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SERS Surfaces Modified with a 4-(2-Pyridylazo)resorcinol Disulfide Derivative: Detection of Copper, Lead, and Cadmium

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We have developed a surface-enhanced Raman spectroscopic technique for the determination of Pb^{2+} , Cd^{2+} , and Cu^{2+} concentration using a 4-(2-pyridylazo)resorcinol (PAR) coating modified with a disulfide. The disulfide provided a strong anchor to a roughened silver substrate. Atomic specificity was demonstrated by the distinct spectral changes that occurred through the interaction of Pb^{2+} , Cd^{2+} , and Cu^{2+} ions with the indicator. The absorption of these metals by the coating was followed with SERS and could be fit to a Frumkin isotherm. Langmuirian behavior was not observed; this is most likely due to the electrostatic repulsions as the metal cations are absorbed. The detection limits at pH 6 for Pb^{2+} , Cd^{2+} , and Cu^{2+} were 522, 50.3, and 1.49 ppb, respectively. Flow experiments using an optical fiber probe indicated instantaneous response to changes in metal concentration.

We have recently demonstrated the ability to detect ionic species¹⁻⁴ and organic compounds in aqueous solutions using chemically modified silver surfaces.^{5,6} The approach we have developed combines surface-enhanced Raman spectroscopy (SERS), analyte-specific affinity coatings, and fiber-optic sensors. The coatings vary from simple alkanes to complex macrocycles with the common theme of containing a thiol or disulfide group to anchor the coating to a silver substrate. Long-chain alkyl disulfides and thiols have been shown to form self-assembled monolayers (SAM) at noble metal surfaces.^{7,8} These surfaces mimic reversed-phase HPLC coatings and provide excellent surfaces for the detection of hydrophobic organic analytes. The same types of intermolecular interactions responsible for self-assembly may not be present in some of the complex metal ion chelating and macrocyclic coatings that we have synthesized for metal ion detection. However, it has been found that these

complex coatings with thiol or disulfide groups are strongly anchored and do not desorb under strongly acidic or basic conditions.

High sensitivity with SERS coatings can be achieved through three sources of signal enhancement. First is the inherent million-fold enhancement available through surface-enhanced Raman spectroscopy. Second, if the coating has accessible electronic absorptions, it is possible to tune the laser to achieve additional resonance Raman enhancements. Last, in solution systems the large partition coefficients allow us to create a locally high concentration of analyte at the metal surface.

In previous metal ion studies we examined Eriochrome Black T (EBT)¹ and a thiol-modified dibenzo-18-crown-6.⁴ The EBT was not chemically bound to the substrate and, therefore, represented an early feasibility study of the potential for SERS in detecting species with no inherent vibrational spectrum (single atoms). SERS of EBT metal complexes exhibited better selectivity between chemically similar metal ions than electronic absorption spectroscopy due to the narrow bandwidth of the SERS spectral features. The bands that showed the strongest Raman signal were features associated with the azo bridge of EBT. These bands shifted in frequency and intensity according to the size and charge of the metal ion. Trace sensitivity was demonstrated with EBT due to the strong resonance enhancement that occurs with green Ar⁺ laser excitation.

More recently, a study with a thiol-modified dibenzo-18-crown-6 illustrated the possibility of enhanced chemical selectivity with SERS coating. Crown ethers are well-known for their atomic radius dependent chemical selectivity.⁹ Diaminodibenzo-18-crown-6 was coupled with mercaptopyridinic acid to form an attachable SERS indicator. It was demonstrated that chemical selectivity could be built into the sensor with a crown ether. However, the sensitivity tended to be lower than EBT due to the lack of resonance enhancement. In this report, we will show results of a surface-enhanced resonance Raman spectroscopic (SERRS) sensor with a disulfide-modified 4-(2-pyridylazo)resorcinol (PAR).

This report discusses the attributes of PAR disulfide (PARDS) as a bound metal ion indicator. The absorption of metal ions was followed by correlating the intensity ratio of a Raman band sensitive to metal coordination and its counterpart in the uncomplexed indicator. This approach provides an internal standard and corrects for changes in laser power or the substrate.

The coordination of PAR with metal ions is very pH dependent. We obtained absorption isotherms at pH 3–6. The isotherms

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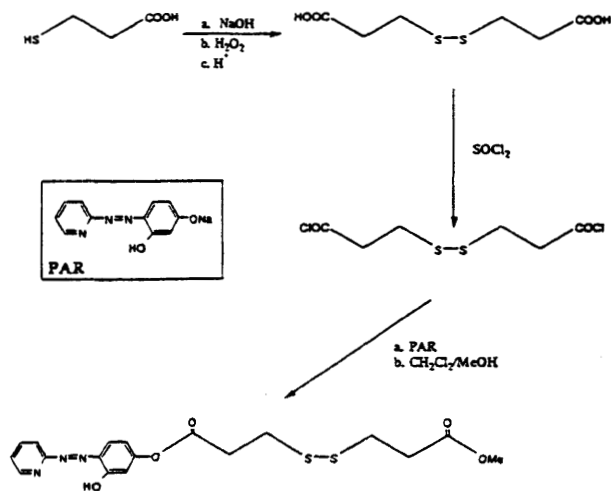


Figure 1. Synthetic route for the addition of a disulfide functionality to 4-(2-pyridylazo)resorcinol.

were fit to a Frumkin isotherm to account for coverage-dependent electrostatic repulsion.¹⁰ In order to combine our results with accepted analytical formulations, we interpreted the data in the form of conditional formation constants. The conditional formation constants allow one to calculate partition coefficients and sensitivities at any pH. The dynamic response of the SERS indicator was tested using a fiber-optic flow system. It was found that the system responded in less than 60 s or in this case the integration time required for the spectroscopic measurement. The use of an optical fiber configuration also demonstrated the potential of this method for in situ monitoring of groundwater metal ion contamination.

The use of an emission spectroscopy for the determination of metal ions is not new. Fluorescence-based metal ion fiber-optic sensors have been investigated by Wangbai,¹¹ Carroll,¹² Bright,¹³ and Lieberman.¹⁴ Detection limits in the micromolar to parts per trillion range were reported. However, while the use of fluorescence provides excellent sensitivity, it may suffer from inadequate selectivity between metal ions. For example, Carroll et al. showed that lifetime discrimination could be used to gain further selectivity.¹² We will demonstrate that the SERRS spectrum of a bound indicator can distinguish between metal ions due to the sharp Raman features of the indicator.

EXPERIMENTAL SECTION

Synthesis. PAR modified with a disulfide functionality (PARDS) was prepared as illustrated in Figure 1. 3,3'-Dithiodipropionic acid was made by oxidizing the thiol group of 3-thiopropionic acid to a disulfide. The oxidation was carried out in an aqueous solution of the thiol made basic with NaOH and treated with H₂O₂. Upon acidification, a white precipitate was filtered, washed with cold water, and dried under vacuum at 50 °C. 3,3'-Dithiodipropionyl

chloride was produced by the reaction of 3,3'-dithiodipropionic acid and thionyl chloride.

PARDS was obtained by combining PAR and 3,3'-dithiodipropionyl chloride in a 1:1 mixture of dichloromethane and methanol. The major product of this reaction was an asymmetric disulfide. It is assumed that an asymmetric disulfide is formed due to steric reasons. The product was isolated using flash chromatography with a mixture of acetonitrile and methanol. The structure was verified by proton and carbon-13 NMR.

Analytes. We chose three metals to test the PARDS for sensitivity and selectivity. These metals were chosen because of their environmental interest and their lack of complicated speciation and hydrolysis in aqueous solutions. Reagent grade Pb(NO₃)₂, Cu(NO₃)₂·2.5H₂O, and Cd(NO₃)₂·H₂O were purchased from Aldrich and used without further purification. Measurements of pH were made with an Orion 520A pH meter. The pH adjustments were made with acetic acid and NaOH. Buffers were not used to avoid complexation of metal ions with buffer components (for example, PO₄³⁻ species). All solutions were made using water obtained from a Millipore system.

Substrate Preparation. Two types of SERS substrates were used in the experiments discussed in this paper. Repetitive testing to determine optimal conditions for the indicators was conducted using silver foil substrates.⁵ Once these conditions were found, experiments were performed with the optical fiber substrates. The foil substrates were prepared by etching 0.1 mm thick silver foil (99.9%, Aldrich) in a stirred solution of 30% nitric acid for 60–90 s. These substrates are known to produce large SERS enhancements.⁵ After rinsing thoroughly with water, the foil was soaked in a 10⁻⁵ M solution of the PARDS in methanol (spectral grade, Baker) for 3–4 h. A methanol wash was necessary to remove any physisorbed PARDS.

The optical fibers were prepared as previously described with soft-clad 600 μm silica core fibers purchased from General Fiber Optics.¹⁵ We found that the UV-enhanced polysiloxane-hexafluorine copolymer cladding and nylon buffer interfered with the detection of metal ions. Our interpretation of this interference is that a small amount of unreacted monomer in the cladding is slightly soluble in methanol and forms an impermeable film over the indicator. The problem was alleviated by removing the cladding from the first centimeter of the fiber by soaking in concentrated sulfuric acid for 15 min. The fiber tip was roughened with 30 μm lapping paper and cleaned in concentrated ammonium hydroxide. The roughened tip was coated with 40 nm of silver (99.9%, Aldrich) by vapor deposition. The probe was fabricated by coating the silvered fiber with PARDS using the same procedure as for the silver foil.

Raman Instrumentation. An Omnichrome Model 532 air-cooled Ar⁺ ion laser provided 514.5 nm excitation for PARDS. Laser power at the sample was maintained at 5 mW throughout these experiments. The Photometrics CCD9000 detector and HR-320 (ISA) spectrograph with 1200 groove/mm grating Raman detection system used has been previously described.⁵ The fiber-optic SERS cell is the same as described in ref 2. Fiber positioning of the transmission fiber before the spectrograph was maintained using a fiber chuck (Newport) placed in a Melles Griot XYZ translator. The laser beam was focused through a glass window on the flow cell onto the SERS fiber using a 50 mm achromat from Melles Griot. The output cone of Raman light was partially

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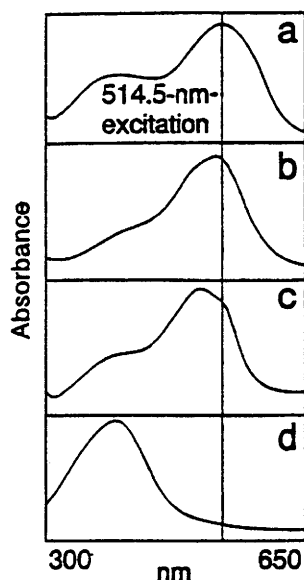


Figure 2. UV-visible absorption spectrum of 2×10^{-4} M PAR complexed with an excess of (a) Pb^{2+} , (b) Cu^{2+} , and (c) Cd^{2+} at pH 6.0; (d) 2×10^{-4} M PAR in water at pH 6.0. The vertical line indicates the laser excitation wavelength.

collimated with an aspheric condenser lens. A plano-convex collection lens was empirically adjusted to focus as much of the Raman scattered radiation through a $10 \mu\text{m}$ slit to just fill the $f/5.4$ mirrors of the HR-320 spectrograph. The fibers were $f/2.2$ and therefore were not $f/\#$ matched to the spectrograph. The $10 \mu\text{m}$ slit width corresponds to 9 cm^{-1} resolution when 514.5 nm excitation is used. An integration time of 60 s was used throughout this study.

RESULTS AND DISCUSSION

Selectivity. The structural sensitivity of Raman spectra allows much greater selectivity than optical techniques founded on electronic absorptions. The electronic absorption spectra of PAR complexed with Pb^{2+} , Cu^{2+} , and Cd^{2+} are shown in Figure 2. It can be seen that the absorption spectra for the different metals are virtually identical. This figure also shows that Raman spectra of PAR-metal complexes will be resonantly enhanced with 514.5 nm excitation.

This use of an absorbing species can lead to short sensor life due to photochemical degradation of the indicator. For example, azo compounds such as PAR are often photosensitive. However, rapid energy transfer occurs at metal surfaces and competes effectively with photochemical degradation.¹⁶ The foil substrates did not show any photochemical degradation. Signals from the optical fibers degraded slightly over 2–3 h of continuous exposure to laser light. This may be a thermal effect since a much tighter focus is used with the optical fiber. Our quantitative studies were carried out with the laser blocked between scans to avoid irreversible laser damage.

Figures 3 and 4 show the spectra observed when the bound PARDS is exposed to a concentrated metal solution at pH 6.0. Figure 3 contains a spectrum of the PARDS- Pb^{2+} complex, a blank of water at the same pH, and the subtraction of the blank from the metal spectra to highlight the changes that occur. The most apparent features for Pb^{2+} (Figure 3) are the shift in

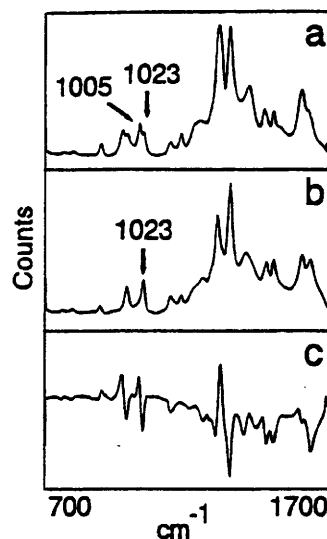


Figure 3. SERRS spectra of (a) PARDS in a 0.01 M Pb^{2+} solution at pH 5.0, (b) PARDS in a blank solution at pH 5.0, and (c) the subtraction (a) - (b).

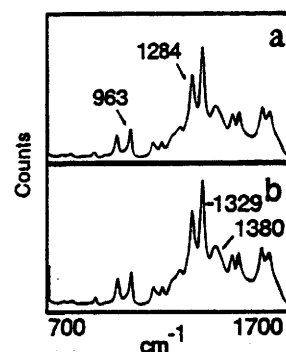


Figure 4. SERRS spectra of (a) the PARDS- Cd^{2+} complex spectrum and (b) the PARDS- Cu^{2+} complex. The bands used in quantitative analysis have been labeled.

frequency of the 969 and 1023 cm^{-1} bands to 951 and 1005 cm^{-1} , respectively. The 1023 cm^{-1} band corresponds to the pyridyl portion of PAR. This would indicate that Pb, the largest of the metals tested, interacts most strongly with the pyridyl nitrogen lone pair. The PARDS in the Cd^{2+} solutions (Figure 4a) shows a slight shift, and there are large intensity changes in the 1284 cm^{-1} band. The most notable difference in the Cu^{2+} solutions (Figure 4b) is the shift of the 1380 cm^{-1} band to 1390 cm^{-1} . Both copper and cadmium affect the azo portion of PAR more strongly than lead. Crystal structures for PAR with these metals have not yet been performed. At this point, we can only assume that the specificity of PAR for metal ions is due to the size of the metal ion and its hard-soft characteristics.

The PARDS spectrum has been assigned as shown in Table 1. The assignments for PARDS were based on a comparison of previous assignments for the unmodified PAR and spectra of PARDS taken in acidic, neutral, and basic solutions and in D_2O .¹⁷ Both the PAR and PARDS show evidence of an azo-hydrazone tautomerism. This tautomerism results in assignments for both $\nu(\text{C}-\text{N}-\text{N}=\text{C})$ and $\nu(\text{N}=\text{N})$.

Adsorption Behavior. Adsorption isotherms were obtained for Pb^{2+} at pH 3–6. The intensity ratio of the 1023 cm^{-1} to the

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Table 1. Assignments for the SERS Spectrum of PARDS

pH 3	pH 7	pH 13	D ₂ O	assignment
			944(sh)	
963	963	963	960	$\nu(\text{C}-\text{C})_{\text{res}} + \delta(\text{N}-\text{H})$
			1002(sh)	
1025	1023	1023	1023	$\nu(\text{C}-\text{C})_{\text{py}}$
1115	1115	1115	1113	$\nu(\text{C}-\text{N}-\text{N}=\text{C}) + \nu(\text{C}-\text{OH})$
1155	1155	1155	1155	$\nu(\text{C}-\text{C})_{\text{res}}$
1260	1284	1284	1286	$\nu(\text{C}-\text{N}-\text{N}=\text{C}) + \delta(\text{N}-\text{H})$
1331	1329	1329	1327	$\nu(\text{C}-\text{C})_{\text{res}}$
1361	1380	1387		$\nu(\text{C}-\text{C})_{\text{res}} + \nu(\text{C}-\text{N}-\text{N}=\text{C})$
		1389		$\nu(\text{N}=\text{N})$
1451	1456	1456	1452	$\nu(\text{C}-\text{C})_{\text{py}}$
1478	1478	1478	1478	$\nu(\text{C}-\text{C})_{\text{py}}$
1575	1579	1579	1577	$\nu(\text{C}-\text{C})_{\text{res py}}$
1605	1605	1607	1605	$\nu(\text{C}=\text{O}) + \nu(\text{C}=\text{N})$

1005 cm⁻¹ Raman band was plotted versus concentration. The ratio at the largest point on the curve was taken as $\theta = 1$, where θ is the fractional coverage. The adsorption was fit to a Frumkin isotherm,

$$\theta = \frac{K_f' e^{2g\theta} C}{1 + K_f' e^{2g\theta} C} \quad (1)$$

where K_f' is the conditional formation constant, C is the solution concentration of the adsorbate, and g is the Frumkin parameter. The Frumkin equation allows for interaction between the adsorbed species. We found a negative value for the Frumkin parameter. The sign of g indicates the presence of a repulsive or attractive force upon adsorption.¹⁰ The negative value observed indicates that a repulsive force is present. This can be interpreted as an initially large electrostatic attraction to the surface from the negatively charged deprotonated PARDS and a decreasing attraction due to neutralization of the surface charge by the positive metal ions.

An example adsorption isotherms for Cd²⁺ is shown in Figure 5. For Cd²⁺ we used the ratio of the 1329 to the 1284 cm⁻¹ Raman band to form the isotherm. The Cu²⁺ isotherm was formed with the 1380 to 1329 cm⁻¹ Raman peak ratios. The conditional formation constants found for the Pb²⁺, Cd²⁺, and Cu²⁺ are listed in Table 2. The conditional formation constant describes the formation of PARDS-metal complexes at a particular pH. The conditional formation constants increase with pH due to a higher population of PARDS⁻ on the surface. The adsorption parameter, g , is also given in Table 2. The value of g becomes more negative with pH. This can be rationalized as the surface going from a negative to positive charge state more rapidly with coverage.

The formation constant (K_f) can be determined from the conditional formation constants by

$$K_f' = \alpha_{\text{PARDS}^-} K_f \quad (2)$$

where α_{PARDS^-} is the fraction of the PARDS in the deprotonated form (PARDS⁻).

This fraction of PARDS⁻ was calculated by use of a speciation program developed at the University of Stellenbosch by Dr. J. J. Cruywagen. The program requires the pK's of the species of interest in order to calculate the fraction metal complex species

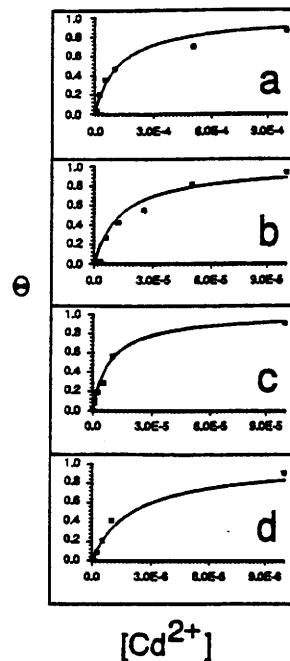


Figure 5. Example of the isotherms obtained for PARDS-metal ions at various pHs. These are the isotherms for Cd²⁺ adsorption at pH (a) 3.0, (b) 4.0, (c) 5.0, and (d) 6.0.

Table 2. Conditional Formation Constants

pH	K_f'	g
For Pb ²⁺ -PARDS Complexes		
3.0	$1.2 \times 10^2 \pm 3.1 \times 10^1$	-0.33 ± 0.12
4.0	$2.2 \times 10^2 \pm 8.1 \times 10^1$	-0.43 ± 0.14
5.0	$4.0 \times 10^3 \pm 3.2 \times 10^2$	-0.61 ± 0.20
6.0	$1.3 \times 10^4 \pm 6.8 \times 10^3$	-0.73 ± 0.27
For Cd ²⁺ -PARDS Complexes		
3.0	$8.5 \times 10^3 \pm 1.0 \times 10^3$	-0.37 ± 0.13
4.0	$7.5 \times 10^4 \pm 4.6 \times 10^3$	-0.43 ± 0.11
5.0	$1.2 \times 10^5 \pm 1.8 \times 10^4$	-0.52 ± 0.18
6.0	$5.0 \times 10^5 \pm 5.0 \times 10^4$	-0.54 ± 0.19
For Cu ²⁺ -PARDS Complexes		
3.0	$2.0 \times 10^5 \pm 1.0 \times 10^4$	-0.27 ± 0.08
4.0	$4.2 \times 10^5 \pm 9.7 \times 10^4$	-0.35 ± 0.13
5.0	$4.0 \times 10^5 \pm 2.0 \times 10^5$	-0.40 ± 0.18
6.0	$5.5 \times 10^6 \pm 7.8 \times 10^5$	-0.67 ± 0.28

present at different pH's. The acid dissociation constants for the unmodified indicator, PAR, are $pK_{\text{NH}} = 3.1$, $pK_{\text{para OH}} = 5.5$, and $pK_{\text{ortho OH}} = 11.9$.¹⁸ In order to show that the disulfide-modified PAR was not significantly different from PAR we found the pK's in solution and on the surface. UV-visible spectra of PARDS were taken from pH 3.0 to 10.0. The spectra were analyzed by use of the SPECFIT program developed by Binstead and Zuberbühler.¹⁹ The SPECFIT program was developed to extract equilibrium data from spectroscopic measurements. The program uses the whole spectrum, UV-visible or Raman, and uses evolving factor analysis to determine concentration profiles.²⁰⁻²³ Equilibrium constants are obtained from the concentration profiles. A $pK_{\text{NH}} = 3.1 \pm$

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0.130 and $pK_{\text{ortho OH}} = 7.13 \pm 0.236$ were determined for an aqueous solution. The shift in the $pK_{\text{ortho OH}}$ to a much lower pK is interpreted as a reduction in the Coulombic attraction between the dianion and the proton in PAR to that of an anion and a proton in PARDS. An additional determination of the pK 's of PARDS was made on the surface using SERRS. The surface pK 's were found by titrating the modified PARDS on a silver substrate. The SERRS spectra change with pH thus allowing the pK 's to be found. The SERRS spectra were analyzed by using the SPECFIT program, and the surface $pK_{\text{NH}} = 3.95 \pm 0.892$ and $pK_{\text{ortho OH}} = 7.55 \pm 0.341$ were found.

The formation constants for Pb^{2+} , Cd^{2+} , and Cu^{2+} complexed with PARDS were found to be 2.8×10^6 , 2.3×10^8 , and $4.3 \times 10^9 \text{ M}^{-1}$, respectively. The formation constants increase in the order $\text{Pb}^{2+} < \text{Cd}^{2+} < \text{Cu}^{2+}$. The surface formation constants would be expected to follow the same order as in solution. The solution formation constants for 1:1 complexes of Pb^{2+} and Cu^{2+} with PAR found in Martell and Smith are 3.9×10^8 and $6.3 \times 10^{14} \text{ M}^{-1}$.¹⁸ A literature value for the formation constant of $\text{PAR}-\text{Cd}^{2+}$ could not be found. Exact correspondence between the solution and the surface would not be expected due to the modification at the para-OH of the resorcinol and possible steric effects due to compact surface coverages.

The detection limit for these metals was determined from the linear region of the adsorption isotherms using

$$DL = \frac{3\sigma}{m} \quad (3)$$

where DL is the detection limit, σ is the standard error of the y estimate, and m is the slope. The standard error of the y estimate was found from

$$\sigma_y = \left[\frac{\sum_{i=1}^N (mX_i - Y_i)^2}{N-2} \right]^{1/2} \quad (4)$$

where N is the number of observations and $N-2$ is the degrees of freedom. The detection limits at pH 6 for Pb^{2+} , Cd^{2+} , and Cu^{2+} at pH 6.0 were 522, 50.3, and 1.49 ppb, respectively. Table 2 shows that the K_f is increasing rapidly with pH. This means that much lower detection limits are possible at pH 7 and above. At this time, our experiments were not carried out at higher pH due to precipitation of the metal hydroxides.

Fiber System. An important application of the work discussed here is the potential in situ monitoring of contaminants or chemical processes using fiber optics. As a simple test we demonstrate the response of the PARDS SERRS system to Pb^{2+} using a silver-coated optical fiber under flowing conditions. The dynamic response of PARDS with Pb^{2+} was tested using a fiber-optic probe. Fibers were first calibrated by use of standard dilutions of Pb^{2+} at pH 5.0. Next, the system response was monitored as a 10^{-3} M

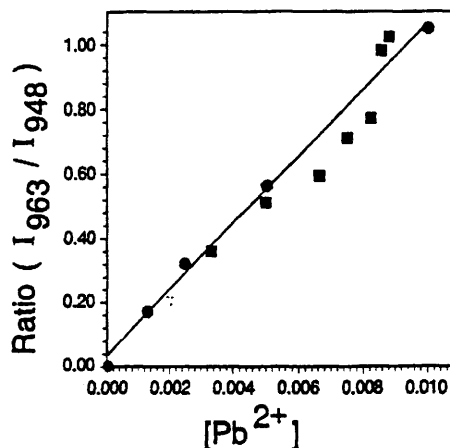


Figure 6. Flow experiment using optical fibers. The circles represent calibration with standard solutions, and the squares are results from the dynamic flow experiment.

Pb^{2+} solution was slowly added to Millipore water at pH 5.0. The water was circulated by the probe tip in a Kel-F flow cell while 10^{-3} M Pb^{2+} solution was added at a rate of 5 mL/min. The concentration of Pb^{2+} in the reservoir at a given time was calculated from the amount of Pb^{2+} added. Figure 6 illustrates the results of the calibration (circles) and the dynamic flow experiment (squares). This experiment demonstrated that the response of the optical fiber system was not limited by chemical on-off rates within the 60 s integrations used in this experiment.

CONCLUSIONS

This work represents an advance in the use of SERRS to detect metal ions. In our previous work with Eriochrome Black T, we extracted the metal ion complex and spun coat it onto a silver surface. Detection limits for Pb^{2+} and Cu^{2+} were 50 and 20 ppb for 100 s integration times, respectively.¹ In this work, we have shown that an indicator can be chemically bound to a surface through a thiolate linkage. The detection limit for Pb^{2+} was higher than EBT (522 ppb in 60 s) and lower for Cu^{2+} (1.49 ppb in 60 s). Future work will be directed at the use of chemometric techniques to utilize the differences in the SERRS spectra to allow both quantitation and speciation of metal ion mixtures.

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