Surface Plasmon Resonance Studies on Molecularly Imprinted Films*

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ABSTRACT: Novel molecular recognition films were prepared from modified polysulfone having perillaldehyde moiety as a side group. The molecular recognition films were obtained from perillaldehyde polysulfone by adopting 9-ethyladenine as a print molecule. The molecular recognition phenomena were studied by surface plasmon resonance (SPR) spectroscopy. Adsorption of adenosine (As) and guanosine (Gs) in the molecularly imprinted film was studied. Dual adsorption isotherms were observed for As in 9-EA imprinted films, while nonspecific adsorption isotherms for Gs in those films. This revealed that the molecular recognition sites toward As were constructed in the films thus prepared. The apparent affinity constant toward As determined by using apparent adsorption isotherms ranged from 7.90×10^3 to 3.31×10^4 mol^{-1} dm^3.
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Key words: adenosine; guanosine; 9-ethyladenine; molecular imprinting; molecular recognition; polysulfone; surface plasmon resonance

INTRODUCTION

Molecular imprinting is regarded as a facile methodology to construct molecular recognition materials, which can be applicable to chromatography, membranes, sensors, catalysts, and so forth.[1–7] One of the authors proposed an alternative molecular imprinting in which polymeric materials are directly converted into molecular recognition materials.[8,9] Various polymeric materials, which can construct given structures and keep their forms, such as synthetic polymers,[10–14] derivative of natural polymers,[15] oligopeptide derivatives,[16–18] and natural polymers,[19] were converted into molecular recognition materials by applying an alternative molecular imprinting. Molecular imprinting efficiency is thought to be dependent on various factors such as molecular imprinting ratio defined as a ratio of the mole number of the print molecule to that of constituting repeating unit of the candidate polymer, combination of print molecule adopted and a candidate material, nature of a given candidate material, and so forth.

In a previous study on polysulfone modified with perillaldehyde moiety, of which chemical structure is given in Figure 1, an undesirable phenomenon occurred with the N-α-benzyloxycarbonyl-D-glutamic acid (Z-D-Glu) or Z-L-Glu imprinted material.[20] In that study, an alternative molecular imprinting was applied to the perillaldehyde polysulfone with a degree of substitution of 0.97 at one fixed molecular imprinting condition of 0.50. Against expectation, only a very low concentration of the perillaldehyde polysulfone was converted into a chiral recognition material. Preliminary experiments showed that 9-ethyladenine is expected to work effectively as a print molecule toward the present perillaldehyde polysulfones. In the present article, the nucleic acid component adenosine was adopted as a target molecule, since recognition of nucleic acid component is of interest and importance in connection with biosensors, drug therapy, genetic engineering, and so forth. To this end, perillaldehyde polysulfone was converted into adenosine recognition material by adopting 9-ethyladenine (9-EA) as a print molecule. The recognition of adenosine/guanosine (As/Gs) was investigated as a model mixture (Fig. 2) by applying surface plasmon resonance (SPR) spectroscopy.

EXPERIMENTAL

Materials

Perillaldehyde polysulfone, modified polysulfone (PPSf-097) having perillaldehyde moiety with a degree of substitution of 0.97 per repeat unit, was prepared by the modification of Radel® R5000 polyphenylsulfone (Solvay Advanced Polymers) (PPSf)

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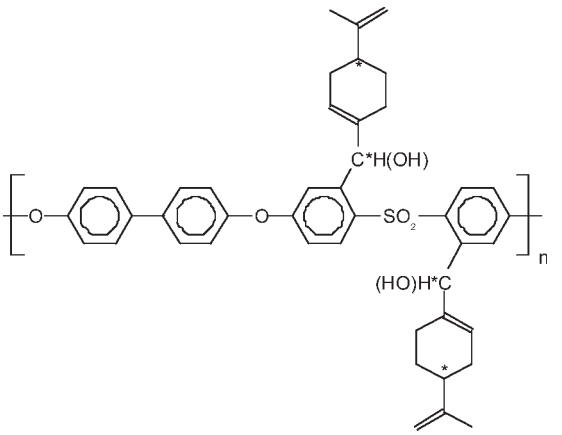


Figure 1 Chemical structure of the polysulfone modified with perillaldehyde moiety, PPSf.

as reported previously.[20] The print molecule 9-ethyladenine (9-EA) was purchased from Sigma Chemical and used without further purification. The substrates, adenosine (As) and guanosine (Gs) were purchased from Seikagaku. *N*,*N*-dimethylformamide (DMF) was purified by the usual method.[21] 1-Octanethiol, ethanol, and sodium azide were used without purification. Distilled water was employed.

Construction of molecular recognition sites

The molecularly imprinted films having molecular recognition sites toward As were prepared as follows: a gold-deposited glass plate was immersed in a 1.0×10^{-5} mol dm^{-3} solution of 1-octanethiol in ethanol for 30 min at ambient temperature prior to the molecular imprinting. The film was prepared by spin-casting a 1.0 g dm^{-3} DMF solution of PPSf-097 onto the pretreated gold-deposited glass plate. The rotation speed for spin casting was 5000 rpm. A prescribed amount of the print molecule 9-EA was dissolved in the spincasting DMF solution for the preparation of molecularly imprinted films. 9-EA was omitted for the preparation of control films. The extraction of the print molecule from molecularly imprinted films was carried out by the buffer flow of the SPR apparatus.

Evaluation of molecular recognition ability of molecularly imprinted films

The molecular recognition of the prepared films toward the target molecule As was evaluated by SPR spectroscopy. The incident light with 670 nm was chosen. The change in incident angle ($\Delta\theta$) responding to the addition of substrates was recorded on the SPR apparatus (SPR670S, Nippon Laser and Electronics Laboratory). During the measurement, 0.02 wt % NaN_3 aqueous buffer was passed over the molecularly imprinted material surface at 5.0 mm^3 min^{-1}. The flow was periodically replaced with solutions of the same buffer containing As or Gs. The experiment was carried out at 27°C.

RESULTS AND DISCUSSION

Adsorption isotherms of As and Gs on the control nonimprinted film

In the present study, adsorption phenomena of molecularly imprinted materials were studied by

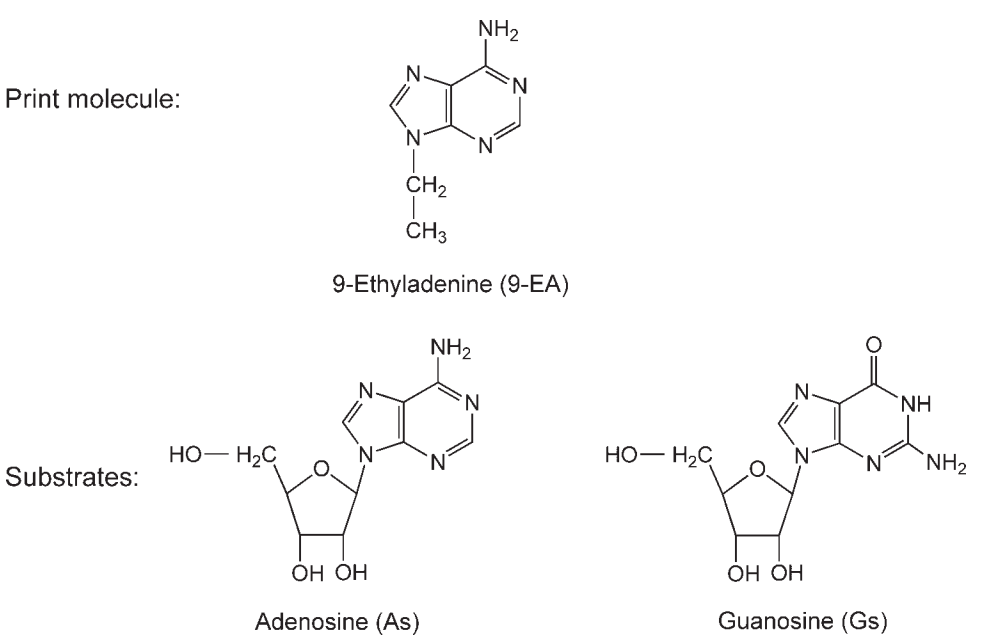


Figure 2 Chemical structures of the print molecule (9-EA) and substrates (As and Gs).

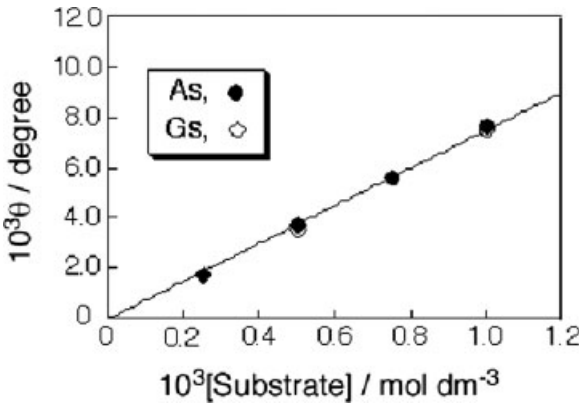


Figure 3 Adsorption isotherms of As and Gs on the control nonimprinted PPSf-097 film.

using SPR spectroscopy. SPR is an optical method to detect changes in the reflective index of the medium close to the gold surface.[22–25] The relationship between reflected intensity and the angle of incidence gives a minimum reflected intensity, corresponding to the excitation of surface plasmons at the gold-solution interface. The value of the incidence angle giving the minimum reflected intensity (θ) shifts with changes in the refractive index of the interfacial region close to the gold surface.[24,25] The shift in θ ($\Delta\theta$) is proportional to the amount of adsorbed substrate at the surface. Using $\Delta\theta$, an apparent adsorption isotherms of a given target molecule can be drawn. In the present study, 9-EA was adopted as a print molecule.

Before studying the molecular recognition ability of 9-EA imprinted PPSf-097 film by SPR, a control film of nonimprinted material (coated on a gold-deposited glass plate in the absence of 9-EA) was measured. $\Delta\theta$ can not be directly converted into the concentration of a given substrate adsorbed in the spin-cast film, even though the value of $\Delta\theta$ is proportional to the amount of the adsorbed substrate. Here, it can be regarded that the multiplication of the substrate concentration adsorbed in the molecularly imprinted film ([Substrate]$_m$) by the factor f gives the $\Delta\theta$

$$\Delta\theta = f[\text{Substrate}]_{\text{m}} \quad (1)$$

where f is the factor converting [Substrate]$_m$ into $\Delta\theta$. The apparent adsorption isotherms of As and Gs can be obtained by plotting the observed $\Delta\theta$ as a function of the substrate concentration and are shown in Figure 3. Both apparent adsorption isotherms for As and Gs are superimposed and not distinguishable. Also, both adsorption isotherms are straight lines passing through the origin, implying As and Gs were nonspecifically adsorbed in nonimprinted PPSf-097 film. In that case, $\Delta\theta$ for the substrate As or Gs, which was nonspecifically adsorbed in the control nonimprinted film can be represented by the following equation

$$\Delta\theta = f[\text{Substrate}]_{\text{m}} = fk_{\text{A,app}}[\text{Substrate}] \quad (2)$$

where $k_{\text{A,app}}$ denotes the apparent adsorption constant, and [Substrate] is the concentration of the substrate in the buffer.

Adsorption isotherms of As and Gs on the 9-EA imprinted film

The apparent adsorption isotherms of 9-EA imprinted PPSf-097 films are shown in Figures 4(a)–6(a). For the molecular imprinting condition, the molar ratio of the amount of 9-EA to that of constituting repeating unit of PPSf-097 was 0.125 for Figure 4, 0.250 for Figure 5, and 0.500 for Figure 6, respectively. Those three types of 9-EA imprinted films gave similar adsorption isotherms; that is, the

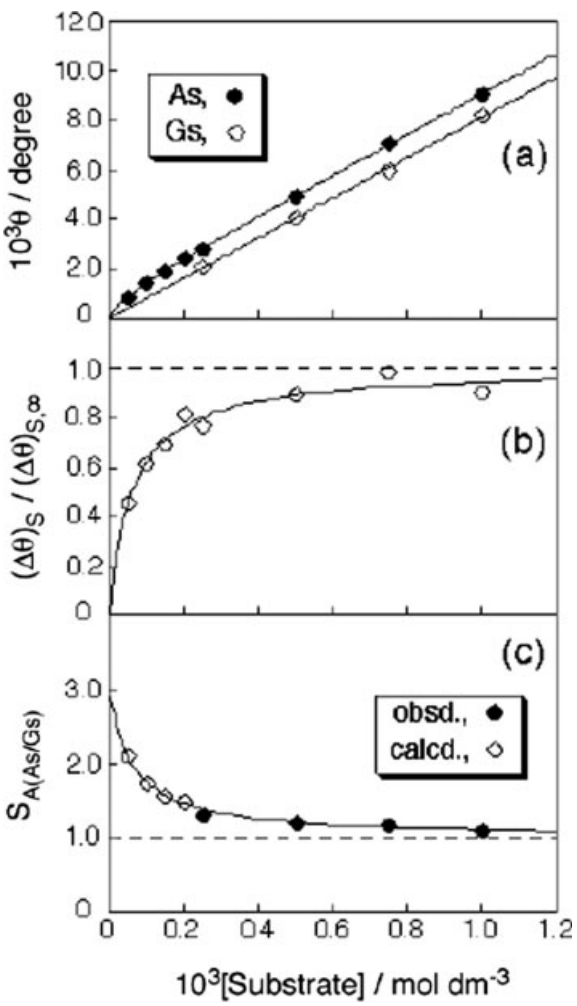


Figure 4 Adsorption isotherms of As and Gs and adsorption selectivity of the imprinted PPSf-097 film. [(9-EA)/(PPSf-097) = 0.125].

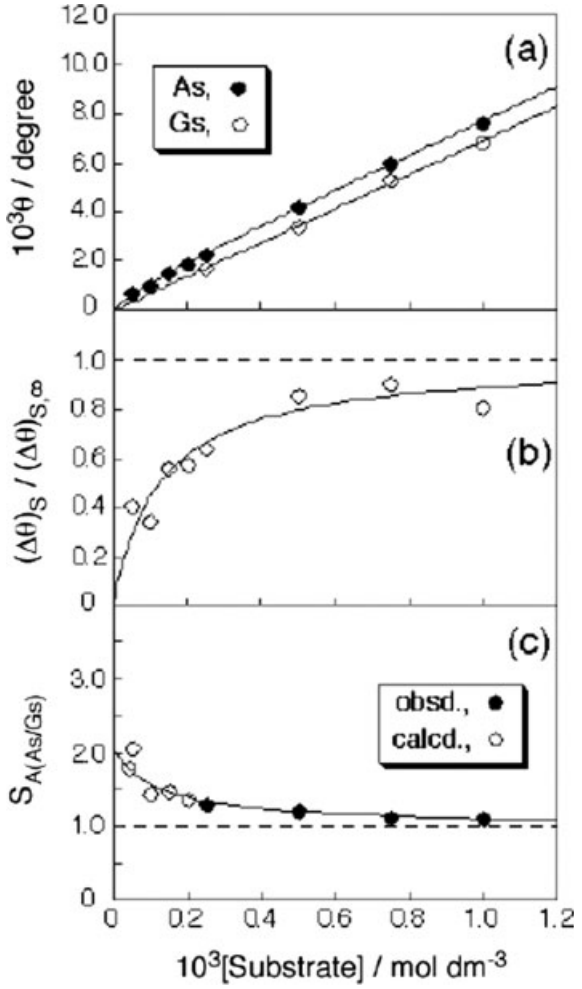


Figure 5 Adsorption isotherms of As and Gs and adsorption selectivity of the imprinted PPSf-097 film. [(9-EA)/(PPSf-097) = 0.250].

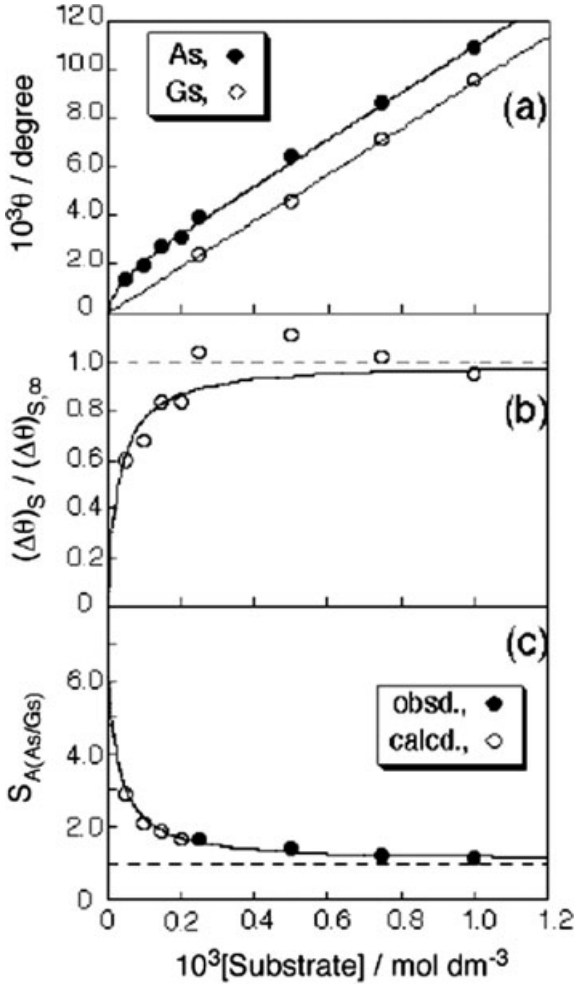


Figure 6 Adsorption isotherms of As and Gs and adsorption selectivity of the imprinted PPSf-097 film. [(9-EA)/(PPSf-097) = 0.500].

adsorption isotherms of Gs are all straight lines passing through the origin like that for nonimprinted film shown in Figure 3. This led to the conclusion that there is no specific recognition site toward Gs in 9-EA imprinted PPSf-097 films. In this case, $\Delta\theta$ for adsorbed Gs in the molecularly imprinted film can be represented by eq. (3)

$$\Delta\theta = f[\mathrm{Gs}]_m = fk_{A,app}[\mathrm{Gs}] \quad (3)$$

where $[\mathrm{Gs}]_m$ is the concentration of Gs adsorbed in the molecularly imprinted film and [Gs] in the buffer.

In contrast to this, dual adsorption isotherms were observed for As in 9-EA molecularly imprinted PPSf-097 films, consisting of nonspecific adsorption and adsorption on an As specific recognition site, like dual sorption of gases.[26–28] The concentration of As adsorbed in the molecularly imprinted film can be represented by eq. (4)

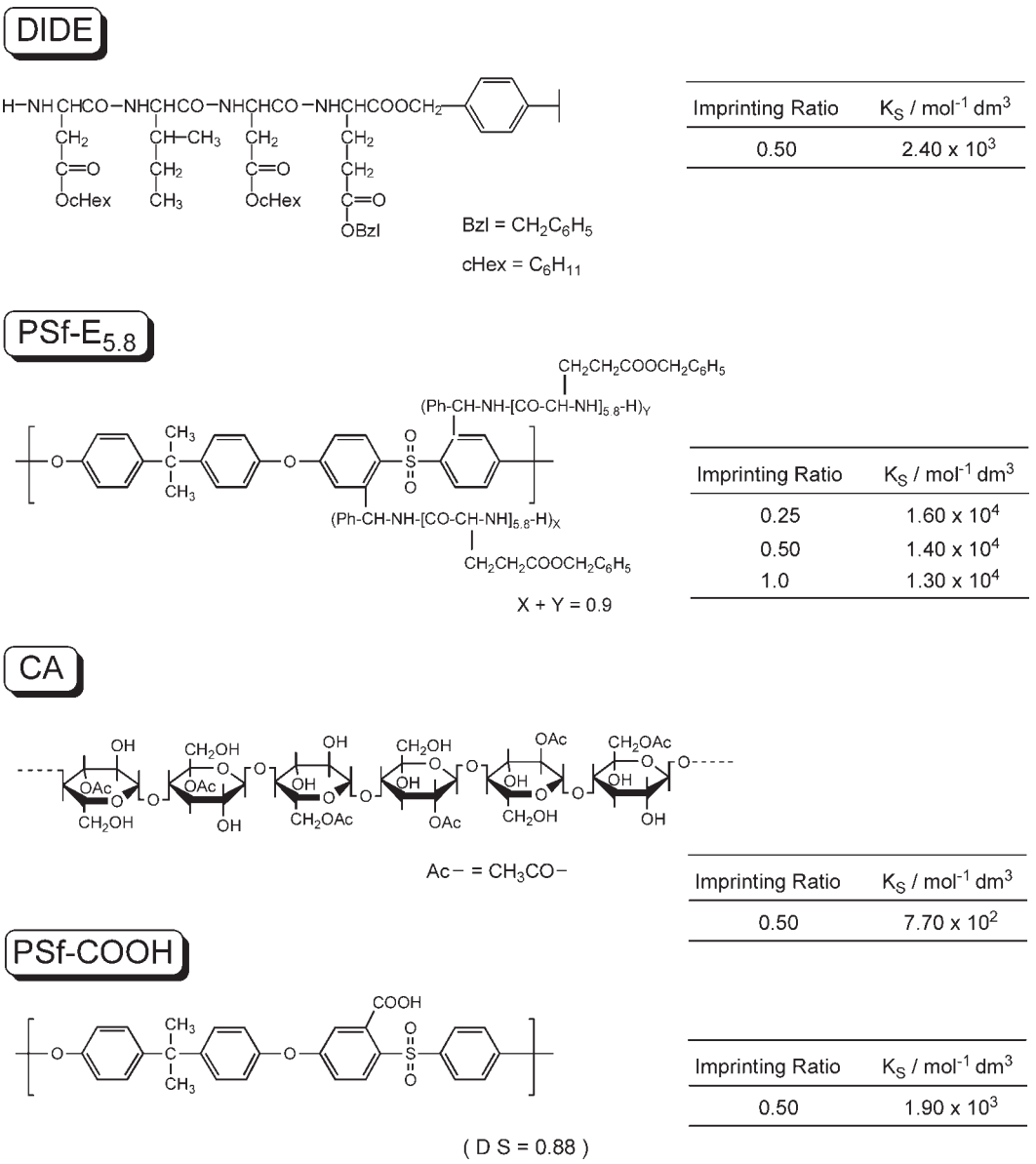
$$\begin{aligned}\Delta\theta &= f[\mathrm{As}]_m \\ &= f\{k_{A,app}[\mathrm{As}] + K_{S,app}[\mathrm{Site}]_0[\mathrm{As}]/(1 + K_{S,app}[\mathrm{As}])\}\end{aligned} \quad (4)$$

where $[\mathrm{As}]_m$ is the total concentration of As adsorbed in the molecularly imprinted film, $K_{S,app}$ denotes the apparent affinity constant between As and the molecular recognition site toward As, $[\mathrm{Site}]_0$ is the concentration of molecular recognition site in the molecularly imprinted film, and [As] is the

TABLE I
Affinity Constant Between the Molecular Recognition Site and As

(9-EA)/(PPSf-097)[a]	$K_{S,app}$ (mol^{-1} dm^3)
0.125	1.61×10^4
0.250	7.90×10^3
0.500	3.31×10^4

[a] Molecular imprinting ratio.



Imprinting Ratio	K_S / mol^{-1} dm^3
0.50	2.40 x 10^3

Imprinting Ratio	K_S / mol^{-1} dm^3
0.25	1.60 x 10^4
0.50	1.40 x 10^4
1.0	1.30 x 10^4

Imprinting Ratio	K_S / mol^{-1} dm^3
0.50	7.70 x 10^2

Imprinting Ratio	K_S / mol^{-1} dm^3
0.50	1.90 x 10^3

Figure 7 Affinity constants between As and molecular recognition sites converted from various candidate materials.

concentration of As in the buffer. The molecularly imprinted film is assumed to have only one type of As recognition site, As–As interactions are assumed not to occur and the system is considered as ideal.[29]

The apparent affinity constants between As and the formed molecular recognition site were determined by the following procedure; the difference in the shift $(\Delta\theta)_S$, between that for As and Gs at a given substrate concentration, which corresponds to the apparent amount of As adsorbed on the As recognition site was obtained. In the case that there was no experimental $\Delta\theta$ value for Gs at a given concentration, the difference between the $\Delta\theta$ for As and the extended straight line for Gs was adopted as $(\Delta\theta)_S$. $(\Delta\theta)_S$ can be correlated with the adsorption equation by the following equation:

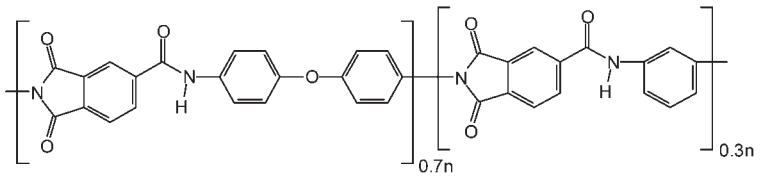
$$(\Delta\theta)_S = f\{k_{S,app}[Site]_0[As]/(1 + K_{S,app}[As])\} \quad (5)$$

A relative shift in θ, the ratio of $(\Delta\theta)_S$ to $(\Delta\theta)_S$ corresponding to the infinite substrate concentration, $(\Delta\theta)_{S,\infty}$ $(= f[Site]_0)$, was plotted as a function of As concentration. $(\Delta\theta)_S / (\Delta\theta)_{S,\infty}$ is correlated with the eq. (6)

$$(\Delta\theta)_S/(\Delta\theta)_{S,\infty} = k_{S,app}[As]/(1 + K_{S,app}[As]) \quad (6)$$

From the relationship between the ratio $(\Delta\theta)_S/(\Delta\theta)_{S,\infty}$ and As concentration, as shown in Figures 4(b), 5(b), and 6(b), the apparent affinity constant for each molecularly imprinted PPSf-097 film was determined. The determined apparent affinity constants, $K_{S,app}$, are summarized in Table I. Molecular

Torlon



Imprinting Ratio	K_S / mol^{-1} dm^3
0.10	2.73 x 10^4
0.20	7.86 x 10^4
0.30	3.80 x 10^4

Hydrophilic water-soluble proteins from *G. thermodenitrificans* DSM465

Imprinting Ratio	K_S / mol^{-1} dm^3
0.10	1.75 x 10^5

Membrane proteins from *G. thermodenitrificans* DSM465

Imprinting Ratio	K_S / mol^{-1} dm^3
0.50	4.62 x 10^4

Figure 7 (*Continued from the previous page*)

recognition sites toward As have been previously constructed from H-Asp(OcHex)-Ile-Asp(OcHex)-Glu(OBzl)-O-CH_2- tetrapeptide derivative (DIDE),[30] polysulfone with oligopeptide derivative of glutamyl residues (PSf-$E_{5.8}$),[31] cellulose acetate (CA) with 40% acetyl content,[30] carboxylated polysulfone (PSf-COOH) with the degree of substitution of 0.88,[30] Torlon (polyamide-imide),[32] hydrophilic water-soluble proteins,[19] and membrane proteins[33] from *Geobacillus thermodenitrificans* DSM465. The affinity constants between As and the molecular recognition sites converted from those candidate materials are summarized in Figure 7 together with the chemical structures of those materials for convenience. Those three affinity constants obtained in the present study show comparable values with those of PSF-$E_{5.8}$, Torlon, and membrane proteins from *G. thermodenitrificans* DSM465. Among those molecular recognition sites toward As, that from hydrophilic proteins from *G. thermodenitrificans* DSM465 gave the highest affinity constant over 1.0×10^5 mol^{-1} dm^3. This might be due to the fact that those water-soluble hydrophilic proteins from *G. thermodenitrificans* DSM465 consisted of natural proteins, in which various functional groups interacting with a target molecule can be found in a high concentration. Moreover such functional groups interact with the print molecule 9-EA cooperatively and flexibly.[31] In the case of PSF-$E_{5.8}$, the apparent affinity constant increased with the decrease in molecular imprinting ratio. This is because multiple oligopeptide derivatives interacted cooperatively with the print molecule. Contrary to the previous results,[31] the apparent affinity constants determined in the present study did not show such tendency. Molecular imprinting conditions, the flexibility of constructed molecular recognition site, the degree of site isolation, and so forth are elucidated as factors leading to such phenomena, though there are no experimental data to explain the observed relationship between apparent affinity constant and the molecular imprinting ratio. At the moment, the affinity constants toward As in the film prepared by an alternative molecular imprinting did not exceed that by a conventional molecular imprinting technique,[34] which was prepared from methacrylic acid, ethylene glycol dimethacrylate, and *N*,*N*′-1,3-phenylenebis(2-methyl-2-propenamide). This might be due to lower flexibility of candidate polymeric materials, which

were adopted in studies summarized in Figure 7, for 9-EA molecularly imprinted films.

In Figures 4(c), 5(c), and 6(c), the calculated adsorption selectivity between As and Gs are given because the experiment for selective adsorption from As/Gs mixture cannot be conducted by SPR spectroscopy. The calculated adsorption selectivity, $S_{A(As/Gs)}$, can be represented by eq. (7)

$$S_{A(AS/Gs)} = \Delta\theta_{As}/\Delta\theta_{Gs} \quad (7)$$

Closed circles in every figure were calculated by using observed $\Delta\theta$ values for both As and Gs, while open ones by using $\Delta\theta$ values for As and extrapolated straight line for Gs. As is observed for the adsorption selectivity profile for materials having a specific recognition site, adsorption selectivity toward As increased with the decrease in substrate concentration.

As described previously,[31,32] SPR spectroscopy provides a rapid and facile evaluation method compared with adsorption experiments usually conducted in the evaluation of molecularly imprinted materials.[10,15–19,30] The combination of molecularly imprinted materials (MIPS) and SPR spectroscopy is a potent analytical method for the detection of a given target molecule[19,31–33,35,36] like that of MIPS and surface-enhanced Raman scattering,[37] that of MIPS and conductimetry,[38] that of MIPS and fluorescence,[39] that of MIPS and quartz crystal microbalance (QCM),[40–42] and so forth.

CONCLUSIONS

Novel molecular recognition films were prepared from modified polysulfone having perillaldehyde moiety as a side group. The molecular recognition films were obtained from perillaldehyde polysulfone by adopting 9-ethyladenine as a print molecule by applying an alternative molecular imprinting. The molecular recognition sites toward adenosine were constructed in the films thus prepared. The molecular recognition phenomena were studied by SPR spectroscopy. The apparent affinity constant determined by using apparent adsorption isotherms ranged from 7.90×10^3 to 3.31×10^4 mol^{-1} dm^3. The present study demonstrates that SPR spectroscopy is a facile method to study molecularly imprinted materials. Also the combination of molecularly imprinted materials and SPR spectroscopy will contribute to the development of sensors for analytical chemistry.

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