Adsorption Property of UV-cured Films Containing Disulfide Bonds in Water

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Abstract

Disulfide bond-containing polymer films were fabricated in a radical UV curing process to adsorb metal cations or proteins. Bi-functional methacryl monomers having a disulfide bond were designed and used for the UV curing. Resulting films showed practical pencil-hardness, H to 3H, with 1 J/cm^2 of irradiation at a wavelength of 365 nm. It was confirmed that disulfide bond-containing films adsorbed colored metal cations such as gold(III) and copper(II) cations. During adsorption experiments, disulfide bond-containing polymer films were not detached from a glass substrate, probably due to relaxation of internal stress by bond exchange reactions between disulfide bonds. Bovine serum albumin seemed to be also adsorbed toward films only in the case that the film had ionic moieties.

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1. Introduction

Separation technology plays quite important role for sustainable chemical industries and our healthful daily lives. Wastewater must undergo removal treatments of various kinds of pollutants, before streaming into rivers and seas. Pesticides are one of major pollutants, and organic dye has been used as a model compound in researches of adsorbents. It was reported that such compounds were adsorbed by clays[1] and metal-organic frameworks (MOFs),[2] while these materials could not adsorb high-molecular-weight molecules due to adsorption sites in angstrom scale. Adsorbents for proteins (up to several dozen nm) and viruses (several dozen to hundred nm) would be desired.

Heavy metal cations are also representative as inorganic pollutants, and their adsorbents have been developed by using inorganic carriers such as mesoporous silica,[3] iron tailings,[4] graphene oxide,[5] and MOFs.[6] Furthermore, abundant organic carrier materials have been used, including pectin- based[7] and polysaccharide-based adsorbents.[8] Inherent carboxy and hydroxy groups in the materials would capture metal cations. Nitrogen- containing moieties would also contribute to the entrapment, while Sulfur-containing ones were not in the limelight. Sulfur moieties are known to work as soft base, and they interact strongly with soft acids including several heavy metal cations. Nakayama reported that mercury(II) and silver(I) cations were adsorbed by interaction with disulfide (SS) bonds in the interlayer space of magnesium–aluminum layered double hydroxide materials.[9] In this case, however, there is a limit of retention ratio of SS bonds to the carrier. On the other hand, organic polymer materials containing SS bonds have been developed in our group as photo- adhesive materials.[10-12] It was found that SS bonds would contribute to strong adhesion toward metal substrates. In this article, we have tried to fabricate organic polymer UV-cured films which contain SS bonds at high density (**Fig 1**), for using as an
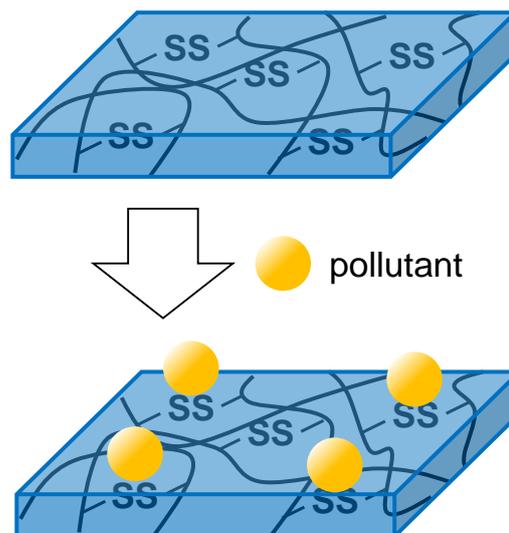
Fig 1. Adsorption of pollutants such as heavy-metal cations and proteins by interaction with disulfide (SS) bonds in a UV-cured film.
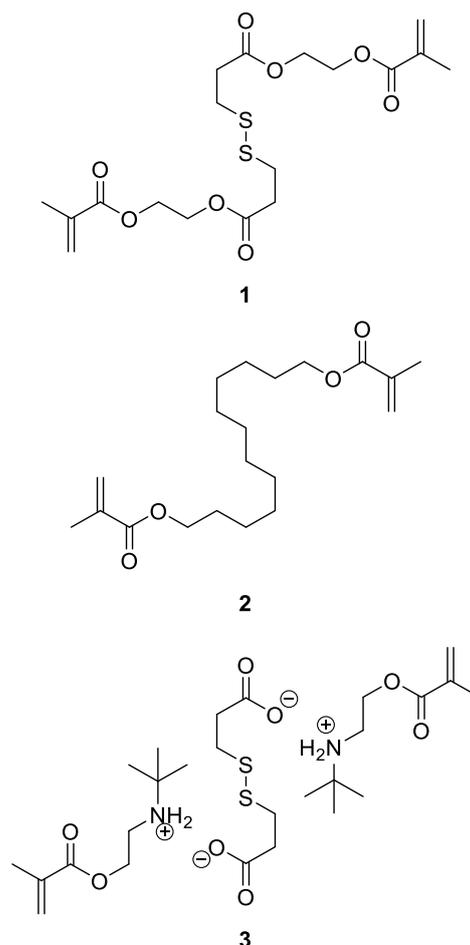
Fig 2. Chemical structures of bi-functional methacryl monomers having a disulfide bond (**1**, **3**), and a control monomer **2**.

adsorbent of heavy metal cations and proteins. Gold(III) and copper(II) cations were chosen in our study, because visual observation could be conducted easily. Furthermore, adsorption of a protein was examined with our UV-cured films. SS bonds in a protein work for structural stability of its tertiary structure.[13] It was expected that proteins were fixed through exchange reactions between SS bonds[14] on the film surface "open space". Bi-functional methacryl monomers (monomers **1** and **3**, **Fig 2**) having a SS bond were prepared and used in radical UV curing.

2. Experimental
2.1 Materials and Equipment

3,3'-Dithiopropionic acid, 2-hydroxyethyl methacylate (HEMA), 2-(*tert*-butylamino)ethyl methacrylate (*t*BuAEMA), 1,12-dodecanediol dimethacrylate (monomer **2**), and Omnirad819 (phenylbis(2,4,6-trimethylbenzoyl) phosphine oxide) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Chloroform ($CHCl_3$) and acetone were purchased from NACALAI TESQUE, Inc. (Kyoto, Japan). Dichloromethane (DCM), sodium tetrachloro-aurate ($NaAuCl_4$, dihydrate), copper(II) chloride ($CuCl_2$, dihydrate), and chloroform-*d* ($CDCl_3$) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Bovine serum albumin (BSA, IgG-free, protease-free) was purchased from Jackson ImmunoResearch Laboratories, Inc. (Pennsylvania, USA). All chemicals were used without further purification.

^{1}H- and ^{13}C-NMR spectra were recorded using a Bruker AVANCE III. ATR-IR spectral measurements were performed using a Perkin Elmer Spectrum100. Radical UV curing was performed with 3UV[TM]-36UVLamp (Analytik Jena AG) and UIT-250/UVD-C365 ultraviolet radiometer (Ushio Inc.). Pencil-hardness of the UV-cured films was evaluated with MJ-PHT tester (SATO SHO-JI Corporation). Static water contact angle of films was measured using an SImage Entry 6 (Excimer Inc.).

2.2 Monomers

Bi-functional methacryl monomer **1** was synthesized from 3,3'-dithiopropionic acid and HEMA as starting compounds in a 21% yield. The synthetic procedure was described elsewhere.[15] Monomer **3** was prepared as follows.

*t*BuAEMA (0.89 g, 4.8 mmol) in DCM (10 mL) was dropped to 3,3'-dithiopropionic acid (0.50 g, 2.4 mmol) in DCM (10 mL), and the solution was stirred under nitrogen atmosphere at room temperature for 178 h. After evaporating, **3** was obtained in a 95% yield as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.29 (18H, s, -C(CH$_3$)$_3$), 1.96 (6H, s, -CH$_3$), 2.64 (4H, m, -C$\underline{H}_2$CH$_2$-O-), 2.95 (4H, m, -CH$_2$C$\underline{H}_2$-O-), 3.09 (4H, m, -CH$_2$C$\underline{H}_2$-S-), 4.41 (4H, m, -C$\underline{H}_2$CH$_2$-S-), 5.62 (2H, m, =CH$_2$), 6.17 (2H, m, =CH$_2$), 7.96 (4H, br, -N$^+$H$_2$-). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 18, 27, 35, 37, 40, 54, 62, 126, 136, 167, 177.

2.3 Fabrication of UV-cured films

Glass substrates were ultrasonicated with acetone and then $CHCl_3$. Films were fabricated by following two methods.

Bar-coat method was used to prepare film samples for adsorption of heavy metal cations. To bi-functional methacryl monomers **1-3** were added HEMA (300-600 mol%), Omnirad819 (4-13 wt% toward monomers), and at least amount of solvent (THF for **1** and **2**, $CHCl_3$ for **3**). Each solution was bar-coated on a glass substrate (4 mil = 101.6 μm in thickness). Films were subjected to UV irradiation (1.5 mW/cm^2, 1 J/cm^2) at a wavelength of 365 nm.

On the other hand, drop-cast method was used to prepare film samples for adsorption of BSA protein. To bi-functional methacryl monomers **1-3** were added HEMA (*ca.* 2500 mol%), Omnirad819 (7 wt% toward monomers), and at least amount of $CHCl_3$. Each solution was drop-cast on a glass substrate. Films were subjected to UV irradiation (1.5 mW/cm^2, 1 J/cm^2) at a wavelength of 365 nm.

2.4 Adsorption of heavy metal cations

Following the procedure of bar-coat method in section 2.3, UV-cured films were fabricated with monomer **1** and **2**. Resulting films were named as **Film-1** and **Film-2**. These film samples were immersed into 30 mM of NaAuCl$_4$ or CuCl$_2$ aqueous solution at room temperature. After 1, 3, 5, and 60 min, films were taken out and washed with distilled water. Air dried film samples were then subjected to visual observation (for Au^{3+} and Cu^{2+} cations), or color image analysis (for Au^{3+} cation).

Color image analysis was conducted by using arbitrary nine pixels for each film image (0, 1, 3, 5 min for **Film-1**, and 0, 1, 3, 5 min for **Film-2**). One pixel was at center of film in the image, and others were within the radius of 60 px from the center pixel. A film in an image was a square, *ca.* 150-210 px each side. Each pixel had a set of standard RGB decimal values, *e.g.* yellow: **Y** (R, G, B) = (255, 255, 0). These values were converted to their ratios *r*, *g* and *b* by equations below,

$$r = \frac{R}{R + G + B}$$

$$g = \frac{G}{R + G + B}$$

$$b = \frac{B}{R + G + B}$$

Values (*r*, *g*, *b*) of representative five colors were calculated as follows, yellow: **Y** (0.5, 0.5, 0), red: **R** (1, 0, 0), green: **G** (0, 1, 0), blue: **B** (0, 0, 1), and white-to-gray: **WG** (0.333, 0.333, 0.333). These five points were plotted in an *r-g* plane as a projection view (**Fig 3**). Total nine sets of (*r*, *g*) were also plotted in a plane for each film image, to show graphically time course of color of each film sample during immersion in Au^{3+} aqueous solution. All sets were plotted in the range of 0.323 < *r* < 0.343 and 0.323 < *g* < 0.343.

2.5 Adsorption of BSA protein

Following the procedure of drop-cast method in
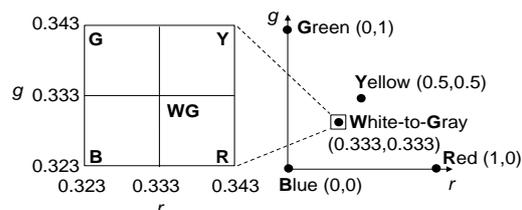
Fig 3. Five representative colors; yellow: **Y**, red: **R**, green: **G**, blue: **B**, and white-to-gray: **WG** in *r-g* plane that is for color image analysis in this study.

section 2.3, UV-cured films were fabricated with monomers **1**, **2** and **3**. Resulting films were named as **Film-I**, **Film-II** and **Film-III**. These film samples were immersed into 7.9 μM of BSA aqueous solution at room temperature. After 60 min, films were taken out and washed with distilled water. Air dried film samples were then subjected to ATR-IR spectral measurements.

3. Results and Discussion
3.1 Fabrication of UV-cured films

Table 1 and **Table 2** show the values of pencil-hardness of UV-cured films. In bar-coat method, the weight ratio of monomer/HEMA was fixed to 1/1

Table 1. Pencil-hardness of UV-cured films fabricated in bar-coat method using monomers **1-3**. **Film-1** and **Film-2** were used for adsorption of heavy metal cations.

Entry	Monomers (mol/mol)[a]	Pencil-hardness
1	**1**/HEMA (1/4)	H (**Film-1**)
2	**2**/HEMA (1/3)	2H (**Film-2**)
3	**3**/HEMA (1/6)	Liq.
4[b]		< 6B

[a] Bi-functional methacryl monomer/HEMA = 1/1, w/w.
[b] 2.9 mW/cm^2, 15 J/cm^2.

Table 2. Pencil-hardness of UV-cured films fabricated in drop-cast method using monomers **1-3**. **Film-I**, **Film-II** and **Film-III** were used for adsorption of BSA protein.

Entry	Monomers (mol/mol)	Pencil-hardness
1	**1**/HEMA (1/24)	2H (**Film-I**)
2	**2**/HEMA (1/25)	H (**Film-II**)
3	**3**/HEMA (1/27)	3H (**Film-III**)
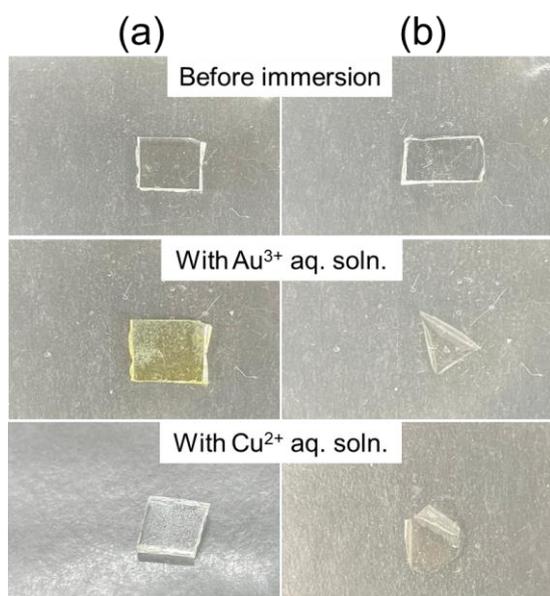
Fig 4. Photographs of UV-cured films (a) **Film-1** and (b) **Film-2**, before immersion (upper), after immersion into 30 mM of Au^{3+} (middle) or Cu^{2+} (bottom) aqueous solution at room temperature for 60 min.

Fig 5. Time course of color of film samples (a) **Film-1** and (b) **Film-2** during immersion into 30 mM of Au^{3+} aqueous solution at room temperature. Longitudinal axis: g, $0.323 \leq g \leq 0.343$, horizontal axis: r, $0.323 \leq r \leq 0.343$. Nine sets of (r, g) are plotted in each r-g plane.

(*see* annotation below the Table). In the evaluation of physical property of films, the hardness of pencil is arranged as follows: 6B (softest), 5B, 4B, 3B, 2B, B, HB, F, H, 2H, 3H, 4H, 5H, 6H (hardest). Practical pencil-hardness was recorded in cases of **Film-1** (2H) and **Film-2** (H). Static water contact angles of **Film-1** and **Film-2** were 83° and 85°, respectively. Films were more hydrophobic than a glass substrate (32°).[12] Films was not cured with monomer **3** under the same condition. Even increasing both intensity (2.9 mW/cm^2) and irradiation energy (up to 15 J/cm^2), the pencil-hardness was less than 6B. Monomer **3** had ionic bonds which could be exchanged readily even in a UV-cured film if the density was high, resulting in softening the film surface.

In drop-cast method, the molar ratio of monomer/HEMA was fixed to *ca.* 1/25, decreasing the density of monomers **1-3** (monomer/HEMA =1/6 to 1/10, w/w). UV-cured films **Film-I**, **Film-II**

and **Film-III** were fabricated successfully to obtain H to 3H of pencil-hardness.

3.2 Adsorption of heavy metal cations

Both films were transparent before immersion into aqueous solution of heavy metal cations. As shown in **Fig 4(a)**, color of **Film-1** became yellow after immersion in Au^{3+} aqueous solution, comparing with color of **Film-2** (**Fig 4(b)**). This indicated adsorption of Au^{3+} cations through interaction with SS bonds. Although such color change was not observed in the case of Cu^{2+} aqueous solution, there was a clear difference between two films where **Film-1** became opaque.

Fig 5(a) showed that color of **Film-1** was changed from cool color to warm one, like pale yellow, within 1 min. Color change was observed in the case of **Film-2**, while the variation range was small (**Fig 5**(b)). **Film-2** was detached from the glass substrate during immersion experiments. Furthermore, it seemed that color dispersion of **Film-1** was larger

than of **Film-2**. This could indicate non-uniformity of the surface in microscopic level. Indeed, **Film-1** after immersion into Au^{3+} and Cu^{2+} aqueous solution, ruggedness was observed visually in **Fig 4**. Adsorption of heavy metal cations would change film surface profiles dynamically, probably due to cross-linking between cations and SS bonds, and swelling with water.

Films were fabricated in a radical process, which was accompanied with film-shrinking.[16] Resulting UV-cured films on a substrate would hold somewhat internal stress (**Fig 6**). In the case of **Film-1** having SS bonds, exchange reactions between SS bonds would be induced by internal stress (**Fig 6(a)**).[17] Consequently, internal stress was relaxed, resulting in remaining cross-linked polymer networks on a substrate. On the other hand, in the case of **Film-2** having no SS bonds, internal stress was not relaxed (**Fig 6(b)**). Stress concentration at the film-substrate interface would result in film-detachment. It was considered that SS bonds in UV-cured films would work for not only adsorption but also fixation of a film on a substrate.

3.3 Adsorption of BSA protein

Film-detachment from glass substrate was not observed in all film samples, even in the case of **Film-II**, after immersion for 60 min. It would result from low-crosslinking density, comparing with the case of **Film-2**. In ATR-IR spectra, intensity of two peaks around 1640 cm^{-1} and 1550 cm^{-1} seemed to increase after the immersion, only in case of **Film-III** (**Fig 7**). These peaks could be assigned to amide I and II bands of secondary acyclic amide compounds.[18] This could be an evidence that BSA proteins were adsorbed on **Film-III**. Such spectral change was not observed in cases of **Film-I** and **Film-II**. These results indicate that BSA proteins would interact electrostatically with ionic moieties rather than SS bonds.
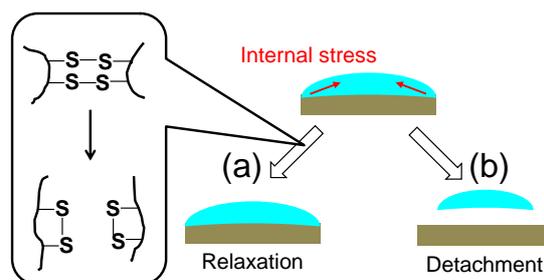
Fig 6. A possible mechanism of (a) film-remaining in cases of **Film-1**, and (b) film-detaching in cases of **Film-2**, during immersion.
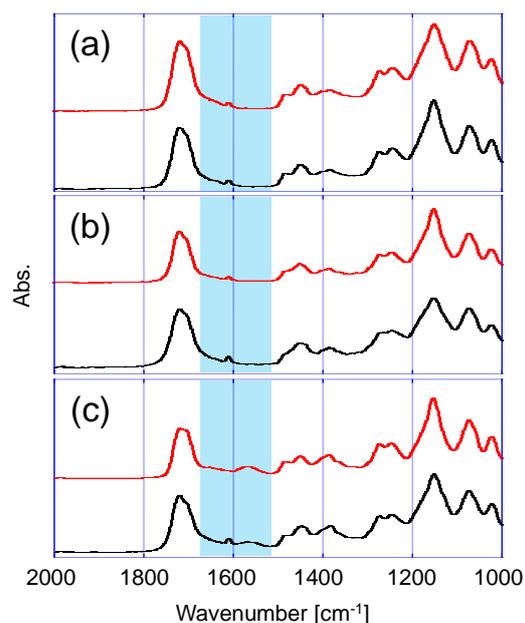
Fig 7. ATR-IR spectral changes of (a) **Film-I**, (b) **Film-II** and (c) **Film-III**. Upper and lower spectra are recorded after and before immersion into 7.9 μM of BSA protein aqueous solution, respectively.

4. Conclusion

UV-cured films were fabricated successfully by using bi-functional methacryl monomers having a SS bond. In adsorption experiments of heavy metal cations, it was found that SS bonds would interact with such cations and that SS bonds could dissipate internal stress through exchange reactions among themselves. Furthermore, UV-cured films having ionic moieties could adsorb proteins probably due to electrostatic interaction. Our SS bond-containing organic polymer material will be a powerful candidate of absorbents. UV coating toward various kinds of substrates and adsorbing ability of the materials are now under investigation.

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