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Determination of Some Metal Ions Using Lichen-Modified Carbon Paste Electrodes

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ABSTRACT

Lichens have long been used as biomonitors of environmental pollution. We therefore investigated the application of lichen-modified carbon paste electrodes (CPEs) for the determination of lead(II) and copper(II) using differential pulse anodic stripping voltammetry. These electrochemical biosensors incorporate the biological selectivity of lichen species such as *Cladonia portentosa* and *Lobaria pulmonaria,* and the genus *Roccella,* with the sensitivity of electrochemical detection. As such, they may offer new reactivity patterns that could be exploited in the determination of trace metal ions in environmental samples and in speciation studies. The voltammetric responses were evaluated with respect to pH of accumulation (carried out under open circuit conditions), pH of electrolyte solution, metal ion concentration, percentage lichen loading in the carbon paste, interferences, and surface renewal.

INTRODUCTION

Lichens are plants formed by the symbiotic association of an alga and a fungus. Their general structure is that of an upper cortex, a protective central fungal medulla (of loosely packed hyphae), and a lower cortex. The algae may form a distinct layer beneath the upper cortex, or they can be dispersed throughout. Most lichens have an extracellular matrix which is a gelatinous secretion containing polysaccharides such as lichenan and isolichenan together with glucans, galactomannose, and lichen acids.

For years, lichens have been known to accumulate metal ions and have been used extensively as biomonitors of environmental pollution [1, 2]. The diversity of lichen species close to a suspected pollution source has been used to assess the levels of gaseous air pollutants, with fewer lichen species being found closer to the emission source [3, 4]. These plants are considered to be useful biomonitors of sulfur dioxide [5, 6], acid rain [7–9], radionuclides [10–12], chlorinated hydrocarbons [13–15], and ozone [16–18]. Both lichens and mosses have been used as monitors of uranium contamination by Boileau *et al.* [19] around two centers of uranium mining in Canada. Samples were analyzed by X-ray fluorescence spectroscopy for metals such as titanium, iron, nickel, lead, and uranium. Richardson and coworkers have also investigated the uptake of lead and uranium by lichens [20, 21].

A review by Richardson [22] on the pollution sensitivity of lichens discusses the effects of sulfur dioxide and acid rain on the lichens, along with the mechanism of airborne elemental accumulation by these plants. Since then, Beck and Ramelow [23] have used lichens enclosed in porous polyvinyl chloride (PVC) tubes as monitors of dissolved metals in natural waters. The PVC tubes were suspended at different sampling points along a river. After 2 weeks, the lichens were analyzed by atomic absorption spectroscopy for a wide variety of metals ions including Pb(II) and Cu(II).

Recently, Wang *et al.* [24] reported on experiments at algae-modified electrodes to investigate the incorporation of anionic and cationic metal complexes by algae. Gardea-Torresday *et al.* [25, 26] then reported on voltammetric measurements at algae-modified electrodes capable of preconcentrating Cu(II) and Au(I). These studies suggested that the development of lichen-modified electrodes might open up a new area of sensor development based on the nonelectrochemical bioaccumulation of metal ions.

This article describes the development of lichen-modified carbon paste electrodes (CPEs) for the detection of lead(II) and copper(II) using differential pulse anodic stripping voltammetry (DPASV). These electrochemical biosensors incorporate the biological selectivity of lichen species such as *Cladonia portentosa* and *Lobaria pulmonaria,* and the genus *Roccella,* with the sensitivity of electrochemical detection.

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EXPERIMENTAL

Working electrodes were prepared by packing glass tubing (3 mm i.d.) with plain carbon paste (mineral oil and graphite powder, 40:60), with a copper wire providing electrical contact. The modified paste (containing lichen) was packed as a thin layer at the sensing end of the electrode and smoothed on a deck of non-oil-absorbing material, such as weighing paper.

Modified pastes were prepared from the three lichens investigated (i.e., *Cladonia portentosa, Lobaria pulmonaria, Roccella*) as follows. Collected lichen samples were cleaned of any nonlichen material and crushed to a fine powder with a mortar and pestle. Appropriate quantities of lichen were mixed with mineral oil, then graphite powder (e.g., 0.2 g of lichen to 0.4 g of mineral oil and 0.4 g of graphite powder) and thoroughly mixed for 15 minutes to ensure equal distribution of the lichen within the carbon paste. Modified pastes were generally made in 1 g batches and stored at room temperature.

Metal ion solutions were prepared from Spectrosol AA standard solutions and stored in polyethylene bottles. Buffers were prepared from potassium dihydrogen phosphate, dipotassium hydrogen phosphate, and sodium acetate (pH adjusted with *o*-phosphoric and acetic acids, respectively). The reference (Ag/AgCl), auxiliary (Pt), and working electrodes were placed in the electrochemical cell through holes in a plastic cover with a fourth inlet for nitrogen, required for purging of the electrolyte. Accumulation was carried out in a separate cell with a magnetic stirrer and stirring bar (7 mm long) providing convective transport.

The working electrode was immersed in the accumulation cell containing a stirred metal ion solution for a preselected length of time, after which it was removed, rinsed with deionized water, and placed in the measurement cell containing the supporting electrolyte.

Accumulation was carried out under open circuit conditions (no applied potential) for a preselected period (accumulation time). The accumulation cell contained the stirred metal ion solution of interest, while the measurement cell contained the electrolyte.

Differential pulse voltammograms were recorded with an EG & G Model 264A Polarographic Analyzer/Stripping Voltammeter and a Houston Instruments Omnigraphic 2000 XY recorder. Deionized distilled water was used throughout to prepare all solutions.

Scans were run after an equilibration time of 15 seconds from a starting potential of −0.80 V for Pb(II) or −0.30 V for Cu(II). The electrode surface was easily renewed by removal of the old surface using a spatula and repacking as before.

RESULTS AND DISCUSSION

Effect of Accumulation Time

The effect of varying the accumulation time for the uptake of Pb(II) at the *Roccella*-modified (20% w/w) working electrode is shown in Figure 1. From this it can be seen that there is an initial rapid increase in the response

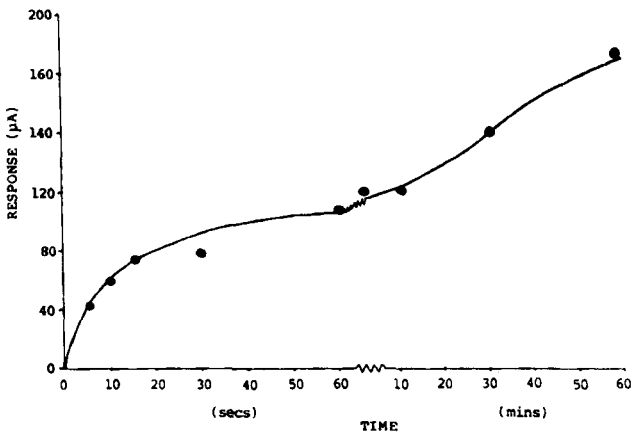


FIGURE 1. Effect of varying the accumulation time for a 1 mM Pb (II) solution (pH_{acc} 6.3, pH_{elec} 4.0 in phosphate buffer, 0.02 M) at a *Roccella*-modified (20% w/w) carbon paste electrode. Pulse amplitude, 100 mV; scan rate, 5 mV/s; scan, −0.80 to −0.20 V.

followed by a plateau and a further increase. The initial uptake could represent either specific or fungal binding, and the later rise in the curve could represent the slower, nonspecific, or perhaps algal, binding of the metal ion.

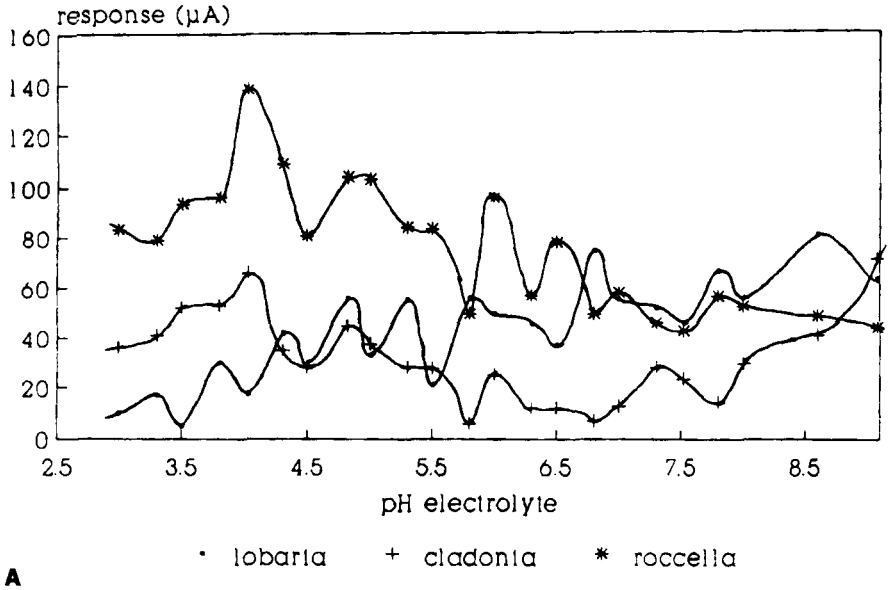
Algae have been shown to follow this pattern of initial rapid uptake and subsequent slow uptake [27]. A similar curve was observed for the uptake of Cu(II) at the *Lobaria*-modified (20% w/w) carbon paste electrode. A two-stage uptake mechanism has also been reported for the moss *Sphagnum* [28]. In the case of Pb(II) accumulation at the *Roccella*-modified electrode, uptake of Pb(II) reached 80% of its equilibrium value at 2 minutes, after which uptake proceeded more slowly.

Effect of pH

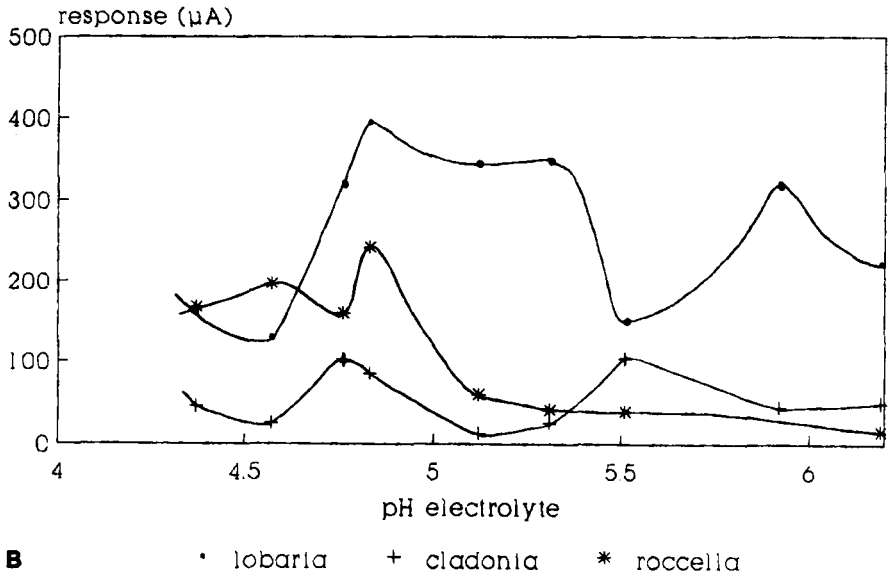
The effect of varying the electrolyte pH for the determination of Pb(II) and Cu(II) at each of the three lichen-modified electrodes is shown in Figure 2. The variation in response is most likely due to the wide range of ligands and binding sites present in each lichen. Each point represents the average of three measurements, where the average coefficient of variation was less than 6%.These patterns are therefore quite reproducible, and the fluctuations are not believed to be due to experimental error.

The functional groups most likely to be involved in binding are thought to contain oxygen and nitrogen donor atoms, such as those listed below, with pK_a values given in parentheses: —PO_3H (2–3), —COOH (4–6), aromatic amines (5–6), phenolic and enolic groups (6–9), —PO_3^{2-} (7–8), and aliphatic amines (7–9) [3].

The effect of varying the pH of the accumulation solution for both Pb(II) and Cu(II) is shown in Figure 3. These patterns were again found to be reproducible and are also thought to reflect the effects of different binding sites on the lichen surface. If one considers Cu(II) binding



A



B

FIGURE 2. Effect of varying the pH of electrolyte for the detection of (**A**) Pb(II) and (**B**) Cu(II) at each lichen-modified carbon paste electrode following accumulation of a 1 mM aqueous solution of Pb(II). Detection conditions for (**A**): electrolyte, 0.02 M phosphate buffer; scan rate, 5 mV/s; pulse amplitude, 100 mV. Detection conditions for (**B**): electrolyte, 0.02 M acetate buffer; scan rate, 5 mV/s; pulse amplitude, 100 mV.

at the *Lobaria*-modified surface, for the determination of Cu(II) concentration, the best response was obtained in an electrolyte of pH 4.8 (Figure 2B). The optimum pH for Cu(II) accumulation at the *Lobaria*-modified electrode was found, however, to be at pH 6.0 (Figure 3B). If we consider these two points (i.e., pH_{acc} 4.8 and pH_{elec} 6.0), it would appear that the binding sites are most likely carboxylate in nature (pK_a in the range 4–6). This is in agreement with the conclusions of Puckett *et al.* [29].

Looking at the responses obtained for Pb(II), it can be seen that the best accumulation occurs in the region 5–7, with a maximum at pH 6.3 for the *Roccella*-modified electrode (Figure 3A). Phenolic and enolic groups are likely candidates for Pb(II) binding, considering the accumulation pH of 6.3. Concurrent peaks in these selectivity patterns may represent similar binding sites in different lichens.

Looking at some of the constituents of these lichens, one finds gyrophoric acid, norstictic acid, and stictic acid in *Lobaria*; and isolichenan, lichenan, roccellic acid, and lecanoric acid in *Roccella*. Considering that carboxylate and hydroxycarboxylate groups are most likely responsible for metal ion binding, it is interesting to note that gyrophoric, roccellic, and lecanoric acids, in particular, are carboxylate by nature.

Finally, comparison of all three lichens for Pb(II) and Cu(II) uptake shows that Pb(II) is preferentially bound by *Roccella* at pH_{elec} 4.0 and pH_{acc} 6.3, whereas *Lobaria* has the greatest affinity for the uptake of Cu(II) at pH_{elec} 4.8 and pH_{acc} 6.0.

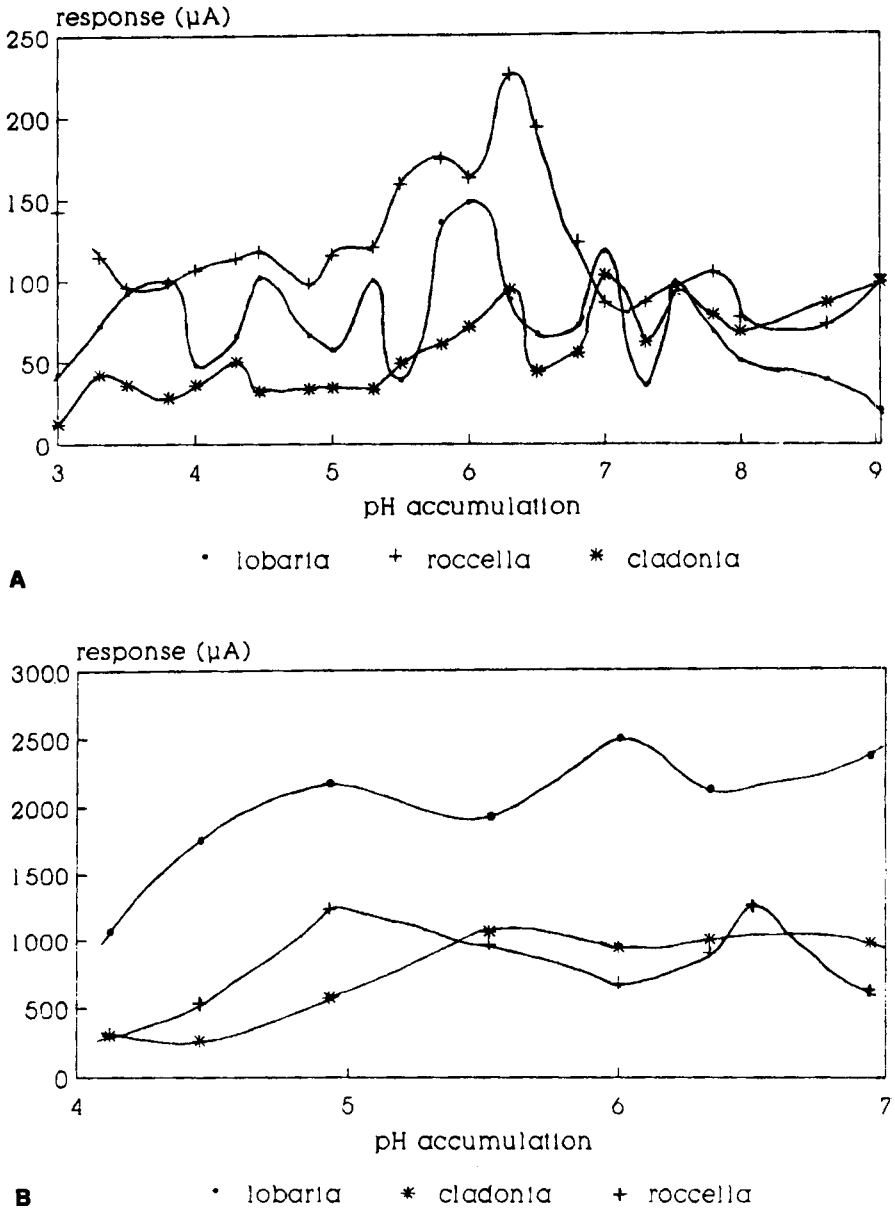


FIGURE 3. Effect of varying the pH of accumulation for the detection of (**A**) Pb(II) and (**B**) Cu(II) at each lichen-modified (20% w/w) carbon paste electrode. Detection conditions for (**A**) as in Figure 2A with the following exceptions: accumulation solution, 1 mM Pb(II) in 0.02 M phosphate buffer; electrolyte, 0.02 M phosphate buffer pH 4.8 (*Cladonia portentosa*), pH 6.8 (*Lobaria pulmonaria*); and pH 4.0 (*Roccella*). Detection conditions for (**B**) as in Figure 2B with the following exceptions: accumulation solution, 1 mM Cu(II) in 0.02 *M acetate* buffer; electrolyte, 0.02 M acetate buffer pH 5.51 (*Cladonia portentosa*), pH 4.76 (*Lobaria pulmonaria*), and pH 4.76 (*Roccella*).

The nature of the binding is not clear, but it is thought to occur via a process of ion exchange [30, 31]. Attempts have been made to identify which fractions of lichen cell walls were responsible for accumulation of metal ions [10]. The main metal binding sites proved to be associated with the protein component of the fungal cell wall. The most likely binding sites are therefore compounds containing carboxylate and hydroxycarboxylate groups, which form part of the protein component of the fungal cell wall [29].

It is possible at this stage only to propose possible ligands based on the comparison of accumulation and electrolyte pH measurements with pK_a values. If the process is one of ion exchange, the uptake of the metal ion may be represented as follows:

$$M^{n+} + H_nR \rightleftharpoons nH^+ + M(R)$$

where R represents the functional group responsible for binding.

On placing the working electrode in the accumulation solution, a slight increase in pH was noted, but generally by no more than 0.3 of a pH unit. For both Pb(II) and Cu(II) uptake (Figure 3), there appears to be a region (5–7) within which greatest accumulation occurs. This is the same as the natural pH of most lichens, reinforcing the view that these species could be used effectively as biomonitors of heavy metal pollution, since there is a direct dose–response effect. The voltammetric responses obtained for Pb(II) at the three lichen-modified electrodes are compared in Figure 4.

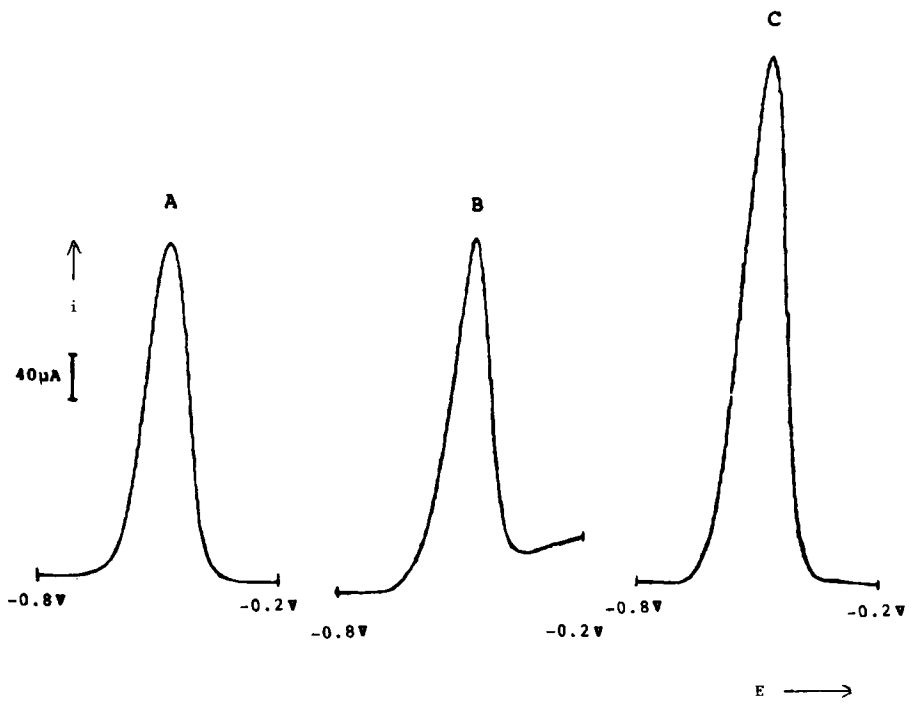


FIGURE 4. Comparison of the DPASV behavior of the three lichen-modified electrodes for the determination of 1 mM Pb(II). (**A**) *Cladonia portentosa:* pH_{elec} 4.8, pH_{acc} 7.0. (**B**) *Lobaria pulmonaria:* pH_{elec} 6.8, pH_{acc} 6.0. (**C**) *Roccella:* pH_{elec} 4.0, pH_{acc} 6.3.

Effect of Lichen Loading

A linear response was obtained for the accumulation of Pb(II) with increasing lichen loading (% w/w) in the carbon paste. As would be expected, the more lichen surface available for binding, the greater the binding capacity, hence the amount of metal accumulated.

Surface Renewal

The possibility of renewing the modified surfaces was investigated by washing the electrodes after use in HCl. For Pb(II) accumulation at the *Roccella*-modified electrode, a 5 minute wash in 0.25 M HCl resulted in almost total removal of complexed Pb(II). A series of 10 accumulations on the same surface yielded a % coefficient of variation of 14%. For Cu(II), a 5 minute wash in 0.25 M HCl resulted in almost total removal of bound Cu(II), and a further scan was found to strip off any remaining metal ion. Increasing the HCl concentration to 0.5 M resulted in total removal of surface-bound metal ion. This experiment confirms that the nature of the binding is via a process of ion exchange, where under very acidic conditions, the bound metal ion is replaced once again by a proton.

Effect of Interferences

The effects of interfering metal ions on both the Pb(II) and Cu(II) responses (using *Roccella*- and *Lobaria*-modified electrodes, respectively), under optimum conditions of accumulation and measurement, were investigated by measuring the percentage of change in the normal response for a 1 mM Pb(II) or Cu(II) solution on addition of 0.5 mM metal ion. The results are shown in Table 1.

It is interesting to note that the Pb(II) response is greatly enhanced in the presence of Hg(II), possibly as a result of the formation of a mercury layer on the surface of the electrode. This would effectively preconcentrate the Pb as an amalgam as in conventional DPASV. No such effect was observed for Cu(II) in the presence of Hg.

Most metals ions interfered with the determination of Cu(II), in particular Pb(II), which produced a 75% decrease in the Cu(II) peak. This is not surprising if the selectivity sequence for the binding of Pb(II) and Cu(II) to carboxylate groups is investigated [29]. Here we see that Pb(II) is preferentially bound over Cu(II) and may in fact displace any Cu(II) that is already bound.

In fact, the effects of different interfering metal ions could possibly be used to classify the nature of the func-

TABLE 1 The Effect of Interfering Metal Ions on Pb(II)and Cu(II) Responses

Interfering Metal Ion	*Change in Response (%)*	
	Pb(II)	*Cu*(II)
Cd(II)	0.0	+13.0
Al(III)	+4.6	−28.0
Fe(II)	0.0	−5.2
Hg(II)	+25.0	−3.9
Zn(II)	0.0	−27.0
Cu(II)	0.0	0.0
Pb(II)	0.0	−75.4

tional groups involved in the binding based on a selectivity sequence. Using the Hg layer may improve the sensitivity of the method.

An interesting point to note is the effect of Cu(II) on the Pb(II) response. While Cu(II) does not affect the height of the Pb(II) peak, there is a broadening of the peak. This most likely represents competition between the ions for binding sites resulting in slower reaction kinetics.

Calibration

Calibration curves were constructed for both Pb(II) and Cu(II) concentrations in the range 0–1 mM. The response for Pb(II) was linear over the range investigated (slope, 113.7 μA/mM; correlation coefficient, 0.9932). At lower concentrations [0–100 μM Pb(II)], linearity was less evident, with a correlation coefficient of 0.9734. A detection limit of 20 μM Pb(II) was calculated based on a signal-to-noise ratio of 3. For detection of Cu(II), there was a slight response at plain carbon paste. Taking this into account, calibrations were linear over the concentration range investigated (0–1 mM) with a slope of 139.8 μA/mM and correlation coefficient of 0.9357.

CONCLUSIONS

This article has demonstrated the possible use of bioselective lichen-modified electrodes for the determination of certain metal ions. Much work still remains to be carried out, in particular on improving the sensitivity of the method. However, it lends feasibility to the use of such devices for on-site analysis, since the surface is easily renewable and highly stable. Because an acid wash removes bound metal, a change of surface may not be necessary.

For the present study, three lichens containing different algae and distribution were chosen. *Cladonia portentosa* contains Trebouxia, the commonest lichen photobiont and is a temperate-zone-based forest species. *Lobaria pulmonaria* contains Myrmecia and has a Western Atlantic distribution, while the lichen *Roccella* contains Trentepohlia and has a typically Mediterranean distribution.

By using one or more lichens to formulate the lichen paste, it may be possible to detect a range of metal ions which are well resolved on a single voltammogram. To further simplify sample analysis and pretreatment, accumulation is carried out under open circuit conditions and is solely dependent on the bioaffinity of the lichen for a particular metal ion.

Extractions of various fractions of the lichens need to be carried out and compared to the response at the total lichen. It may also be possible to carry out speciation studies, since accumulation of the metal ions is highly dependent on pH, using careful selection of pH conditions and other parameters.

Work is continuing in our laboratory with the aim of understanding more clearly the mechanism of operation of such sensors, increasing the number of metal ions that can be determined using this approach, and finding ways to increase the sensitivity and selectivity of the method.

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