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Adsorption Behavior of Metal Ions onto a Bovine Serum Albumin (BSA) Membrane Monitored by Means of an Electrode-Separated Piezoelectric Quartz Crystal

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The interaction between metal ions and bovine serum albumin (BSA) was studied by using a piezoelectric quartz crystal (PQC) arranged in the electrode-separated configuration. A silanized surface of the PQC was coated with a BSA membrane *via* a coupling reaction with glutaraldehyde. The frequency shifts obtained from PQC coated with a BSA membrane suggested that various kinds of metal ions could be adsorbed onto the BSA membrane from aqueous solutions containing a low concentration of metal ions (2 or 10 μmol dm^{-3}), only when the BSA was denatured with an alkaline solution. Anionic species of Pt(IV) and Au(III) were adsorbed onto the denatured BSA membrane from an acetic acid solution at pH 2.2, and cationic species of Cd(II), Zn(II), Co(II), Ni(II), Cu(II), and Ag(I), and cations, such as Ca^{2+}, Ba^{2+}, and Mg^{2+}, were adsorbed from ammonia buffer at pH 9.5, whereas Al(III), Cr(III), Fe(III), Hg(II), and Pb(II) were hardly adsorbed. The adsorption mechanisms of these metal ions are discussed, based on the electrostatic interaction between the metal ions and the denatured BSA membrane, and complex formation between the metal ions and amino acid residues of the denatured BSA. Further, the PQC coated with a denatured BSA membrane was applied to the determination of Pt and Cd, using large frequency shifts for Pt(IV) and Cd(II).

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Introduction

In recent years, a membrane-coated piezoelectric quartz crystal (PQC) has been frequently used as a chemical sensor for investigating interactions among various biological substances.[1–8] The results have been applied to immunoassay[5,7,8] and to detection of a trace amount of an environmental pollutant, such as imitated dioxins.[6] In previous work, we investigated the adsorption of metal ions onto a PQC coated with a membrane of chitosan, or its derivatives, and applied chitosan-coated PQC to the determination of metal ions in aqueous solution.[9,10] In addition, the adsorption of surfactants, which are contained in soap, onto skin was researched by using a PQC coated with a collagen membrane, serving as imitated skin.[11] In the present work, the adsorption of metal ions onto a membrane of bovine serum albumin (BSA) was studied by using a PQC coated with a BSA membrane (a BSA-coated PQC). It is well-known that albumin, abundantly existing in living bodies, plays an important role for the transport and preservation of metal ions. Indeed, it has been reported that Cd(II), Cu(II), Zn(II), and $[PtCl_4]^{2-}$ strongly interact with albumin.[12–17] Hence, it is assumed to be worthwhile to study the adsorption of metal ions onto a BSA membrane with a BSA-coated PQC for understanding the interaction between metal ions and BSA.

A membrane-coated PQC is usually prepared by immobilizing a monolayer of mercaptoalkylcarboxylic acid on a gold electrode attached to a PQC, and then chemically bonding DNA, proteins, and sugar on the monolayer *via* an Avidin-Biotin method.[1] On the other hand, a PQC has been used in three different configurations in aqueous solution.[18,19] The first is a normal (conventional) configuration, in which a PQC with two gold electrodes on both sides is used in an experiment. The second is a one-electrode-separated configuration, in which a PQC with one gold electrode and a platinum board electrode are separately arranged at a short distance. In the last configuration, called an electrode-separated configuration, a PQC is placed between two platinum board electrodes apart from each other. In the last two configurations, the electrical contact parts in the PQC assembly never touch the aqueous sample solutions, that is, the parts are hardly corroded, and moreover, the assembly can be readily assembled or disassembled. Thus, the one-electrode-separated and electrode-separated configurations are superior to the normal configuration with respect to chemical durability and treatment of the PQC assembly. Making use of such advantages of the one-electrode separated and electrode-separated configurations, several PQC assemblies have been proposed for the trace determination of metal ions.[20,21] In this work, a PQC was coated with a BSA membrane *via* an Avidin-Biotin method, and the BSA-coated PQC was used in an electrode-separated configuration. This paper describes not only the interaction between metal ions and BSA, but also the applicability of BSA-coated PQC arranged in the electrode-separated configuration for the determination of metal ions.

Experimental

Apparatus

A PQC arranged in the separated-electrode configuration is illustrated in Fig. 1. A PQC of 8 mm in diameter (Kyushu

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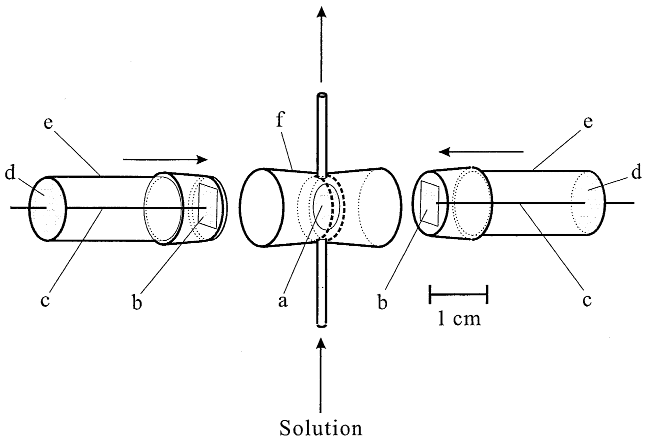

Fig. 1 Piezoelectric quartz crystal (PQC) arranged in the separated-electrode configuration. (a) PQC, (b) platinum board electrodes, (c) lead wires for the electric contact, (d) epoxy cement for fixing the lead wires, (e) an inner glass joint, (f) an outer glass joint.

Dentsu Production, AT cut, 9 MHz) was placed between two platinum board electrodes apart from each other in a homemade glass flow cell. The experimental setup used in this work is shown in Fig. 2. Electrode terminals of the two platinum board electrodes were linked to a transistor oscillator, and a DC voltage of 5.0 V was applied between the terminals with a power supply. The resonant frequency of the PQC was continuously monitored with a frequency counter (Advantest Corp., TR5822), and stored in a personal computer (NEC, PC-9801). Each solution was pumped into the flow cell at a constant flow rate of 4 cm^3 min^{-1} with a peristaltic pump (Tokyo Rikakiki, MP-3).

Reagents

Bovine serum albumin (BSA), γ-aminopropyltriethoxylsilane (γ-APTES), and glutaraldehyde (70% of solution) were purchased from Wako Pure Chemical Industries, Ltd. The other chemicals used were of reagent grade from Wako Pure Chemical Industries, Ltd. Storage solutions of metal ions, except for Fe^{3+}, Al^{3+}, and Hg^{2+}, were prepared by dissolving their nitrates or chlorides in deionized water, whereas storage solutions for Fe^{3+}, Al^{3+}, and Hg^{2+} were prepared with 10 mmol dm^{-3} nitric acid. The metal concentration of the storage solutions was 10 mmol dm^{-3}. Each storage solution was diluted to a proper concentration when used. The pH and electric conductivity of solutions were measured with a pH meter (TOA Electronics Ltd., HM-35 V) and a conductivity meter (HORIBA, DS-8M), respectively.

Coating of a PQC with a BSA membrane

A PQC was coated with a BSA membrane according to the following procedures.[10] First, two gold electrodes attached on the PQC were completely removed with aqua regia. After the PQC was cleaned with a 2 mol dm^{-3} sodium hydroxide solution for 30 min, it was thoroughly rinsed with deionized water and acetone. The PQC was immersed into a 10% γ-APTES acetone solution for 3 h, and then dried in an oven at 100°C for 1 h. After drying, the PQC was immersed in a 10% glutaraldehyde solution buffered at pH 7.0 with 0.1 mol dm^{-3} phosphate buffer for 3 h, and was subsequently treated in a 50 mg cm^{-3} BSA solution overnight. Before each experiment, the BSA-coated PQC was conditioned with acetate buffer at pH 5.8 for 20 min in the flow cell, and was stored in the acetate buffer when not in use.

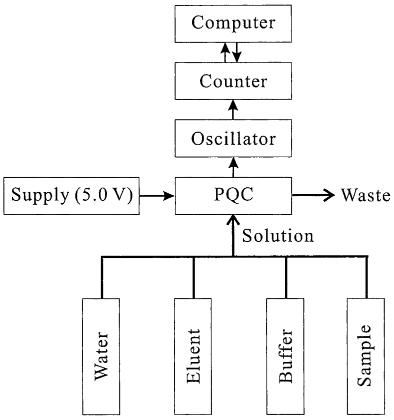

Fig. 2 Experimental setup.

Duration of the BSA-coated PQC

The BSA-coated PQC, in which the BSA membrane was denatured as described later, could be continuously used for *ca.* two weeks. After use for two weeks, the resonant frequency slowly increased with time and became noisy. However, dipping the PQC into a 2 mol dm^{-3} hydroxide solution for one day allowed the PQC to take back the frequency immediately after denaturation.

Resonant-frequency measurements

Measurements of the resonant frequency of the BSA-coated PQC were carried out in a flow system (Fig. 2). After pumping a blank solution (buffer solution) into the flow cell until the BSA-coated PQC showed a constant frequency (F_1), a sample solution was pumped for the adsorption of metal ions onto the BSA membrane. The resonant frequency was continuously monitored while pumping the blank and sample solutions, and was read out 5 min after introducing the sample solution (F_2). The flow cell was conditioned with the blank solution again for a subsequent measurement. The frequency shift was defined as $\Delta F = F_1 - F_2$, which was usually positive when adsorption occurred.

When a PQC is used in a liquid, the frequency shift generally depends on not only an increase in mass due to the adhesion of substances onto the PQC, but also due to some physicochemical properties of the liquid, such as the viscosity, density, and electric conductivity.[22] In this work, the electric conductivity was expected to give an additional change to the frequency shift. Consequently, the buffer solutions used were prepared so that their conductivities were kept constant at 1.5 mS cm^{-1}.

Results and Discussion

Adsorption of metal ions onto a native BSA membrane

Frequency shifts were measured for sample solutions containing metal ions at a concentration of 10 μmol dm^{-3} with a PQC coated with a native BSA membrane (a native BSA-coated PQC) in acetate (pH 5.8) and borax (pH 7.9) buffers. Among the metal ions tested, only Al(III), Fe(III), and Hg(II) gave small decreases in the resonant frequency of *ca.* 100 Hz in the acetate buffer and of *ca.* 200 Hz in the borax buffer. However, the adsorption of these metal ions onto the BSA membrane was merely due to the physical deposition of their hydroxides, but not due to a chemical interaction between the metal ions and the native BSA. In fact, no frequency shifts were obtained in a 1

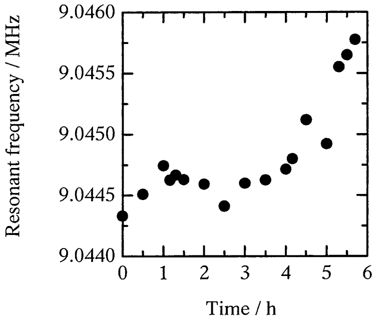

Fig. 3 Time course of the resonant frequency of a BSA-coated PQC during a denaturation treatment with ammonia buffer (pH 11.5).

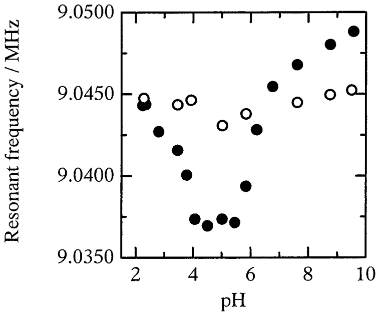

Fig. 4 pH characteristic of native and denatured BSA membranes. The open and solid circles refer to the native and denatured membrane, respectively.

mol dm^{-3} acetic acid solution (pH 2.2, 1.5 mS cm^{-1}).

Denaturation of BSA membrane and adsorption of metal ions onto the membrane

Denaturation of a native BSA membrane. Since the frequency shift was hardly obtained for adsorption of metal ions onto a native BSA membrane, we tried denaturing the native BSA by immersing the native BSA-coated PQC into ammonia buffer (pH 11.5, 1.5 mS cm^{-1}). In order to examine the denaturation process of the native BSA membrane, the resonant frequency of the BSA-coated PQC was continuously monitored for 5.7 h in an ammonia buffer solution flowing at a flow rate of 2 cm^3 min^{-1}. Incidentally, the resonant frequency of the bare PQC changed by only several tens of Hz. Figure 3 shows the time course of the resonant frequency. The frequency began to rise rapidly after *ca.* 3 h, and then increased by *ca.* 1.4 kHz after 5.7 h. The first 3-h period, for which the frequency was not so changed, is assumed to be a time for hydroxide ions to soak into the membrane, that is, the induced time of the denaturation process. Considering the increase in the resonant frequency after 3 h on a basis of the operational principle of PQC, the increase can be simply interpreted as a decrease in the mass of the BSA membrane, meaning dissolution of the membrane into the ammonia buffer. However, it was difficult to check the decrease in mass between before and after the denaturation treatment, because of a poor sensitivity of the analytical balance. As described above, there are some factors to give rise to a change in the frequency.[22] Kanazawa and Gordon have reported from a theoretical consideration about a shear wave on a PQC in a liquid that the frequency shift (decrease), Δf, is expressed by $\Delta f = f_0^{3/2}(\eta\rho/\pi\mu_Q\rho_Q)^{1/2}$, where f_0 is the resonant frequency, η and ρ are the viscosity and density of the liquid, and μ_Q and ρ_Q are the shear modulus and density of the PQC.[23] The expression expects that a decrease in viscosity and density of the BSA membrane can lead to an increase in the resonant frequency, provided that the membrane is regarded as being the liquid in contact with the PQC. The denaturation treatment is capable of unbinding a rigid structure of the native BSA, and thereby the density and viscosity of the BSA membrane may be reduced. Consequently, it is likely that the increase in the resonant frequency after 3 h resulted from a decrease in the density and viscosity of the BSA membrane during the denaturation treatment. In this work, it was required to denature the native BSA membrane equally for every BSA-coated PQC used. For this purpose, we treated the native BAS membrane with the ammonia buffer, until the increase in the resonant frequency of the BSA-coated PQC reached 1 kHz.

pH characteristic of the native and denatured BSA membranes. In order to investigate the pH characteristic of the native and denatured BSA membranes, the resonant frequencies of both PQCs coated with the native and denatured BSA membranes were measured in solutions of different pHs and of a constant conductivity of 1.5 mS cm^{-1}. As can be seen in Fig. 4, the resonant frequency for the native membrane was almost constant all over the pH region tested. This is probably because a rigid structure of the native BSA can protect the amino acid residues from hydrogen and hydroxide ions in the buffer solutions. On the other hand, the frequency for the denatured membrane largely decreased in the pH region between 3 and 6.5 and showed values greater than those for the native membrane at pHs more than 7. Although the frequency became noisy in the pH region between 3 and 6.5, the decrease was reproducible. Such a pH dependence can also be interpreted, based on a change in the density and the viscosity of the BSA membrane. Since an isoelectric point of BSA falls in the pH region between 4.7 and 4.9,[24] an electrostatically attractive interaction can operate between positively charged amino groups and negatively charged carboxyl groups of the amino acid residues in this pH region. As a result, the BSA membrane becomes tighter, that is, the density and viscosity of the membrane are increased, which leads to a decrease in resonant frequency. On the other hand, in pH regions of more than 8 and less than 3, the denatured membrane is negatively and positively charged due to the dissociation and protonation of the carboxyl and amino groups, respectively. Consequently, in both pH regions, an electrostatically repulsive interaction causes a decrease in the density and viscosity of the BAS membrane, and hence the frequency is increased, compared with that in the pH region between 3 and 6.5.

Adsorption of metal ions onto the denatured BSA membrane at pH 2.2 and 9.5. In order to understand the interaction between metal ions and the denatured BSA, the adsorption of various kinds of metal ions onto the membrane was investigated in a 1 mol dm^{-3} acetic acid solution at pH 2.2 and in ammonia buffers at pHs from 7.5 to 10.5. Table 1 summarizes the frequency shifts at pH 2.2 and at pH 9.5. In addition, frequency shifts for the bare PQC were rarely observed. In the acetic acid solution, only hexachloroplatinate(IV) ($[PtCl_6]^{2-}$) and tetrachloroaurate(III) ($[AuCl_4]^-$) gave frequency shifts. On the other hand, in the ammonia buffer at pH 9.5, frequency shifts could be obtained for most of metal ions that are expected to exist as cationic species. As shown in Table 1, the frequency shifts decreased at pH 9.5 in the order of Cd(II) >> Zn(II) > Co(II) > Ni(II) > Cu(II) > Ca^{2+} > Ba^{2+} > Mg^{2+} > Ag(I) >> Al(III) > Pb(II) > Cr(III) ≈ Fe(III) ≈ Hg(II) ≈ 0. However, as shown in Fig. 5, Cu(II) showed a peculiar pH dependence compared with

Table 1 Frequency shifts for the adsorption of metal ions onto a denatured BSA membrane in a 1 mol dm^{-3} acetic acid solution (pH 2.2)[a] and an ammonia buffer (pH 9.5)[b]

Metal	Added as	C/ µmol dm^{-3}	ΔF at pH 2.2/ Hz	ΔF at pH 9.5/ Hz	ΔF/a.w.[c]/ Hz
Pt	H_2PtCl_6	2	527	—[d]	1.29
Au	$HAuCl_4$	2	68	—	0.20
Cd	$CdCl_2$	10	—	1545	13.8
Zn	$Zn(NO_3)_2$	10	—	642	9.82
Co	$Co(NO_3)_2$	10	—	591	10.0
Ni	$Ni(NO_3)_2$	10	—	534	9.10
Cu	$Cu(NO_3)_2$	10	—	370	5.83
Ca	$Ca(NO_3)_2$	10	—	300	7.48
Ba	$Ba(NO_3)_2$	10	—	229	1.67
Mg	$MgCl_2$	10	—	150	6.17
Ag	$AgNO_3$	10	—	108	1.00
Al[e]	$Al(NO_3)_3$	10	—	44	1.6
Pb[e]	$Pb(NO_3)_2$	10	—	26	0.24
Cr	$Cr(NO_3)_3$	10	—	—	—
Fe	$Fe_2(SO_4)_3$ $(NH_4)_2SO_4$	10	—	—	—
Hg	$HgCl_2$	10	—	—	—

Flow rate, 4.0 cm^3 min^{-1}; reaction time, 5 min; temperature, 25°C.

a. The electrical conductivity was 1.5 mS cm^{-1}.

b. The ammonia buffer solution contained 17 mmol dm^{-3} ammonia and 17 mmol dm^{-3} ammonium chloride. The electrical conductivity was 1.5 mS cm^{-1}.

c. The term "a.w." indicates atomic weight.

d. The symbol "—" indicates that a frequency shift was hardly obtained.

e. The precipitates could be observed by the naked eye.

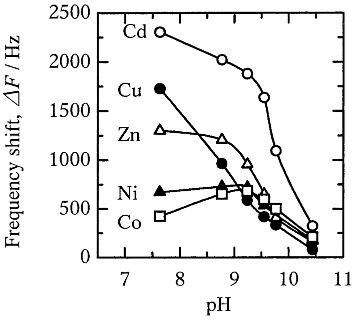

Fig. 5 pH dependence on the adsorption of various metal ions onto the denatured BSA membrane. The concentration of the metal ions was 10 µmol dm^{-3}.

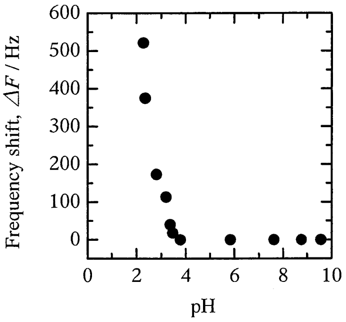

Fig. 6 pH dependence on the adsorption of $[Pt(IV)Cl_6]^{2-}$ onto the denatured BSA membrane. The concentration of $[Pt(IV)Cl_6]^{2-}$ was 2 µmol dm^{-3}.

the other metal ions. Further, Al(III) and Pb(II) formed precipitates, which could be observed by the naked eye, and the metal ions, except for $[AuCl_4]^-$, could be readily eluted, when acetate buffer of pH 5.8 was pumped for 5 min as an eluent.

In general, the following mechanisms are assumed to be responsible for adsorption of the metal ions onto the denatured BSA membrane: (1) physical adsorption onto the membrane, (2) adsorption through an electrostatic interaction between the metal ions and the membrane, and (3) chemical adsorption through complex formation between the metal ions and the amino acid residues of BSA. Mechanism (1) complies with the adsorption of Al(III), Pb(II), Cr(III), Fe(III), and Hg(II). These metal ions are easy to form hydroxides without any electric charges, and hence to produce precipitates. Since the precipitates can be only physically adsorbed onto the membrane, it is reasonable that the frequency shifts for these metal ions were very small or almost zero. Mechanism (2) is dominant to the adsorption of charged species. Since the denatured BSA membrane is charged positively at pH 2.2 and negatively at pH 9.5, it is expected that anions and cations can be adsorbed onto the membrane at pH 2.2 and at pH 9.5, respectively. In fact, anions, such as $[PtCl_6]^{2-}$ and $[AuCl_4]^-$, gave relatively large frequency shifts at pH 2.2, whereas cations such as Cd(II), Zn(II), Co(II), Ni(II), Cu(II), and Ag(I), which probably exist as ammine complexes in the ammonia buffer, and Ca^{2+}, Mg^{2+}, and Ba^{2+}, showed significant frequency shifts at pH 9.5. However, the frequency shifts were largely dependent on a kind of metal ion. Hence, complex formation is also expected to operate between the metal ions and the amino acid residues of BSA (Mechanism (3)) in addition to the electrostatic interaction. The frequency shifts for Cd(II), Zn(II), Co(II), Ni(II), and Cu(II) were larger than those for Ca^{2+}, Mg^{2+}, and Ba^{2+}. This fact suggests that complex formation with the amino acid residues partially contributes to the adsorption of metal ions onto the denatured BSA membrane. Indeed, it was reported that there exists chemical interactions between Cd(II), Zn(II), Ni(II), and Cu(II), and amino acid residues of albumin, such as histidine and aspartic acid.[12,17] The relatively small frequency shift for Ag(I) may result from its electric charge of +1 and a stable formation of the ammine complex in the buffer solution. Figure 5 indicates that the adsorption of Cd(II), Zn(II), Ni(II), and Co(II) became rapidly weak with increasing pH of more than pH 9. This is probably due to an increasing formation of hydroxides and/or amine complexes with the concentration of ammonia in the buffer solutions. The peculiar pH dependence for Cu(II) is assumed to be a case in which the formation of amine complexes successively effected adsorption of Cu(II). Further, it should be noted that the frequency shifts divided with the atomic weights for Mg^{2+} and Ca^{2+} (6.17 and 7.48, respectively) are much larger than that for Ba^{2+} (1.67). It is inferred from this fact that Mg^{2+} and Ca^{2+} have some weak chemical interaction with the denatured BSA. On the other hand, the anions of $[PtCl_6]^{2-}$ and $[AuCl_4]^-$ could be adsorbed onto the denatured BSA membrane at pH 2.2. We have, therefore, concluded that the adsorption for these ions dominantly results from the electrostatic interaction. However, $[AuCl_4]^-$ could not be eluted with acetate buffer of pH 5.8, as described above, and further, the frequency shift was comparable to those for Al(III) and Pb(II), which were hardly adsorbed. This fact implies a chemically or sterically special interaction between $[AuCl_4]^-$ and the denatured BSA rather than the simple electrostatic interaction.

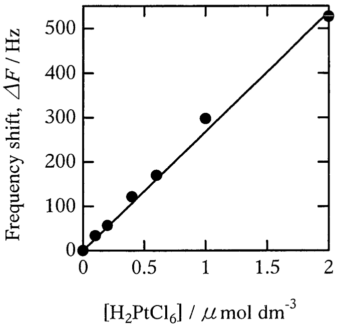

Fig. 7 Relationship between the frequency shift and the concentration of H_2PtCl_6 in a 1 mol dm^{-3} acetic acid solution at pH 2.2. The solid line was obtained from a method of least squares. The correlation coefficient was calculated as 0.998

Table 2 Effect of foreign ions on the frequency shift for the adsorption of $[Pt(IV)Cl_6]^{2-}$ onto denatured BSA-coated PQC in a 1 mol dm^{-3} acetic acid solution[a]

Ion	Added as	Concentration/ μmol dm^{-3}	Error, %
$AuCl_4^-$	$HAuCl_4$	8	13.5
$EDTA^-$	Na_2EDTA	10	13
CO_3^{2-}	Na_2CO_3	100	0.3
NO_3^-	$NaNO_3$	100	–2
SCN^-	NaSCN	100	–2.5
SO_4^{2-}	Na_2SO_4	100	–2.7
HPO_4^{2-}	Na_2HPO_4	100	–4.7
Cd^{2+}	$CdCl_2$	100	3.3
Fe^{3+}	$Fe_2(SO_4)_3(NH_4)_2SO_4$	100	3
Mg^{2+}	$MgCl_2$	100	0.9
Na^+	CH_3COONa	200	–1.4
K^+	KCl	200	–1.6
Cu^{2+}	$Cu(NO_3)_2$	100	–3.3
Zn^{2+}	$Zn(NO_3)_2$	100	–4.5
Ba^{2+}	$Ba(NO_3)_2$	100	–4.6
Ca^{2+}	$Ca(NO_3)_2$	100	–5.9
Pb^{2+}	$Pb(NO_3)_2$	100	–6.5
Co^{2+}	$Co(NO_3)_2$	100	–8.9
Cr^{3+}	$Cr(NO_3)_3$	100	–9.6
Ni^{2+}	$Ni(NO_3)_2$	100	–11.5
Hg^{2+}	$HgCl_2$	100	–15.9
Ag^+	$AgNO_3$	100	–16.5

Flow rate, 4.0 cm^3 min^{-1}; reaction time, 5 min; temperature, 25˚C; eluent, acetate buffer (pH 5.8, 1.5 mS cm^{-1}).

a. The concentration of $[Pt(IV)Cl_6]^{2-}$ was 2 μmol dm^{-3}.

Application of the denatured BSA-coated PQC to the determination of Pt and Cd. As can be seen in Table 1, the anion, $[PtCl_6]^{2-}$ gave a large frequency shift, and all of the cations tested did not give frequency shifts in an acetic acid solution. Such experimental results allow the application of the denatured BSA-coated PQC to the determination of Pt. We investigated the pH dependence of the frequency shift for $[PtCl_6]^{2-}$ in detail, and constructed a relationship between the frequency shift and the concentration. Figure 6 shows the pH dependence. The frequency shift increased rapidly with decreasing pH in the pH region below 4, but no frequency shift was obtained in the pH region above 4. At pH values less than 2, which were adjusted with hydrochloric acid, the denatured BSA-coated PQC stopped oscillating, probably due to a higher conductivity of the hydrochloric acid solution. This pH dependence clearly indicates that the adsorption of $[PtCl_6]^{2-}$ is strongly dominated by positive charges on the denatured BSA membrane, coming from protonation of the amino groups of the BSA. Figure 7 shows the relationship between the frequency shift and the concentration in a 1 mol dm^{-3} acetic acid solution at pH 2.2. A correlation coefficient of 0.998 was obtained in the concentration range to 2 μmol dm^{-3}, and $[PtCl_6]^{2-}$ could be detected down to at least 0.1 μmol dm^{-3} (19.5 ppb as Pt). In addition, the relative standard deviation was 1.9% for 7 repetitive measurements for 2 μmol dm^{-3}. On the other hand, the effect of foreign ions on the frequency shift for 2 μmol dm^{-3} $[PtCl_6]^{2-}$ was examined in the presence of a large excess of the foreign ions. The effect is listed in Table 2. The positive and negative errors indicate an increase and a decrease in the frequency shift for 2 μmol dm^{-3} $[PtCl_6]^{2-}$, respectively. Minor negative errors of less than several percentages result from an increase in the conductivity by adding foreign ions. However, several foreign ions, such as Pb^{2+}, Co^{2+}, Cr^{3+}, Ni^{2+}, Ag^+, and Hg^{2+}, gave relatively large negative errors. While all of the negative errors by these metal ions can not be explained, the errors by Ag^+ and Hg^{2+} probably come from their withdrawing ability of chloride ions from $[PtCl_6]^{2-}$, since the two ions easily react with chloride ions to precipitate. On the other hand, anions, such as $[AuCl_4]^-$ and $EDTA^-$, gave large positive errors. These errors clearly come from additional adsorption onto a positively charged membrane.

Many metal ions can be adsorbed onto a denatured BSA membrane at pH 9.5, as shown in Table 1. However, it is assumed to be useful to examine the linearity and sensitivity of a calibration curve for metal ions. The calibration curve was made for Cd(II) as a representative, since Cd(II) gave a frequency shift much larger than that of the other metal ions. As a result, the calibration curve suggested that the frequency shift was proportional to the concentration to 10 μmol dm^{-3} with the correlation coefficient of 0.999. The detection limit (S/N = 3) was 80 nmol dm^{-3} (9 ppb as Cd).

Conclusion

A PQC coated with the native BSA membrane was only slightly sensitive to the adsorption of metal ions. However, once the BSA membrane was denatured with an ammonia buffer solution at pH 11.5, the coated PQC could operate as a sensitive chemical sensor to many metal ions, in particular, the hexachloroplatinate(IV) ion in an acidic media and Cd(II) in a basic medium. The frequency shifts of PQC coated with a denatured BSA membrane suggest that the adsorptions of metal ions occurred through electrostatic interactions and complex formation between the metal ions and amino acid residues of the denatured BSA. Thus, it has been shown that the denatured BSA-coated PQC is a useful tool for examining the interaction between the metal ions and the BSA. Further, the applicability of the denatured BSA-coated PQC for the determination of Pt and Cd has been proved.

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