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Electrochemical Investigation of Pb^{2+} Binding and Transport through a Polymerized Crystalline Colloidal Array Hydrogel Containing Benzo-18-crown-6

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The transport of Pb^{2+} through a sensory gel, a polymerized crystalline colloidal array hydrogel with immobilized benzo-18-crown-6, is important for understanding and optimizing the sensor. Square wave voltammetry at a Hg/Au electrode reveals many parameters. The partition coefficient for Pb^{2+} into a control gel (no crown ether), K_p, is 1.00 ± 0.018 (errors reported are SEM). The porosity, ϵ, of the gel is 0.90 ± 0.01. Log K_c for complexation in the gel is 2.75 ± 0.014. Log K_c in aqueous solution for Pb^{2+} with the ligand 4-acryloylamidobenzo-18-crown-6 is 3.01 ± 0.010 with dissociation rate $k_d = (8.34 \pm 0.45) \times 10^2$ s^{-1} and association rate $k_f = (8.79 \pm 0.025) \times 10^7$ M^{-1} s^{-1}. The partition coefficient of the ligand 4-acryloylamidobenzo-18-crown-6 into the control gel, $K_{p,L}$ is 2.07 ± 0.15. The diffusion coefficient of Pb^{2+} in the control gel is $6.72 \times 10^{-6} \pm 0.12$ cm^2/s. For the sensor gel, but not control gel, diffusion coefficients are location dependent. The range of diffusion coefficients for Pb^{2+} in the probed locations was found to be $(6.11-12.60) \times 10^{-7}$ cm^2/s for 0.91 mM $Pb^{2+}$and $(2.84-9.39) \times 10^{-7}$ cm^2/s for 0.35 mM Pb^{2+}. Lead binding in the sensor gel is slightly less avid than in solution. This is attributed, in part, to the demonstrated affinity of the ligand 4-acryloylamidobenzo-18-crown-6 to the gel. Diffusion coefficients determined for the sensor gel were found to be location dependent. This is attributed to heterogeneities in the crown concentration in the gel. Analysis of diffusion coefficients and rate constants show that diffusion and not chemical relaxation will limit the time response of the material.

Pb^{2+} is a toxic heavy metal that accumulates in soil and natural waters. Devices that will detect this metal at the trace level are the aim of numerous research groups. Crown ethers and, in particular, 18-crown-6 have emerged as a potential recognition element in these devices for Pb^{2+} detection. Crown ethers are known to bind selectively to cations based on the macrocycle effect.

Hydrogels are cross-linked hydrophilic polymers that can absorb large amounts of water and swell, while maintaining their three-dimensional structure. When this swelling is engineered to be the result of a specific stimulus, the resulting material can serve as a sensor, drug delivery device, actuator, chemical memory device, etc.[1−6] The hydrogels investigated in this paper contain a crystalline colloidal array (CCA) with benzo-18-crown-6 incorporated into the hydrogel network.[3−6] The CCA diffracts visible light according to Bragg's law with the wavelength of light diffracted dependent on the spacing of the particles in the CCA.[7] As a result, the crown-containing hydrogels optically report on changes in Pb^{2+} concentration by the volume changes that occur when Pb^{2+} binds to the crown moieties altering the spacing in the polymerized crystalline colloidal array (PCCA).[4] This material finds application as both a Pb^{2+} sensor[3] and a Pb^{2+} optrode.[5,6]

While the general, qualitative features of this sensor material are understood, the dynamics in the gel are not understood quantitatively. It is important to understand diffusion, chemical kinetics, and binding strength in the gels in order to discover the parameters that limit sensitivity, dynamic range, and response time. The use of voltammetry to study metal complexation[8] and normalized chronoamperometry to study diffusion[9−12] is well established and seems particularly well suited to determining these important parameters in the PCCAs. Indeed, Murray has investigated cytochrome *c* transport in polymer films[13] and Tl^{1+}

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in crown-containing polymers[14] on electrode surfaces. In the former study, transport rates for the protein were shown to depend on water content. In the latter study, the voltammetry revealed the binding of the thallous ion in the film. Thus, there is both the need and the means to determine parameters of these sensing gels.

We have used electrochemical methods to study the interaction between Pb^{2+} and two types of hydrogels. One contains a benzo-18-crown-6 moiety that is covalently bound to the hydrogel network; the other does not. From thermodynamic and mass balance principles, and voltammetric measurements of free Pb^{2+}, we determined the partition coefficient, K_p, of Pb^{2+} into the hydrogels. We also determined the equilibrium binding constant K_c for the Pb^{2+} with the pendant crown ether. Using normalized chronoamperometry at a microelectrode, we measured the diffusion coefficients for Pb^{2+} through these hydrogels.

EXPERIMENTAL SECTION

Reagents and Instrumentation. All reagents were of analytical grade and used as received. Solutions were prepared with 18 MΩ purified water from a Millipore Synthesis A10 water purification system (Billerica, MA). $Pb(NO_3)_2$ (Baker) was used as the source of Pb^{2+} in all experiments. Except where noted, the electrolyte used was 0.10 M $LiNO_3$ (Baker). Triple-distilled mercury (Bethlehem Instruments) was used to prepare the working electrodes. All working electrodes were made in-house using either 0.025- (Goodfellow Metals Ltd., 99.99+% purity) or 0.5-mm-diameter (Goodfellow Metals Ltd., 99.95% purity) gold wire. All electrochemical experiments were carried out using a BAS Epsilon computer-controlled potentiostat with accompanying BAS software. UV−visible measurements were made on an Hewlett-Packard 845x UV−visible system and accompanying software. All errors are presented as standard errors of the mean (SEMs).

Preparation of the PCCA Hydrogels. Hydrogels were prepared according to the previously reported procedure.[4,5] For the sensor gels, gels containing benzo-18-crown-6, 0.1499 g of acrylamide (Fluka), 0.0141 g of bisacrylamide (Fluka), 0.0338 g of 4-acryloylamidobenzo-18-crown-6 (AAB18C6, Acros Organics), and 0.0138 g of 2,2-diethoxyacetophenone (Acros Organics) were dissolved in 3 mL of an 8% (by weight) dispersion of diffracting polystyrene colloids. The mixture was injected into a quartz cell with a 500-μm spacer and photopolymerized with UV light from a Blak Ray (365 nm) mercury lamp for a minimum of 1 h. The resulting PCCA hydrogels were placed in large quantities of purified water and allowed to equilibrate for several days. For the control gels, the AAB18C6 was omitted. We also prepared a clear hydrogel by omitting the diffracting polystyrene colloids.

Binding and Partitioning Experiments. *Cyclic Voltammetry in Solution and on PCCA Hydrogels.* A three-electrode configuration was used with a mercury film on gold working electrode, an Ag/AgCl reference electrode, and Pt wire counter electrode. The working electrode was prepared by dip coating a 3-mm gold electrode in elemental mercury. Excess mercury was manually removed. Both a control gel and sensor gel were transferred to an aqueous solution of 0.1 M $LiNO_3$ containing 1.0 mM $Pb(NO_3)_2$. With the electrodes suspended in bulk solution above the gels, a cyclic voltammogram was obtained at a scan rate of 500 mV/s. Measurements were taken with the electrodes held in place on the control and sensor gels as described in the text. Solutions were degassed with argon prior to use to remove O_2.

Partition Studies of Pb^{2+} into Control and Sensor Gels. Square wave voltammetry (SWV) was used to measure the current from $Pb(NO_3)_2$ reduction in a known volume of 0.10 M $LiNO_3$. The square wave pulse (E_{sw}) was 30 mV, step height (ΔE) was 2 mV, and the square wave period (τ) was 10 ms. The concentration of Pb^{2+} was varied between 40 (or 80 μM in some experiments) and 320 μM. Measurements were repeated in the same total volume containing pieces of a control PCCA hydrogel. Prior to the first scan and between stepwise additions of Pb^{2+}, the sample was stirred and degassed with argon for 15 min. Electrochemical measurements were made in the bulk solution above the gel. Solutions were deoxygenated with argon prior to use.

Partition Studies of AAB18C6 into Control Gel. UV spectrophotometry was used to monitor AAB18C6 concentration in a 1-cm cuvette containing 512 μL of a control hydrogel. The instrument kinetic mode was used to monitor the disappearance in absorbance due to AAB18C6 with time. Experiments were carried out at concentrations of 20.0, 60.0, and 100.0 μM AAB18C6 in purified water. Experiments were repeated in 6.5 mM $LiNO_3$. AAB18C6 partitioning from purified water into a blank hydrogel (no colloidal array) was also investigated.

Determination of Crown Ether Concentration in Hydrogel. A 512-μL sensor gel was placed in 20 mL of 7.68 mM $Pb(NO_3)_2$ in water. After 3 days, the gel was removed, excess solution was drained off, and the gel was transferred to a solution (0.1 M KNO_3 (J. T. Baker)) that displaced the Pb^{2+} by competitively binding to the crown. After 2 days, 5.0 mL of the displacing solution supernatant was analyzed for Pb^{2+} concentration using SWV (E_{sw} = 30 mV, ΔE = 2 mV, τ = 10 ms). The 5.0-mL sample was spiked with a known concentration of $Pb(NO_3)_2$ and analyzed by SWV with the same SW parameters.

Binding and Rate Studies of Pb^{2+}/AAB18C6 in Solution. Complexation was studied by preparing series of solutions containing 50 mM Pb(II), 20 mM $LiClO_4$, and varied concentrations of AAB18C6 (13−80 excess of crown). After transferring into the electrochemical cell and prior electrochemical tests, solutions were deoxygenated with argon for 15 min. Prior to all experiments, the mercury film electrode was tested with SWV for reversible behavior by monitoring the current as a function of $(1/t)^{1/2}$ in the absence of ligand. Differential pulse voltammetry (DPV) was used for determination of binding constant (ΔE_s = 1 mV, t_p = 50 ms, T = 200 ms, ΔE_p = 25 mV, line period sampling). SVW was used for kinetic measurements (E_{sw} = 30 mV, ΔE = 2 mV; square frequency, f, was varied randomly between 10 and 150 Hz). For the kinetic studies, the concentration of Pb(II) was 100 mM, $LiNO_3$ was 0.100 M, and AAB18C6 was 2.72 mM.

Diffusion Experiments. Chronoamperometry at a microelectrode was carried out to determine the diffusion coefficients for Pb^{2+} in solution, in the sensor gel, and in the control gel. To study diffusion through solution, data were collected for 15 s for both the reduction and oxidation pulses. For the studies through the gels, data were collected for 60 s for each pulse. The initial potential was −250 mV, and the final potential was −550 mV (vs

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Ag/AgCl) for all experiments. The aqueous solution contained 0.91 mM $Pb(NO_3)_2$ in 0.1 M $LiNO_3$. To probe diffusion through the gel, a piece of the control gel was added to the 0.91 mM $Pb(NO_3)_2$ solution containing 0.1 M $LiNO_3$. The working microelectrode was placed on the surface of the gel and held with a micromanipulator (Narishige model M152). Bulk solution existed above the gel. Data were acquired at several places on the gel. A minimum of three repeats was carried out at any given spot on the gel. Between moving the electrode to another location on the gel, the response of the electrode was checked by collecting chronoamperograms in the bulk solution. For the sensor gel, 0.91 and 0.35 mM Pb^{2+} were tested. For all experiments, a two-electrode configuration was used with an Ag/AgCl (BAS) reference electrode and a 25-μm-diameter Au/Hg working electrode. The Au/Hg electrodes were prepared by attaching the gold wire to a tinned copper wire with silver conducting paint and sealing in glass with Torr-Seal (Varian). Immediately prior to use, electrodes were polished (0.05-μm alumina) and dip-coated with mercury. The resulting film electrodes were polished on a wet polishing cloth, and the magnitude of the steady-state current was checked via cyclic voltammetry (scan rate 10 mV/s) to ensure planar geometry of the electrode.

RESULTS

Equilibria and Chemical Kinetics. The crown ether recognition element is incorporated into the sensor gel by copolymerization of AAB18C6 during preparation of the gel. Prior to studying the sensor gel material, the binding constant and chemical kinetics at 20 °C for the Pb(II)/AAB18C6 system were determined in aqueous electrolyte using DVP and SWV, respectively. Concentration of free lead in solution can be calculated from the shift of the DPV peak potential (relative to crown-free solution) through

$$[Pb]_f = e^{(nF/RT)\Delta E}[Pb]_T \quad (1)$$

The equilibrium constant K_C was calculated according to (see also eq 6)

$$K_C = \frac{[Pb]_T - [Pb]_f}{[Pb]_f ([L]_T - [Pb]_f)} \quad (2)$$

Averaging constants at various crown excess ratios yields $\log(K_c) = 3.01 \pm 0.010$. Rate constants were determined using SWV to monitor changes in ΔI_{max} values over a range of experimental time scales. ΔI_{max} values are normalized and compared to a computer-generated working curve as previously described.[15,16] From these data, the dissociation rate constant, k_d, is calculated to be $(8.34 \pm 0.45) \times 10^2$ s^{-1}, and the association rate constant, k_f, is calculated to be $(8.79 \pm 0.025) \times 10^7$ $M^{-1}s^{-1}$ at 20 °C.

The partitioning of Pb^{2+} between water and a control PCCA hydrogel can be investigated by square wave voltammetric measurements of $[Pb^{2+}]$ in an aqueous solution containing a control gel. Figure 1 shows SW voltammograms of 120 μM Pb^{2+} in solution with and without control gel. Such data can be used

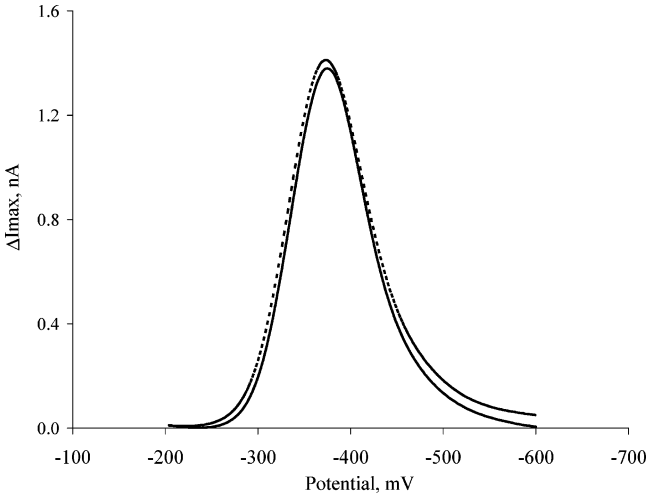

Figure 1. SWV for reduction of 120 μM $[Pb^{2+}]$ in solution. Solid line is for pure aqueous sample (2.50 mL total volume). Dashed line is for sample containing 20.5% hydrogel (2.50 mL total volume; 90% of gel is water).

to determine the partition coefficient quantitatively. The partition coefficient, K_p, is defined as

$$K_p = \frac{[Pb^{2+}]_g}{[Pb^{2+}]_s} = \frac{n_{Pb^{2+},g} V_s}{V_V n_{Pb^{2+},s}} \quad (3)$$

where $n_{Pb^{2+},g}$ is the moles of Pb^{2+} in the gel void volume, $n_{Pb^{2+},s}$ is the moles of Pb^{2+} in solution outside of the gel, V_V is the volume of the void space in the hydrogel, which equals the product of the porosity, ϵ, and the total gel volume, V_g, and V_s is the volume of the solution outside of the gel.

Then $[Pb^{2+}]_g$ can be expressed as

$$[Pb^{2+}]_g = n_{Pb^{2+},g}/\epsilon V_g \quad (4)$$

Using eqs 3 and 4 and mass balance ($n_{Pb^{2+},T} = n_{Pb^{2+},g} + n_{Pb^{2+},s}$), an expression can be derived that relates $[Pb^{2+}]_s$ to $n_{Pb^{2+},T}$:

$$[Pb^{2+}]_s = n_{Pb^{2+},T}/(K_p \epsilon V_g + V_s) \quad (5)$$

Therefore, if $[Pb^{2+}]_s$ measurements are made over a range of known added $n_{Pb^{2+},T}$, plots can be made according to eq 5, which gives a slope of $1/(K_p \epsilon V_g + V_s)$.

Using this approach, K_p can be determined if V_g, ϵ, and V_s are known. The volume of the gel, V_g, can be calculated from the area of the gel and spacer height used in fabrication. The porosity, ϵ, was estimated by the change in weight of the hydrated gel compared to the fully dehydrated gel. The value of ϵ was thus determined to be 0.90 ± 0.01 (SEM, $n = 3$). The solution volume, V_s, was approximated as a constant based on the mean value (4568.0 μL) used from the stepwise additions of Pb^{2+} (giving less than 2% error in solution volume, which is within experimental error). $[Pb^{2+}]_s$ in the presence of control gel was determined from a calibration plot made in the absence of gel. For the measurements taken in the presence of gel, the electrodes were suspended in bulk solution above pieces of the gel. The total volume of sample

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was either 5.0 or 2.5 mL. The measurements of $[Pb^{2+}]_s$ as a function of $n_{Pb^{2+},T}$ yielded straight lines ($R^2 > 0.991$) with zero intercepts as predicted by eq 5. K_p from the three repeats was found to be 1.00 ± 0.018 (SEM, $n = 3$) at 20 °C.

In a similar manner, the binding constant for the complex in the gel can be determined using SWV. K_c is defined as

$$K_c = [Pb^{2+}]_b/[Pb^{2+}]_f[L]_f \quad (6)$$

with

$$[Pb^{2+}]_b = n_{Pb^{2+},b}/V_T$$

$$[Pb^{2+}]_f = n_{Pb^{2+},f}/V_T$$

$$[L]_f = n_{L,free}/V_T$$

where $n_{Pb^{2+},b}$ is the moles of bound Pb^{2+}, $n_{Pb^{2+},f}$ is the moles of free Pb^{2+}, $n_{L,free}$ is the moles of uncomplexed ligand, and V_T is the total sample volume, i.e., $V_g + V_s$.

Weber has shown that the correct volume to use for calculating effective concentrations of species bound to a surface (e.g., surface-bound species in a sensor) is the total liquid volume available to freely diffusing species.[17] Here we use the total volume (including the polymer and colloidal particles) as Giddings has done[18] for experimental convenience (measuring V_T is easy). A more rigorously correct binding constant is derived below, which is directly proportional to K_c and is only 1% different from it. Using mass balance, $[Pb^{2+}]_T = [Pb^{2+}]_f + [Pb^{2+}]_b$, and eq 6, an expression for $[Pb^{2+}]_f$ is derived in terms of $[Pb^{2+}]_T$:

$$[Pb^{2+}]_f = [Pb^{2+}]_T/(K_c[L]_f + 1) \quad (7)$$

Over a range of Pb^{2+} concentrations (20−320 μM), plots of $[Pb^{2+}]_f$ versus $[Pb^{2+}]_T$ are linear ($R^2 > 0.989$, $n = 3$) as predicted by eq 7. The slopes of the plots are equal to $1/(K_c[L]_f + 1)$. To arrive at a value for K_c, we determined the value of $[L]_T$ as described in the Experimental Section to be 15.2 mM in the gel. Given the range of $[Pb^{2+}]$ used, $[L]_f$ can be approximated by $[L]_T$. Log K_c was calculated to be 2.76 ± 0.014 (standard error of the measurement, SEM) giving a K value of 571 ± 18 (SEM) at 20 °C. This is somewhat less than log K_c for the complex in aqueous solution.

If the free energy of the bound ligand is lower than in solution because of a noncovalent interaction between the ligand and some portion of the gel, then a lower K_c would be expected. Therefore, we investigated the partitioning of AAB18C6 into the control gel using UV−visible spectrophotometry. The partition coefficient, $K_{p,L}$, for AAB18C6 into the control gel is defined as

$$K_{p,L} = \frac{n_{L,g}/\epsilon V_g}{n_{L,s}/V_s} \quad (8)$$

where $n_{L,g}$ is the moles of AAB18C6 in the gel and $n_{L,s}$ is the moles of AAB18C6 in solution outside the gel.

When the solution is made in purified water, $K_{p,L}$ is 2.07 ± 0.15 (SEM, $n = 3$). When the solution is 6.50 mM $LiNO_3$ (giving the same ratio of Li^+/AAB18C6 as used in SWV experiments), $K_{p,L}$ is 2.17 ± 0.18 (SEM, $n = 3$). When this experiment is repeated with a clear acrylamide hydrogel that does not contain colloidal particles, $K_{p,L}$ is 2.12 ± 0.18 (SEM, $n = 3$).

Diffusion. Chronoamperometry can be used to investigate the diffusion of Pb^{2+} through the hydrogels by analyzing normalized transient currents obtained at a mercury film on gold microelectrode. Normalized current at a disk microelectrode is given by[9]

$$i(t)/i_{ss} = (\pi^{1/2}/4)r(Dt)^{-1/2} + 1 \quad (9)$$

where $i(t)/i_{ss}$ is the normalized transient current, r is the radius, D is the diffusion coefficient, and t is time. A plot of $i(t)/i_{ss}$ versus $t^{-1/2}$ has an intercept of 1 with a slope, S, equal to $(\pi^{1/2}/4)rD^{-1/2}$. With a known radius, D can be determined:[9]

$$D = \pi r^2/16S^2 \quad (10)$$

This approach is chosen, as opposed to potential-step chronoamperometry at a planar macroelectrode, because it allows diffusion coefficients to be determined independent of analyte concentration. This has obvious advantages for measurements made on the sensor gel.

To minimize the error in D, the response of the working electrode was investigated to ensure planar geometry of the mercury film on the gold substrate. Cyclic voltammograms at a scan rate of 10 mV/s were obtained for Pb^{2+} reduction in 0.1 M $LiNO_3$ after the film was prepared by dip coating the gold electrode in elemental mercury. When CVs were collected immediately after coating, the current response exceeded the value calculated for a planar electrode, but exactly matched the value for a hemisphere (13.0 nA, $I = 2\pi rnFDC$). Electrodes were then polished on a clean, wet polishing pad. Steady-state currents taken after this treatment agreed with theory for a planar disk (8.0 vs 8.3 nA from theory, $I = 4rnFDC$, with $D = 9.45 \times 10^{-6}$ cm^2/s at 21 °C). Therefore, this treatment removes excess mercury. Polishing after dip-coating has become standard procedure (see Figure 2).

Chronoamperometry was carried out in aqueous solution, and the transient current was normalized to determine the diffusion coefficient of Pb^{2+} in solution. Normalized currents were plotted versus $t^{-1/2}$. In accordance with eq 4, the normalized current intercept for these plots should be 1. As eq 4 is a simplification, and a more exact though harder to use expression exists,[9] we checked the results from the slopes of the $i-t^{1/2}$ plots. Cottrell behavior is observed at short times. The diffusion coefficient in free solution calculated from short-time data and from eq 4 are in agreement. The value obtained for the diffusion coefficient of Pb^{2+} in free solution, D_s, was (9.45 ± 0.31) × 10^{-6} cm^2/s (SEM, $n = 4$) at 21 °C (for all repeats, the normalized current intercept is in the range 0.94−0.95, $R^2 > 0.990$).

To evaluate the diffusion coefficients of Pb^{2+} in the control and sensor gels, measurements needed to be made of the Pb^{2+} species in the gels, as opposed to solution species. Cyclic voltammetry was used to investigate whether this could be done by placing

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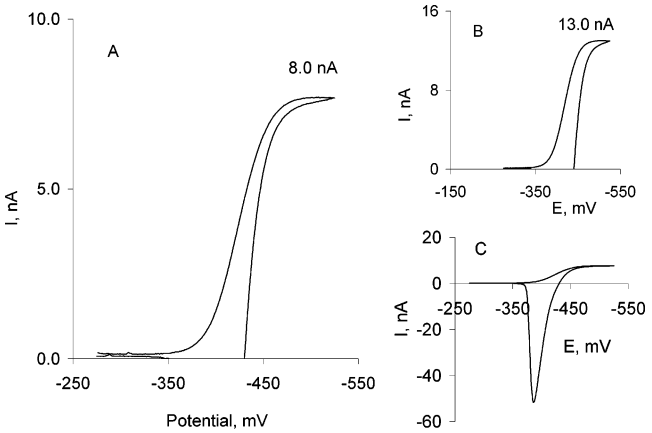

Figure 2. (A) Reduction wave from steady state (scan rate 10 mV/s) cyclic voltammogram of 0.91 mM $Pb(NO_3)_2$ in 0.1 M $LiNO_3$ after excess mercury is removed from Hg/Au microelectrode ($d = 25\ \mu m$). (B) Reduction wave prior to removal of excess Hg from Hg/Au microelectrode. (C) Entire signal for reduction wave given in (A).

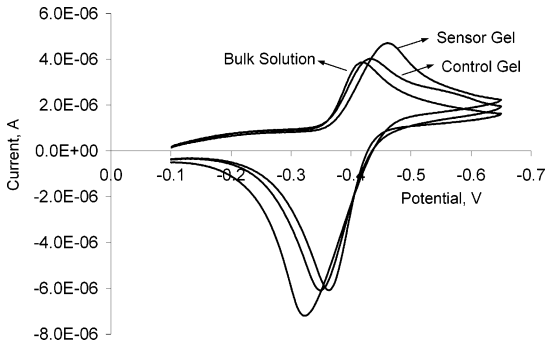

Figure 3. CVs for the reduction of 1.0 mM $Pb(NO_3)_2$ in 0.1 M $LiNO_3$ at a 3 mmHg/Au working electrode. Scan rate 500 mV/s. Labels indicate where electrodes are located at the time of measurement.

the electrodes on the gel surface. The mercury film on a 3-mm-diameter gold microelectrode and the reference electrode were placed directly onto the control gel and held in place with a micromanipulator. The counter electrode was placed along the edge of the gel. A cyclic voltammogram was obtained at a scan rate of 500 mV/s. The electrodes were moved to the sensor gel in an analogous configuration, and a cyclic voltammogram was obtained. The electrodes were also suspended in bulk solution above the gels, and a cyclic voltammogram was obtained (see Figure 3). The solution and control gel CVs are fairly similar. The signal obtained from the sensor gel is increased in magnitude, and the reduction wave is shifted along the potential axis in the negative direction. The increased magnitude is consistent with the sensor gel having a higher concentration of Pb^{2+}, and the shift indicates the electron transfer is preceded by dissociation of the complex. Analysis of the CVs confirmed movement through the gel could be monitored by placing the electrodes on the gels.

Chronoamperometry at a microelectrode was then used to probe the diffusion coefficients for Pb^{2+} moving through the PCCA hydrogel containing benzo-18-crown-6 and through the control gel. A 0.91 mM Pb^{2+} sample was prepared in 0.1 M $LiNO_3$ containing the control gel. The working and reference electrodes were placed on the gel with bulk solution above the gel. Chronoamperometry measurements were obtained. For these measurements, 60-s pulse times were used. In the gels, convection is less likely to occur on this time scale as compared to bulk solution. Increasing the pulse times allows for closer approximation of the steady-state current, which gives a better fit for $i(t)/i_{ss}$ versus $t^{-1/2}$ (intercept more closely approaches 1) compared to the 15-s pulse times used for the solution work. This is necessary because the diffusion through the gel is slower as a result of the tortuosity of the gel matrix, and eq 10 is optimal for $t > r^2/D$.[9,12]

Table 1. Regression Data for Plots of $i(t)/i_{ss}$ versus $t^{-1/2}$ for Chronoamperometry Experiments with Electrodes Placed on Control Gel in 0.91 mM $Pb(NO_3)_2$ with 0.1 M $LiNO_3$[a]

spot on gel	intercept	R^2	$D/10^{-6}$ cm^2 s^{-1}
A	>0.94	>0.99	6.67 ± 0.30
B	>0.94	>0.99	6.66 ± 0.30
C	>0.94	>0.99	6.68 ± 0.30
D	>0.94	>0.99	6.85 ± 0.30

[a] Intercept and R^2 values are given for the lowest value in three repeats. Error in D_{cg} is SEM.

Table 2. Regression Data for Plots of $i(t)/i_{ss}$ versus $t^{-1/2}$ for Chronoamperometry Experiments with Electrodes Placed on Sensor Gel in 0.91 mM $Pb(NO_3)_2$ with 0.1 M $LiNO_3$[a]

spot on gel	intercept	R^2	$D/10^{-7}$ cm^2 s^{-1}
A	>0.90	>0.99	6.11 ± 0.18
B	>0.93	>0.99	12.60 ± 0.53
C	> 0.90	>0.99	6.95 ± 0.87

[a] Intercept and R^2 values are given for the lowest value in three repeats. Error in D_{sg} is SEM.

Four spots on the control gel were tested with three measurements made at each spot. Between switching locations on the gel, solution measurements were made to verify the integrity of the working electrode. Regression data for plots of $i(t)/i_{ss}$ versus $t^{-1/2}$ for the control gel measurements are given in Table 1. Note that the measurements for the control gel are very consistent regardless of the location sampled on the gel ($D_{cg} = (6.72 \pm 0.24)$ (SEM) $\times 10^{-6}$ cm^2/s at 21 °C).

This procedure was repeated for the sensor gel. Three spots on the gel were sampled. Regression data for plots of $i(t)/i_{ss}$ versus $t^{-1/2}$ are given in Table 2. Notice the diffusion coefficients measured in the sensor gel are location dependent. For the three spots sampled, the range of diffusion coefficients was (6.11−12.60) $\times 10^{-7}$ cm^2/s. This is significantly less than the values obtained for the control gel. This is attributed to the interaction that occurs between the Pb^{2+} and crown as the Pb^{2+} moves through the gel. The concentration of Pb^{2+} for these measurements was 0.91 mM. Because the crown concentration was 15.2 mM, it was desirable to work at lower Pb^{2+} concentrations. For this reason, the experiment was repeated with 0.35 mM Pb^{2+}. At this concentration of Pb^{2+}, a higher percentage of crown is uncomplexed and therefore free to interact with diffusing Pb^{2+}. Diffusion coefficients should be lower at this concentration. Regression data for plots of $i(t)/i_{ss}$ versus $t^{-1/2}$ for this concentration range are given in Table 3. For the three spots sampled, the range of diffusion coefficients was (2.84−9.39) $\times 10^{-7}$ cm^2/s. This range of diffusion coefficients is lower than the range observed with 0.91 mM Pb^{2+}.

Table 3. Regression Data for Plots of $i(t)/i_{ss}$ versus $t^{-1/2}$ for Chronoamperometry Experiments with Electrodes Placed on Sensor Gel in 0.35 MM $Pb(NO_3)_2$ with 0.1 M $LiNO_3$[a]

spot on gel	intercept	R^2	$D/10^{-7}$ cm^2 s^{-1}
A	>0.93	>0.99	8.62 ± 0.70
B	>0.93	>0.99	9.39 ± 0.30
C	>0.85	>0.99	2.84 ± 0.22

[a] Intercept and R^2 values are given for the lowest value in three repeats. Error in D_{sg} is SEM.

DISCUSSION

Mercury film electrode preparation is very important to arrive at good data. The procedure described in the Experimental Section does not always give usable electrodes, and therefore, they need to be carefully tested prior to use. An alternative electrode preparation method is given in Supporting Information. A comparison of AAB18C6/Pb(II) binding constant determination by three different methods (electrochemistry, UV absorbance, ^{1}H NMR) is also presented in Supporting Information. This comparison was prompted by a disagreement between early experiments that were done by titrating a solution of Pb^{2+} with small volumes of a concentrated solution of crown and data reported here in which a series of solutions was analyzed independently. Now, this disagreement is attributed to the limited solubility of AAB18C6 in water.

In our investigation of the gels, several interesting things have been discovered. K_p for Pb^{2+} in the control gel was found to be exactly 1.00. This implies that, for the Pb^{2+} species, the environment in the hydrogel appears to be purely aqueous despite the presence of the colloidal particles and the polymer network.

We reported above that the binding constant between Pb^{2+} and AAB18C6 in the gel is 571 ± 18 M^{-1}. This binding constant can be more rigorously calculated to take into account the portion of gel that is not solvent, i.e., $(1 - \epsilon)V_g$. (By invoking a porosity, ϵ, the physical picture of porous media is called to mind. Others[19] have used the porous model for low-density polymer particle materials. One should not, however, envision anything like hard-walled, cylindrical pores existing in these materials.) The corrected binding constant, $K_{c,corrected}$, is given by

$$K_{c,corrected} = [Pb^{2+}]_{b,g}/[Pb^{2+}]_{f,g}[L]_{f,g} \qquad (11)$$

where the concentrations are now in terms of the liquid volume in the gel as indicated by the subscript "g".

$$[Pb^{2+}]_{b,g} = n_{Pb^{2+}b,g}/\epsilon V_g$$

$$[Pb^{2+}]_{f,g} = n_{Pb^{2+}f,g}/\epsilon V_g$$

$$[L]_{f,g} = n_{L,b,g}/\epsilon V_g$$

As the two ratios $[Pb^{2+}]_b/[L]_f$ (eq 4) and $[Pb^2]_{b,g}/[L]_{f,g}$ (eq 11) are equal, the only difference between eqs 6 and 11 is $[Pb^{2+}]_{f,g}$ and $[Pb^{2+}]_f$. The latter includes moles in the solution and the gel, while the former includes moles only in the gel. To resolve the difference, we use the mass balance relationship

$$n_{Pb^{2+},f} = n_{Pb^{2+},f,s} + n_{Pb^{2+}.f,g} \qquad (12)$$

and the partition coefficient written as eq 3, then

$$K_{c,corrected} = K_C\left(\frac{\epsilon V_g + V_S/K_p}{V_T}\right) \qquad (13)$$

For our system, $K_{c,corrected}$ is 565 ± 18 differing from K_c by 1% (K_c = 571).

K_c in free solution was found to be $10^{3.01}$. This equilibrium binding constant is in the same range as for similar 18-C-6 crown-Pb^{2+} complexes measured in solution.[16,20−22] Thus, the affinity of the crown-modified acrylate gel was not expected to be different from that for freely diffusing crown ether, and indeed they are very comparable ($10^{2.75}$ in the gel vs $10^{3.01}$ in solution). One possible explanation for the slight decrease of affinity is that the activity coefficient of the crown is lower in the gel than in solution. This would be the case, for example, if there were attractive interactions between the crown and the gel network. If this were the case, we would expect to find a partition coefficient of the crown that is greater than 1.0. The partition coefficient for AAB18C6 into the control gel, $K_{p,L}$, was measured. $K_{p,L}$, is 2.07. Because the control gel contains both polymer and the colloidal array, an additional partition coefficient was determined for AAB18C6 into a plain acrylamide-based gel with no colloidal particles. This partition coefficient was found to be 2.12, indicating the interaction is with the polymer matrix. This magnitude of $K_{p,L}$ is adequate to account for the entire decrease in binding strength in the gel. Therefore, crown−gel interactions may be a factor in modulating Pb^{2+}/crown binding in gels. Other possible causes are conformational constraints on the ligand and inaccessible ligand molecules as a result of the polymerization. Our measurement of the concentration of the ligand in the gel was accurate, but it allowed for long-time relaxation of the gel network. Thus, by this method described in the Experimental Section, we may count ligands that are not instantaneously accessible in the normal operating time scale. As what we truly measure in the laboratory is the product $K_C[L]_T$, an overestimate of $[L]_T$ will lead to an underestimate of K_C.

To determine diffusion coefficients for Pb^{2+} through the gels, normalized chronoamperometry at a microelectrode was used. This technique has two advantages for our work. First, diffusion coefficients can be determined independently of Pb^{2+} concentration. This is useful for measurements in the sensor gel where concentration of Pb^{2+} is unknown. Second, this technique has enhanced spatial resolution compared to bulk transport experiments. Our measurements are made at a 25-μm-diameter electrode. The electrode "sees" 10r, or 125 μm. As a result of this resolution, the diffusion coefficients in the sensor gel were discovered to be location dependent (2.84 × 10^{-7}, 8.62 × 10^{-7},

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and 9.39×10^{-7} cm^2/s, for three separate locations; see Table 3). This is attributed to heterogeneities in crown ether concentration throughout the gel, and not heterogeneities in cross-link density, as diffusion coefficient measurements in the control gel are homogeneous. In applications, this material reports on Pb^{2+} concentration by changes in the wavelength of diffracted light. Heterogeneities in ligand concentration in the material would broaden the diffraction response.

It may be that damage to the gel occurs during measurements. We cannot absolutely rule this out; however, the following observations are relevant. The measurements at any given spot are reproducible. If damage were occurring over time or if the perturbation of the electrode caused the gel to deform slowly, a time-dependent response would be seen. The measurements made in solution between spots are reproducible and quantitatively correct, so if there is damage, it is not associated with strong electrode−polymer interactions which would result in fouling. It may be that the electrode, which of course is not absolutely planar, is not in intimate contact with the gel and that we are measuring in a liquid film over some of the electrode surface. When measurements are made in the gels, the theory and data agree most of the time. On occasion, there are two "breaks" in the current−time transient that we have interpreted as being caused by the presence of a liquid film between the gel and the electrode surface. In these cases, subtle repositioning of the electrode leads to a normal response. One may hypothesize that the spot-to-spot heterogeneity in the crown-containing gels is caused by variability in the intimacy of the contact between the gel and the electrode. However, the spot-to-spot variability of the control gel is negligible. Thus, while we cannot prove that the gel and the electrode are in intimate contact, all indications are that the contact is good. It is especially revealing that in some cases the electrode was obviously not in intimate contact and that a predictable response resulted from repositioning the electrode.

Some information about the gel structure can be obtained from analysis of the diffusion coefficient of Pb^{2+} in the control gel. The tortuosity λ decreases the diffusion coefficient (D_{cg}) in the gel network in comparison to the diffusion coefficient in free solution (D_s) according to eq 14[23,24]

$$D_{cg} = D_s/\lambda^2 \tag{14}$$

where the tortuosity λ is related to the ratio of step lengths in a random walk model of the gel versus free solution. Tortuosity values are higher the more twisted and convoluted the medium is. Typical values for λ of gases in highly porous media like silica and alumina can be much greater than unity,[21,22] while for a more open network like a methacrylate hydrogel, values of λ in the range of 1.04−1.09 are found.[25,26]

The diffusion coefficient for Pb^{2+} through the control gel, D_{cg}, is 6.72×10^{-6} cm^2/s. The diffusion coefficient for Pb^{2+} through the solution, D_s, is 9.45×10^{-6} cm^2/s. Using eq 18, λ is calculated to be 1.19. For our system, it is known that no interaction between the Pb^{2+} and the gel exists ($K_p = 1.00$), so any decrease in diffusion coefficient in the gel is certain to be strictly geometrical. Thus, λ in the PCCA gels is slightly higher than reported λ values for macroporous methacrylate hydrogels ($\lambda = 1.04-1.09$[25])We attribute this to the presence of the polystyrene latex spheres.

Evaluation of the diffusion coefficients obtained for Pb^{2+} in the sensor gel (D_{sg}) was done by calculating theoretical values based on the mass-weighted average of the value obtained for the diffusion coefficient through the control gel:

$$D_{sg} = \frac{[Pb^{2+}]_b D_b + [Pb^{2+}]_f D_{cg}}{[Pb^{2+}]_b + [Pb^{2+}]_f} \tag{15}$$

where the subscript "b" indicates Pb^{2+} bound to the crown ether.

Ideally, diffusion of lead in the control gel would be carried out at infinite dilution of Pb^{2+}, because the sensor gel is saturable. Due to signal-to-noise ratio constraints, we have not been able to do this, so we determined diffusion coefficients at two fairly low concentrations of Pb^{2+}.

Using the equilibrium binding expression and mass balance, $[Pb^{2+}]_f$ and $[Pb^{2+}]_b$ were calculated to be 3.72×10^{-5} and 3.13×10^{-4} M, respectively, for $[Pb^{2+}]_T = 3.50 \times 10^{-4}$ M and 9.97×10^{-5} and 8.10×10^{-4} M for $[Pb^{2+}]_T = 9.10 \times 10^{-4}$ M. D_{cg} was taken as 6.72×10^{-6} cm^2/s, the experimentally determined value for Pb^{2+} diffusion through the control gel. D_b was taken as zero. $[Pb^{2+}]_b$ cannot diffuse as it is bound to the immobilized ligand. Solving eq 15 gave $D_{sg} = 7.14 \times 10^{-7}$ cm^2/s for $[Pb^{2+}]_T = 3.50 \times 10^{-4}$ M and 7.36×10^{-7} cm^2/s for $[Pb^{2+}]_T = 9.10 \times 10^{-4}$ M. These values are well within the range of experimentally determined diffusion coefficients for the sensor gel. We can get some idea of the range of ligand concentration heterogeneity from the range of diffusion coefficients. Using the relevant equilibrium expression, eq 17 becomes

$$D_{sg} = D_{cg}\frac{1}{1 + K_c[L]} \rightarrow \left(D_{cg}\frac{1}{1 + K_c[L]_T}\right)_{Pb^{2+}\rightarrow 0} \tag{16}$$

The term $K_c[L]$, representing bound/free ratio of Pb^{2+}, is ~10 in the experiments just described, which yield $D_{sg} \sim 7 \times 10^{-7}$. Data in Table 2 show values ranging from $\sim 6 \times 10^{-7}$ to 13×10^{-7} cm^2/s corresponding to a factor of ~2 range in $[L]_T$.

Equation 16 can be used to find D_{sg} at infinite dilution, which is calculated to be 7.01×10^{-7} cm^2/s. This value can be used to evaluate the approximate time for Pb^{2+} to diffuse through the sensor gel. If we accept 1 min as a "time to 90%" response, then we can calculate the thickness of the gel required. According to Crank,[27] ~90% of the equilibrium is attained when the whole thickness, l, is $(4\ Dt)^{1/2}$. A 1-min response corresponds to a 130-μm-thick gel.

To determine whether the rate of the chemical reaction will limit the material response time, rate constants for Pb^{2+} complexation and dissociation with AAB18C6 in aqueous solution are used as an approximation. In solution, k_d is 8.34×10^2 s^{-1}, and k_f is 8.79×10^7 M^{-1}s^{-1} at 20 °C (see above). The chemical relaxation

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time, $1/(k_d + k_d K_c[L_T])$, is 1.23×10^{-4} s. The gel thickness at which the diffusional relaxation time equals the chemical relaxation time is only ~200 nm. The PCCA hydrogels need to be a few micrometers thick to diffract light. Therefore, chemical kinetics will never limit the response of a device based on this system.

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SUPPORTING INFORMATION AVAILABLE

An alternative method for mercury film electrode preparation, determination of AAB18C6/Pb(II) binding constant in solution by UV−visible and NMR spectroscopy. This material is available free of charge via the Internet at http://pubs.acs.org.

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