], Indo 1 [9], Quin 2 [9], Rhod 2 [10]), zinc ion sensitive molecules (dansylaminoethyl-cyclen [11], TQS [12], zinquin ethyl ester [13]), and chloride ion sensitive molecules (MQAE [14], SPQ [15]) are well known in the field of bioscience. If these functional molecules are introduced in the case of hybridization, the hybrid materials having the character of these molecules will be obtained. In this article, we propose the novel functionalization method for the organic-inorganic polymer hybrid materials. 2',7'-Bis(carboxyethyl)-4- or 5-carboxyfluorescein (BCECF) was employed as a pH sensitive molecule for organic-inorganic polymer hybrids (Scheme 1). The hybridization of poly(acrylate), silica gel derived from alkoxysilane, and BCECF may produce pH sensitive organic-inorganic polymer hybrids.



Scheme 1. Synthesis of pH sensitive organic-inorganic polymer hybrids.

Experimental

Materials

2-Hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) were distilled and stored under nitrogen. Tetramethoxysilane (TMOS) methyltrimethoxysilane (MeTMOS) were distilled and stored under nitrogen. 2,2'-Azobis(isobutyronitrile) (AIBN) was recrystallized from ethanol. Fluorescence probe was purchased from Dojindo Laboratories. The other solvents and reagents were used as supplied.

Measurements

^{1}H NMR spectra were recorded on a JEOL JNM-EX270 spectrometer. The FT-IR spectra were obtained on a Perkin-Elmer 1600 infrared spectrometer. GPC measurement of poly(MMA) was performed with a Shodex K-803 in chloroform at 25 ^{o}C by use of polystyrene as standard samples. GPC measurements of poly(HEMA) and poly(HEMA-*co*-MMA) were carried out on TSK gel α-3000 by using DMF containing LiBr (10 m*M*) as an eluent at 40^{o}C after calibration with polystyrene as standard samples. Fluorescence emission spectra were recorded on a Perkin-Elmer

LS50B luminescence spectrometer. All pH values were measured with a HORIBA D-21 pH meter. Scanning electron microscopy (SEM) measurements were conducted using a JEOL JNM-5310/LV system. Thermogravimetric analysis (TGA) was performed using a TG/DTA6200, SEIKO Instruments, Inc., with a heating rate of 10 ℃ min^{-1} in air.

Homopolymerization of Poly(HEMA)

HEMA (20.00 g, 0.15 mol) and AIBN (1.26 g, 4.67 mmol) were dissolved in a mixed solvent (200 mL) of methyl ethyl ketone (MEK) and propanol (PrOH) (v/v, 7/3) under nitrogen atmosphere. After 24 h at 60 ℃, the reaction mixture was poured into diethyl ether and the precipitated polymer was dried *in vacuo*. The obtained polymer was characterized by ^{1}H NMR and IR spectra. Yield: 93%, GPC (eluent: DMF containing LiBr 10 m*M*): M_n=7800 (M_w/M_n=3.34).

Homopolymerization of Poly(MMA)

MMA (10.00 g, 0.10 mol) and AIBN (0.82 g, 4.99 mmol) were dissolved in a mixed solvent (100 mL) of MEK and PrOH (v/v, 7/3) under nitrogen atmosphere. After 24 h at 60 ℃, the reaction mixture was poured into methanol and the precipitated polymer was dried *in vacuo*. The obtained polymer was characterized by ^{1}H NMR and IR spectra. Yield: 46%, GPC (eluent: chloroform): M_n=18800 (M_w/M_n=1.11).

Copolymerization of HEMA and MMA (Typical Procedure)

HEMA (6.50 g, 49.95 mmol), MMA (5.00 g, 49.94 mmol) and AIBN (0.82 g, 4.99 mmol) were dissolved in a mixed solvent (100 mL) of MEK and PrOH (v/v, 7/3) under nitrogen atmosphere. After 24 h at 60 ℃, the reaction mixture was poured into diethyl ether and the precipitated polymer was dried *in vacuo*. The obtained poly(HEMA-*co*-MMA) was characterized by ^{1}H NMR and IR spectra. The unit ratio of the copolymer was calculated from ^{1}H NMR spectra (HEMA/MMA=51/49). Yield: 85%, GPC (eluent: DMF containing LiBr 10 m*M*): M_n=8600 (M_w/M_n=3.50).

Synthesis of pH Sensitive Organic-Inorganic Polymer Hybrids from Poly(HEMA) and TMOS (Typical Procedure)

Poly(HEMA) (0.50 g) was dissolved in a mixed solvent (20 mL) of MEK and PrOH (v/v, 7/3) with TMOS (1.27 g), 0.1 *M* HCl aqueous solution (10 equiv to alkoxysilane), and 50 μL of BCECF methanol solution (0.5 m*M*). After being stirred at room temperature for 1 h, the mixture was placed in a polypropylene vessel covered with a wiping paper and left in air at 40 °C for 5 days. The obtained polymer hybrid was dried *in vacuo* at 60 °C for 2 days.

Measurements of Internal Fluorescence Intensity of Polymer Hybrids

The pH sensitive organic-inorganic polymer hybrids were placed into the fluorescence cell. The fluorescence cell was filled with the potassium dihydrogenphosphate-sodium hydroxide buffer solution. It can be seen that fluorescence at excitation wavelength near 450 nm is relatively insensitive to pH, while fluorescence above 490 nm increases directly as function of pH. The pH insensitive region allows the construction of a fluorescence ratio (fluorescence at sensitivity λ / fluorescence at insensitivity λ) which retains pH sensitivity but tends to correct for variations in excitation light intensity or dye concentration. Therefore, the fluorescence spectra were recorded at two excitation wavelengths, 490 and 450 nm and the ratio of the fluorescence intensities at 530 nm (490 nm/ 450 nm) was calculated. The pH values of solutions were checked using a pH meter calibrated with standard buffers of pH 6.86 and 4.01 (Wako Pure Chemical Industry).

Results and Discussion

The pH sensitive organic-inorganic polymer hybrids were prepared *via* acid-catalyzed sol-gel reaction of TMOS or MeTMOS in the presence of poly(acrylate) derivatives such as poly(HEMA), poly(HEMA-*co*-MMA), or poly(MMA). The polymer hybrids from poly(acrylate) derivatives were found to be transparent and homogeneous in a wide range of HEMA unit (x unit) / MMA unit (y unit) ratios. As shown in Table 1, the transparent polymer hybrids were obtained when TMOS was employed as a starting material for sol-gel reaction. In contrast, the polymer hybrid became turbid when MeTMOS was employed. The result might be attributed to methyl groups of MeTMOS, which are suspected to interrupt hydrogen bonding interaction.

Table 1. Effect of unit ratio of copolymer on the homogeneity.[a)]

R-Si(OMe)$_3$		Unit Ratio in Organic Polymer	Appearance	Ceramic Yield (%)[b)]			T_{d10}
R-	(g)	(x unit / y unit)		obs	calc	obs/calc	
OMe	1.277	100/0	transparent	42.5	50.2	84.6	324.7
OMe	1.292	82/18	transparent	43.6	50.5	86.3	316.8
OMe	1.271	75/25	transparent	42.8	50.1	85.4	312.7
OMe	1.420	51/49	transparent	44.7	52.9	84.5	307.4
OMe	1.275	0/100	transparent	40.4	50.2	80.5	270.2
Me	1.143	100/0	turbid	47.4	56.1	84.5	211.5

a) Conditions: 0.50 g of polymer was dissolved in 20 mL of MEK/PrOH (7/3, v/v) with alkoxysilane and 0.1 *M* HCl aq. 0.6 mL (10 eq.). The mixture was heated at 40 ^{o}C for 5 days. BCECF methanol solution (0.5 m*M*, 50 μL) was added.

b) Ceramic yield = (weight percent of ceramic in polymer hybrid observed by TGA) / (calculated value of weight percent of ceramic).

The polymer content calculated was almost the same as that measured from TGA in the polymer hybrid prepared from poly(MMA) and TMOS, T_{10} was observed at 270.2 ^{o}C. While in the polymer hybrids using the copolymer with high HEMA unit content, T_{10} was shifted to higher temperature. These results show that the thermal stability was increased with an increase of the inorganic content.

As shown in Figure 1, the polymer hybrid prepared from TMOS showed no recognizable segregation at this level in the SEM image. On the other hand, phase separation appeared in the polymer hybrid prepared from MeTMOS.

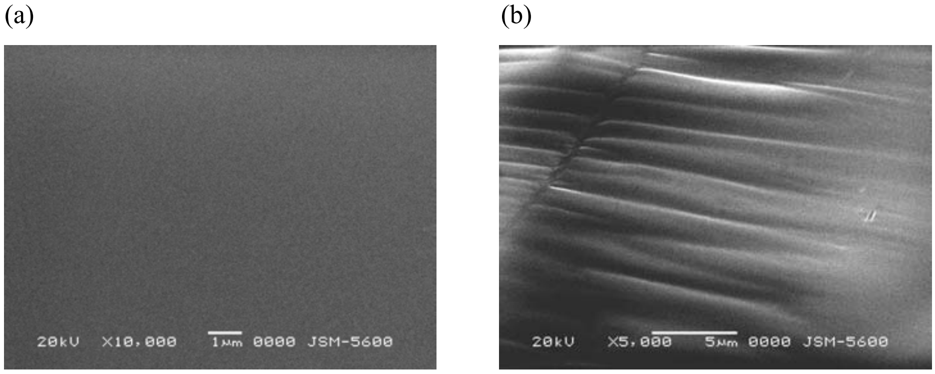


Figure 1. Scanning electron micrographs of (a) the polymer hybrid prepared from poly(HEMA) and TMOS (b) the polymer hybrid prepared from poly(HEMA) and MeTMOS.

In the obtained organic-inorganic polymer hybrids, the response to pH was evaluated by fluorescence measurement. The organic-inorganic polymer hybrid prepared from poly(HEMA), TMOS, and BCECF was placed into the fluorescence cell. Then the fluorescence cell was filled with the buffer solution in which the pH value was predetermined. Subsequently, fluorescence measurements were performed. Figure 2 demonstrates the lineality of the relative fluorescence intensity with the external pH value. However, when the same measurement was repeated, the slope of a straight line became gradual. Since poly(HEMA) has a hydrophilic character, the fluorescence probe might seep out from the polymer hybrid.

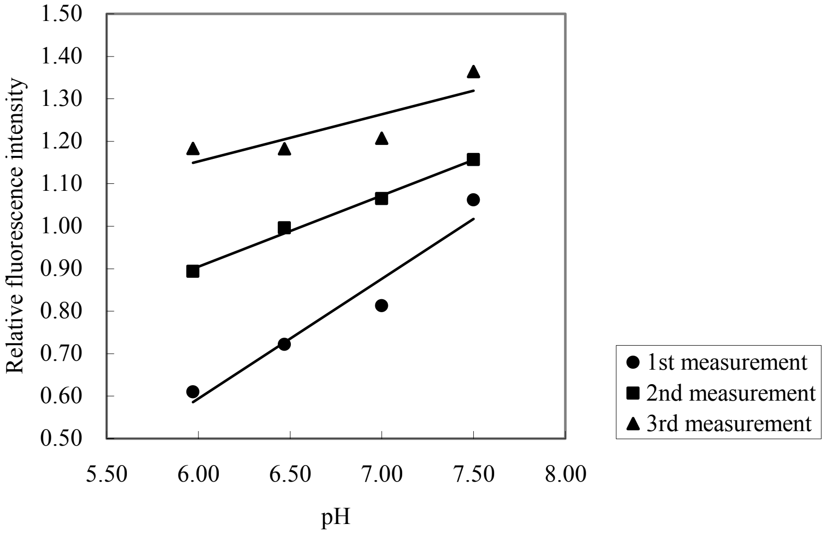


Figure 2. pH-response profile for the polymer hybrid prepared from poly(HEMA) and TMOS.

To overcome the above seeping problem, the synthetic conditions for the water resistant polymer hybrids were examined. When poly(MMA) or poly(HEMA-*co*-MMA)s was used as an organic polymer segment, the water resistant property was much improved. However, the higher content of MMA unit than 25% caused the poorer pH response due to too hydrophobicity. Thus, the polymer hybrid consisting of poly(HEMA-*co*-MMA) (x unit / y unit = 82 / 18), TMOS and BCECF was employed for the measurement of the change of fluorescence intensity depending on pH values.

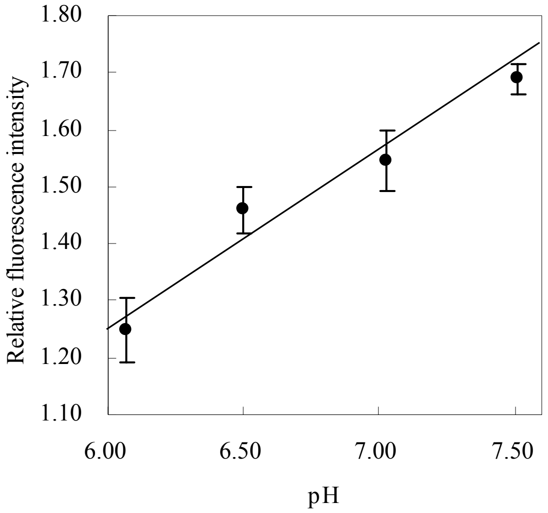


Figure 3. pH-response profile for the polymer hybrid prepared from poly(HEMA-*co*-MMA) (x unit / y unit =82/18) and TMOS. After soaking a hybrid in buffer solution (pH=6.07) for 30 min, the fluorescence measurements were performed. Error bars show the errors of the observed values three times between 0 and 30 min.

In this case, the relative fluorescence intensity was found to be on a linear response depending on the pH value between 6.07 and 7.51. The fluorescence measurements were carried out every 5 min in the buffer solution. This result shows that the linear response was accomplished without depending on the time. It should be also emphasized in this case that the fluorescence probe was hardly eliminated from the hybrid.

The pH sensitive organic-inorganic polymer hybrids are expected in many fields such as environmental analysis, biological field, and medical field. Furthermore, the pH sensitive organic-inorganic polymer hybrid might be stable even under the condition of soaking in the sample solution. The information of pH value can be readout by the fluorescence measurement. The preliminary results presented here indicate a potential functionalization of the organic-inorganic polymer hybrid by the doping with functional molecules. Other functional organic-inorganic polymer hybrids can be produced by using this methodology.

Conclusion

We have demonstrated the synthesis of pH sensitive organic-inorganic polymer hybrids. The polymer hybrid prepared from poly(HEMA), TMOS, and BCECF did not have good water resistance property. The polymer hybrid prepared from poly(HEMA-*co*-MMA) (x unit / y unit =82/18), TMOS, and BCECF, showed good pH response. These polymer hybrids have a potential as various sensors.

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