The Use of a Glass Fiber Filter Functionalized with a Double-layer PEI/HA Coating for Removing Trace Levels of Copper from Artificial Seawater

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To avoid degrading the performances related to fuel consumption and maneuverability, an antifouling paint that contains Cu2O as a source of biocidal Cu(II) are applied to the bottom of a ship. However, increasing the Cu(II) concentration around a dockyard during ship maintenance6 has been regarded as a serious environmental issue. The ability of humic acid (HA) and polyethyleneimine (PEI) for complexes with heavy metal ions was examined for removing Cu(II) from contaminated seawater. A glass fiber filter was functionalized by coating it with PEI and HA by taking advantage of the electrostatic character of these substrates. The resulting coated filter proved to be highly effective for removing Cu(II) from artificial seawater by simple filtration. The capacity and efficiency on Cu(II) removal for a filter coated with PEI was increased by further coating with HA. The removal of Cu(II) was dependent on the effectiveness of the coated area, suggesting that this functionalizing method could be applied to other forms of glass fiber materials.

Keywords Humic acid, polyethyleneimine, copper, glass filter, antifouling paint

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Introduction

Fuel consumption and maneuverability are critical issues in the shipping industry. The accumulation and growth of organisms on ship hulls increases the frictional resistance, which causes problems, such as an increase in fuel consumption and degraded maneuverability. According to the accumulation and growth of fouling organisms, the hulls of most ships are treated with antifouling paint containing pigments that can leach some biocidal chemical components.1,2 Tributyltin had been used as the most successful compounds against biofouling; however, its use as an antifouling paint has been prohibited internationally since 2003 because of its severe negative impact on the marine ecosystem.2,3 Cu(II) has an adverse effect on the growth of organisms, even when its level is low. Thus, cuprous copper oxide (Cu2O) has been used for the main component of antifouling paint as a source of biocidal cupric copper ions since the 1980s.1

In recently, the contamination by copper derived from antifouling paint constitutes an environmental problem in enclosed coastal areas. The average concentration of copper in seawater was reported to be 4 nM;4 however, the copper concentration was higher around a dockyard located in an enclosed coastal area (6 – 150 nM).5 Around a shipyard located in an enclosed coastal area, the copper concentration in seawater was higher (8 – 370 μM).5 In particular, copper contamination in areas around a dockyard has been expected to be higher than that in shipyards because antifouling paint particles are released from a dockyard during ship maintenance.6 In sediment around a dockyard, fine antifouling paint particles are accumulated.7,8 Because of this, seawater around a dockyard would be easily polluted by copper that is eluted from the particles.1,4,7–9 For example, the growth of a brown algal sporophyte and a mussel could be inhibited by the higher copper concentration (>0.1 μM).10,11 Hence, even if the concentration of Cu(II) is lower than 1 μM, the removal of Cu(II) could be necessary to preserve coastal ecosystems. However, the concentrations of copper ions are significantly lower than the levels of other cations that are dissolved in natural seawater. For this reason, the removal of trace amounts of contaminating copper from seawater poses a serious and difficult problem.

It is well known that humic acid (HA) forms complexes with heavy metals.12–15 Polyethyleneimine (PEI) contains a large amount of amine groups that could serve as binding sites for Cu(II).16,17 Hence, a combination of HA and PEI would be expected to serve as an adsorbent that could be used to remove Cu(II) from seawater.17–19 To create an efficient removal system, these components should be in an insoluble form. It is possible for PEI, a positively charged polymer, to be adsorbed to a glass filter that has been treated with a negative electrical charge. However, the removal of trace amounts of contaminating copper from seawater poses a serious and difficult problem.

Keywords: Humic acid, polyethyleneimine, copper, glass filter, antifouling paint

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Experimental

Materials

Branched PEI (average Mw, 1800 Da) was obtained from Wako (Japan), and 1% PEI was prepared as a stock solution by dissolving it in a 0.1 M borate buffer at pH 8.00 ± 0.02. A stock solution of 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (Bathocuproine, BCP, 96% purity) (Sigma-Aldrich, USA) was prepared by dissolving it in dichloromethane (≥99.5% purity) (Wako, Japan) at a concentration of 100 μM. A stock solution of Cu(II), which was used as a source of copper, was prepared by dissolving CuSO₄ ⋅ 5H₂O (≥99.5% purity) (Wako, Japan) in 0.02 M aqueous HCl at a concentration of 1 mM. A stock solution of Zn²⁺, which was used as a source of zinc, was prepared by dissolving ZnCl₂ (98% purity) (Nacalai, Japan) in ultrapure water at a concentration of 10 mM. An aqueous solution of 1 M L(-)+-ascorbic acid (Wako, Japan), which was used as a reducing agent, was prepared freshly for each experiment day. Simplified artificial seawater, which was composed of major salts in ASP medium,²⁰ was prepared by dissolving the following salts in 1000 g of pure water: NaCl 28.5 g; MgSO₄ ⋅ 7H₂O 7.00 g; MgCl₂ ⋅ 6H₂O 4.00 g; CaCl₂ ⋅ 2H₂O 1.48 g; KCl 0.70 g. The pH of artificial seawater was buffered at 8.00 ± 0.01 using 0.05 M Tris–HCl buffer (pH 8.00 ± 0.01) (Nacalai, Japan). A 0.05 M Tris–HCl buffer was used as an artificial freshwater, and its pH was buffered at 8.00 ± 0.01. Hardwood bark compost was used as a source of HA, and was obtained from the Sanyo Chip Industry Co., Ltd. (Japan). The compost was freeze-dried and filtered through a 2-mm mesh stainless-steel sieve, and compost particles of less than 2 mm were used for the extraction of HA. All other reagents used were obtained from Nacalai Tesque (Japan) as analytical reagent grade, unless described.

Humic acid

Humic acid was extracted from dried powdered compost according to previous reports.¹⁸,²¹,²² The precipitated HA fraction was recrystallized three times with 0.1 M NaOH and concentrated HCl to decrease the ash content. The resulting precipitated HA fraction was subsequently transferred into a SpectroPore® 6 dialysis tube (MWCO: 1000 Da) and purified by dialysis against pure water. After dialysis, powdered HAs were prepared by freeze-drying. Powdered HA was dissolved at a concentration of 1000 mg L⁻¹ in 0.1 M aqueous NaOH; before use, the solution was diluted to 20 mg L⁻¹ with 0.1 M borate buffer (pH 8.00 ± 0.02).

Preparation of layer coated filters

GF75 types of 25 mm diameter glass fiber filter were obtained from ADVANTEC (Japan). The nominal pore size of the filter is informed to 0.3 μm as product information. In a typical experiment, a filter was placed in a filter folder, SWINNEX® (Millipore, USA), and 50 mL of 0.1 M aqueous HCl was passed through it to remove impurities. After rinsing with 50 mL of 0.1 M borate buffer (pH 8.00 ± 0.02), the filter was pre-coated with PEI by passing 50 mL of 1% PEI through the filter. The resulting filter was noted to be a mono-layer coated filter with PEI (PEI-MLC). After rinsing with a 50-mL borate buffer, a second layer of HA was applied by passing 50 mL of a 20 mg L⁻¹ solution of HA through the filter. The resulting filter was noted to be a double-layer coated filter with PEI and HA (PEI/HA-DLC). After rinsing the filter by passing 50 mL of artificial seawater through it, it was used to the remove Cu(II).

The adsorption behaviors of PEI or HA were investigated using a GF75 glass filter based on changes in the UV absorptivity (cm⁻²) of the filtrate at 240 nm from those of the initial solutions, 1% PEI or 20 mg L⁻¹ HA diluted in the borate buffer. After adsorption experiments, the desorption behaviors of PEI or HA were also examined by passing artificial seawater. 1% PEI and 20 mg L⁻¹ HA diluted in artificial seawater were used as reference solutions in the desorption experiments. The changes were measured by the absorptivity of 5 mL filtration by each time. These preparations were carried in approximately 2 mL s⁻¹ of filtering speed, unless described.

Removal of Cu(II)

To investigate an effective filter for this study, a 10-mL portion of artificial seawater containing 1 μM Cu(II) was passed through non-treated or the coated filter (2 mL s⁻¹), and the concentration of total copper in the filtrate was analyzed. The relationships between the filtration frequency and the Cu(II) removal efficiency for non-treated and the coated filter were investigated by filtration 200 mL of artificial seawater containing 1 μM by 10 mL each time (2 mL s⁻¹). The total copper concentrations of filtrates were analyzed. The filtration for removal of copper was also carried out under the condition of different ratios of seawater content (%). A medium in which the ratio of seawater content was varied to 0, 25, 50, 75 and 100% was prepared by mixing artificial freshwater (0.05 M Tris-HCl, pH 8.00 ± 0.02) and artificial seawater. Then, 100 mL of the medium containing 1 μM Cu(II) was passed through the coated filters at a 2 mL s⁻¹ filtration velocity, and a 10-mL aliquot of the filtrate was used for quantification of the total copper.

The selectivity of the filter for copper removal was examined by the filtration of artificial seawater containing copper and zinc. The copper removability was evaluated under the condition of the initial concentration of Cu(II), which was kept to a constant of 1 μM; that of Zn(II) was varied to 0, 1, 10 and 100 μM. After filtration of 100 mL artificial seawater in 2 mL/s, a 10-mL aliquot of the resulting filtrate was used for the quantitative analysis of copper.

The effect of the filtering speed to the copper removability was investigated by the filtration of 100 mL of artificial seawater that contained 1 μM Cu(II) in 1, 1.5 and 2 mL s⁻¹. The concentration of the total copper in a 10-mL aliquot of the filtrate was analyzed.

Quantification of total Cu(II) in artificial seawater

The concentration of total copper in the filtrate was measured by a method using BCP as a colorimetric agent.¹⁸,¹⁹,²³ The cupric copper in the filtrate was reduced to a cuprous copper species by adding a 500-μL aliquot of 1 M ascorbic acid. The Cu(I) was concentrated and 1 mL aliquots of 100 μM of BCP dissolved in dichloromethane were added, and the dichloromethane phase then isolated. After three successive isolations, the solvent of the resulting extract was removed with a stream of N₂ gas at 75°C and the dried fraction was dissolved in 1 mL of 50% EtOH. After centrifuging (3000 rpm, 3 min), the concentration of the Cu-(BCP)₂ complex in the resulting supernatant was determined based on the absorbance at 476 nm using a UV-vis spectrophotometer (Jasco V-630, Japan) with a 1-cm pass length micro-cuvette.

Results and Discussion

HA coating on the PEI-MLC filter

The adsorption and desorption behaviors of PEI on the GF75
glass filter are shown in Fig. 1. Approximately 5% of the PEI in the initial solution was adsorbed at the first filtration; however, the ratio of adsorption has been around 1% after the second filtration. In a desorption experiment, approximately 3% of PEI was desorbed, and the desorption ratio was decreased under 0.5% after the second filtration. The adsorption and desorption behavior of HA on the GF75 glass filter are shown in Fig. 2. In the case of a non-treated filter, the ratio of the adsorption of the first filtrate was 3 - 4%, and the color of the filtrate was not changed after the second pass. In the case of PEI-MLC, approximately 70% of HA in the initial solution was adsorbed after the first filtration. The ratio of the adsorption was gradually reached to 0% after the second pass. Passing artificial seawater through the filters after the adsorption experiment, the filtered seawater at the initial stage was colored lightly, indicating that a part of the HA on the filter was desorbed. After the desorption experiments, the resulting PEI/HA filter passed HA had a light-yellow/brown color, and microscopic observations indicated that no large trapped particulate or aggregated HA was observed on the filter (Fig. S1, Supporting Information), suggesting that HA was adsorbed on the PEI-MLC. PEI is able to adsorb on the glass fiber by electrostatic interaction; thus, the glass fibers of the filter could be coated by PEI, and the surface of fiber was electrified to be positive. In a weak alkaline condition, HA has a negative charge due to the deprotonation of acidic functional groups in it, such as carboxylic and phenolic hydroxyl groups. Therefore, passing HA through the filter charged positive, HA could be adsorbed on the surface of the glass fiber by electrostatic interaction between PEI and HA (Fig. S1). From this mechanism, the filter could be succeeded in double-layer coating with PEI and HA. From the results, we decided that 50 mL is a sufficient filtration volume to coat the filter with HA and PEI or to rinse it.

The amount of coated PEI and HA was estimated by subtracting the amount of disrobed HA from the value for the adsorbed HA, as shown in the following equation:

\[
\text{Amount of coated PEI or HA} = \left( \sum_{n=1}^{10} A_i \right) - \left( \sum_{n=1}^{10} D_i \right) \times C \times V,
\]

where \( n, A_i, \) and \( D_i \) represent the filtration frequency, for the value of each plot (%) for the adsorption and desorption experiments (Figs. 1 and 2), respectively. \( C \) and \( V \) are the initial concentration of PEI or HA and the filtration volume per one pass (5 mL), respectively. It was estimated that approximately 40.2 \( \pm \) 15 mg of PEI could be coated on the filter, and approximately 125 \( \pm \) 36 \( \mu \)g of HA could be coated on PEI-MLC. The results also indicate that HA was not trapped on the non-treated filter, and also show that the molecular size of HA was smaller than the pore size (>0.3 \( \mu \)m) of the GF75 filter.

**Cu(II) removability of DLC filter**

A function of the glass filter was evaluated based on the percent of the removal efficiency (RE%). The changes in the RE% were investigated by filtering the total volume of 200 mL of artificial seawater containing 1 \( \mu \)M Cu(II), and the Cu(II) removal efficiencies were evaluated for each 10 mL fractions that were eluted (Fig. 3). Cu(II) was not removed from artificial seawater by a non-treated filter, indicating that the effective removal of dissolved Cu(II) would be not be expected to be directly influenced by the pore size of the filters (>0.3 \( \mu \)m). In the case of the PEI-MLC filter, the RE% was drastically decreased with increasing filtration volume; the RE% after the filtration of 100 mL decreased to about 30%. The RE%, in the case of the PEI/HA-DLC, also decreased with the increasing frequencies of filtration. However, the decrease was more gradual compared to that in the case of PEI-MLC; the RE% for the PEI/HA-DLC filter was maintained at approximately 65%, even after filtering a volume of 200 mL. In the presence of a large amount of competitive cations, the specific binding sites favorable for binding Cu(II), such as amine groups and phthalic
hydroxylic groups, contributes to the removal of Cu(II) from seawater.13,18,24 The result in Fig. 3 suggested that almost all amine binding sites in PEI would be occupied after several tens of mL of filtration. In the case of PEI/HA-DLC, the binding site in HA would also serve an increasing of the Cu(II) removal capacity.

The salts concentration around coastal areas could be varied because the mixing of seawater and freshwater from river flow would occur; thus, the RE% was examined to the 100 mL of a variety ratio of the artificial seawater content (Fig. 4). In this experiment, the initial cupric copper concentration was also varied to 1, 5 and 10 μM, and the RE% for a non-treated filter was less than 3% in all cases, regardless of the ratio of the artificial seawater content. In all cases, the values of RE% for PEI/HA-DLC were higher than those for PEI-MLC due to the copper binding capacity would increase due to HA coating. In the case of PEI-MLC, the value of RE% was to be independent of the ratio of seawater content. However, the values of RE% decreased with increasing the initial copper concentration. In the case of PEI/HA-DLC, the value of RE% was the highest in artificial freshwater, and decreased upon the mixing of artificial seawater. Cu²⁺ ion is a borderline Lewis acid based on the HSAB theory, carboxylates in HA serve a binding site of cupric copper ions.13 However, carboxylates, which is a hard base, prefer to associate with hard acids such as Ca²⁺ and Mg²⁺.13 A large amount of competitive cations, Ca²⁺ and Mg²⁺, inhibit the binding Cu(II) to the hard bases. For 1 and 5 μM of the initial concentration of Cu(II), the values of RE% did not change under the ratio of artificial seawater content was 25 to 100% (Figs. 4(A) and 4(B)). The phenomenon may be due to the carboxylate sites occupied by Ca²⁺ and Mg²⁺ at 25% of artificial seawater content. For 10 μM of the initial concentration of Cu(II), the RE% for PEI/HA-DLC was decreased with increasing the ratio of seawater content (Fig. 4(C)). The result suggests that, under the condition of the high concentration of Cu(II), a small amount of Cu(II) was able to bind with the hard bases in HA, even in the presence of a large amount of Ca²⁺ and Mg²⁺.

The concentration of Zn(II) could also increase in coastal areas polluted by antifouling paint;19,20 thereby, the influence of Zn(II) as a competitive metal on the copper removal was investigated. Figure 5 shows the removal efficiencies for 1 μM of initial Cu(II) in the presence of a variety concentration of Zn(II). For PEI-MLC and PEI/HA-DLC, the values of RE% in the 1:0 and 1:1 ratio of Cu(II):Zn(II) did not differ, suggesting that the binding sites in the filters prefer to adsorb Cu(II) than Zn(II). When the initial concentration of Zn(II) increased to 10 and 100-times that of Cu(II), the values of RE% for PEI-MLC decreased drastically. The binding of Zn(II) with PEI compete to that of Cu(II) with PEI because of Zn(II) is a borderline Lewis acid, the same as Cu(II). Thereby, the values of RE% decreased with increasing Zn(II) concentration. The RE% values for PEI/HA-DLC decreased with increasing the initial Zn(II) concentration, however, the filter performed approximately 30% of RE% even in the Cu(II):Zn(II) ratio of 1:100.

Influence of filtration velocity
The removal techniques in the previous reports have required 1 to 3 days for the effective removal of trace level of Cu(II) (1 - 3 μM of the initial concentration) from artificial seawater (80 - 90% RE%).18,19 The required time for effective removal by the filtration technique has greatly improved compared with
The removal performance was found to be dependent on the scale of the effective coated area; thus, this type of functionalizing procedure would be applicable to many other types of glass fiber materials.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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