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Hybrid materials based on lichen–polysiloxane matrices: application as electrochemical sensors

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Chemically modified electrodes based on the incorporation of lichens in a rigid matrix obtained by sol–gel process show higher ruggedness and stability than conventional carbon paste electrodes. The lichen ion-exchange properties in the uptake of metal ions are combined with the improved physical characteristics of the synthesized materials. SEM, EIS and AS-LSV techniques have been applied to characterize the sol–gel/lichen materials and to evaluate their ability in the construction of electrochemical devices. The coating of solid graphite electrodes with the lichen–polysiloxane materials by means of a spin-coating process allows the development of robust electrochemical sensors. Such devices exhibit a long-time stability and a suitable electroanalytical performance in the amperometric determination of heavy metal ions in water samples. The impedancimetric response of the sensor towards sodium ions has been also demonstrated.

Introduction

Chemically modified electrodes (CMEs) have exhibited rapid development over the last few years, especially in the electroanalytical field in order to provide fast and direct methods of analysis. They result from the bulk- or surface-modification of a conductive material to produce an electrode suited to a particular function.[1] The synthesis of composite materials including modifiers for the chemical recognition of target analytes and its application to the preparation of electrochemical sensors allows the development of new CMEs with improved physical and chemical properties.

Lichens, a symbiotic association of fungus and alga, are able to accumulate heavy metals in their tissue and, therefore, they are commonly used as environmental pollution biomonitors.[2] As indicated by Dempsey *et al.*,[3] the retention of heavy metal ions seems to take place in the lichen functional groups containing oxygen and nitrogen donor atoms, most likely carboxylic groups that form part of the fungal cell wall, by means of an ion-exchange process.[4] Trace element determination is possible by combining the preconcentration step of metal ions in the active sites of the lichen tissue with their quantitative determination using different techniques, such as X-ray fluorescence spectrometry, emission spectrometry, atomic absorption spectrometry, neutron activation analysis or voltammetry.[5,6]

Lichens and other biomasses, for instance, mosses or algae, have been incorporated into carbon paste electrodes (CPEs) where they act as the sensing phase for the recognition of toxic cations.[3,7–10] Following the bioaccumulation process in biomass-modified CPEs, the determination of cations has been carried out by means of anodic stripping combined with voltammetric techniques such as cyclic, differential pulse or square wave voltammetry. In spite of the advantages found for CPEs,[11] for instance, their simplicity of preparation, the use of these sensors is limited because of their lack of ruggedness, instability in organic solvents or in hydrodynamic conditions and low reproducibility. For this reason, rigid networks with improved mechanical and chemical stability are promising materials for the preparation of electrochemical sensors. The most commonly employed sensors of this type are based on epoxy composites[12] as well as plasticized PVC membranes incorporating ionophores, such as macrocyclic compounds, for ionic recognition.[13–15]

Rigid silica matrices obtained by sol–gel technology have been also widely employed incorporating different sensing agents, mainly discrete molecules, for instance, crown-ethers.[16–19] In some cases, more complex systems, such as biological species like enzymes or cells, have been incorporated in these types of matrices.[20,21] However, the entrapment of larger elements, such as macromolecular aggregates and biological tissues, still remains difficult since their huge volume affects the polymerization processes, providing silica matrices with fractures that can not be used in the building of sensor devices. In this work, we have optimized the processing of this material thereby obtaining improved polysiloxane composites, which act as excellent matrices to include the lichen tissues. We have chosen a mixture of suitable precursor alkoxysilanes and have optimized their molar ratio in order to obtain a matrix that shows enough flexibility for the embedding of large size lichen particles. The lichen *Pseudocyphellaria hirsuta* (Cochrane, Chile) has been incorporated into the resulting polysiloxane matrix and this modified material has been used to prepare electrochemical sensors. A spin-coating process was employed to spread the lichen-modified polysiloxane network onto the surface of solid graphite electrodes. The incorporation of lichens into sol–gel matrices is here reported for the first time, as it is important to point out that the entrapment of lichens in the preparation of electrochemical sensors has only been previously reported for CPEs. Scanning electron microscopy (SEM) was carried out to characterize the lichen-modified material. Electrochemical impedance spectroscopy (EIS) and anodic stripping linear scan voltammetry (AS-LSV) were employed in order to evaluate the electrochemical behaviour of the sensor and its ability to act as an amperometric sensor for the determination of lead in aqueous solutions.

Experimental

Reagents

The lichen sample (*P. hirsuta* from Cochrane, Chile) was washed with purified water and, after drying, treated with liquid

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nitrogen, ground and sieved (mesh-size 53 μm, *ca.* 250 mesh). Graphite rods (6 mm diameter, 99.999%, low density) were purchased from Aldrich. Tetramethoxysilane (TMOS) 98%, 3-(trimethoxysilyl)propyl methacrylate (MAPTS) 99%, methanol 99.8%, and HCl 32% were purchased from Fluka and acetic acid 100% from Merck. Lead(II) nitrate and sodium chloride were provided by Riedel-de Haën. Silicon carbide discs were obtained from Electron Microscopy Sciences. Deionized water (resistance of 18.2 MΩ) was obtained with a Maxima Ultra Pure Water system from Elga.

Sensor preparation

Graphite rods were inserted in methacrylate tubes, sealed with epoxy resin and finally polished consecutively using 600 and 1200 grit silicon carbide discs. An electrical contact was established with a copper wire for the construction of the graphite electrode (geometrical area 0.28 cm^2). MAPTS (1.19 ml) and TMOS (0.74 ml) (1 : 1 molar ratio) were mixed thoroughly with 1.215 ml of methanol. Then, 0.505 ml of water were added, the mixture was stirred for 2 min and the sol was left to cure at room temperature for 3 days prior to the lichen incorporation, with the aim of avoiding a long-term contact between the lichen particles and the methanol. The final silane : methanol : water molar ratio was 1 : 3 : 3. An aliquot of 100 μl of gel with 13.7 mg of lichen (20%) was deposited on the surface of the graphite electrode and spun at 1300 rpm for 180 s. The resulting layer was left to dry at room temperature for several days. The electrode surface was conditioned by soaking in supporting electrolyte overnight before electrochemical measurements were made.

Instrumentation

SEM was performed with a ZEISS DSM-950 microscope working at 20 kV. The samples were treated with liquid N_2 to cut them in order to image a transverse section. EIS measurements were carried out using a Solartron 1255 frequency response analyser coupled to a PAR 273A potentiostat from EG&G, controlled with M398 software. LSV measurements were performed with a PAR 273A potentiostat controlled with M270 software. In both electrochemical techniques, a conventional three-electrode configuration was employed. A home-made sensor acted as the working electrode, a coiled platinum wire served as the counter electrode and all potentials were measured against an Ag/AgCl reference electrode.

Electrochemical procedures

The sensor impedancimetric characterization was performed by EIS in NaCl and $Pb(NO_3)_2$ aqueous solutions with concentrations of between 10^{-6} and 10^{-2} M, over the 100 kHz–1 Hz frequency range and applying a sinusoidal signal with amplitude ±10 mV. AS-LSV for the determination of lead included three steps: (1) preconcentration was carried out in 5 ppm Pb^{II} in 10 mM acetate buffer, pH 5.0, for 20 min at open circuit; (2) after thoroughly rinsing with deionized water, the sensor was transferred to the measurement cell containing 0.1 M HCl; first, a reductive potential of −1.0 V was applied for 10 s and then the potential was swept between −1.0 and +1.0 V at a scan rate of 500 mV s^{-1} to induce the anodic stripping of the reduced lead; (3) finally, immersion of the sensor in 0.5 M HCl for 2 min assured the surface renewal, with the lichen active sites available for new measurements. All experiments were conducted under ambient conditions.

Results and discussion

The Chilean lichen *P. hirsuta* was incorporated in a rigid silica matrix in order to develop rugged amperometric sensors with better physical and chemical characteristics than conventional ones. The strategy employed is based on the incorporation of lichen particles in a polysiloxane matrix obtained by sol–gel through the hydrolysis and polycondensation reactions of the precursor alkoxysilanes MAPTS and TMOS. The methacrylate group in the MAPTS structure endows the resulting network with better mechanical characteristics than matrices based only on TMOS.[17–19] A combination of these alkoxysilanes in an optimal molar ratio provides a flexible matrix that makes easy the entrapment of the bulky lichen tissue, avoiding the presence of fissures in the resulting material. The lichen-modified network is then deposited on the surface of a polished graphite electrode by means of a spin-coating process. A spin rate of 1300 rpm and a spin time of 3 min were chosen in order to obtain a uniform electrode surface, since the quality of the sol–gel films is mainly affected by these parameters.[22,23] The resulting layer was left to dry at room temperature for 5 days to avoid cracking the film, which takes place at higher temperatures. The thickness of this film was estimated by measuring an analoguous layer deposited on a glass slide following the same spin-coating procedure. Five measurements of the film thickness were carried out with a profilometer and an average thickness value of 35 ± 9 μm was obtained.

The study of the lichen-modified material by means of SEM confirms a homogeneous distribution of lichen particles in the polysiloxane matrix (Fig. 1). Only the fungal structure of the lichen (hyphae), which is mainly involved in the uptake of lead as well as other heavy metal ions[3,10] is distinguished, as revealed by the presence of tubular-shaped elements. However, the alga tissue is not visible because of the significant shrinkage that takes place during the drying step in the lichen treatment. Fig. 1a shows the SEM image of a transverse section of the modified-material, where the hyphae are clearly observed as well as the tracks left by other hyphae during the material division. The absence of fissures in the sol–gel matrix is also remarkable, as can be observed in the lichen-free zones. The SEM image of the modified-material surface (Fig. 1b) confirms that the lichen hyphae are completely embedded in the sol–gel network.

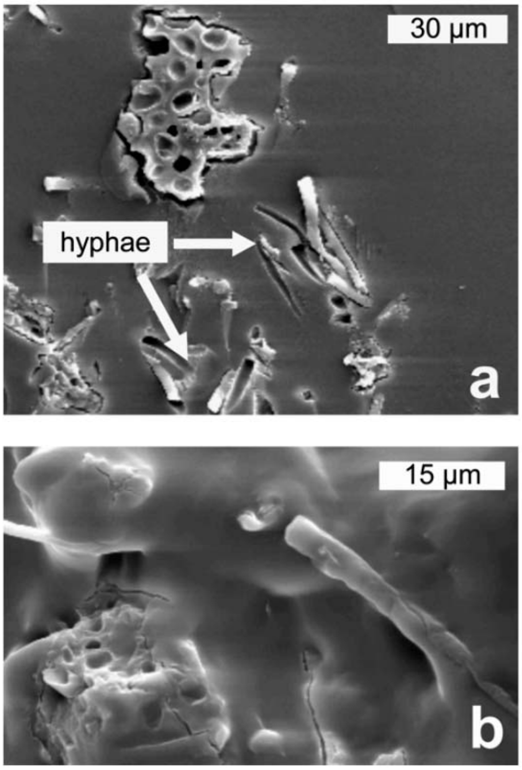

Fig. 1 SEM images of (a) the transverse section and (b) the surface of the lichen-modified polysiloxane matrix.

The background current measured in 0.1 M HCl with the resulting lichen-modified polysiloxane matrix based sensor was considerably lower than that obtained for the bare graphite electrode. This significant decrease in the capacitative current takes place when the electrode surface is covered with a low dielectric layer. The obtained sol–gel matrix yielded a specific surface area of 26.8 $\pm$ 0.8 $m^2\ g^{-1}$ (BET, N_2), which is in good agreement with surface area values reported by Jiménez-Morales[24] for similar polysiloxanes resulting from mixtures of the same alkoxysilane precursors. The reduced accessibility of N_2 molecules used as adsorbate in the BET measurements, reveals the low porosity of the membranes. According to the electrochemical results, the mass transport within the film is explained by the permeability of the thin polysiloxane layer allowing the metal ions to reach the electrode surface.

In order to evaluate the electrochemical behaviour of the resulting sensor, the impedancimetric characterization was performed by means of EIS in 10^{-2} M NaCl and 10^{-2} M $Pb(NO_3)_2$ aqueous solutions, as described in the Experimental section. The obtained impedance data is represented in the complex impedance plane as Nyquist diagrams (Fig. 2). The general form of both diagrams includes two overlapped semicircles followed by a linear tail (Warburg diffusion). The proposed equivalent circuit is represented in the inset of Fig. 2. Relevant information about membrane behaviour and its influence in the global sensor response can be obtained from the first semicircle, which can be related to the ionic resistance, R_i, and the geometric capacitance, C_g, of the polysiloxane matrix. These parameters are represented as a resistive element in parallel with a capacitive element in the equivalent circuit. The parameters corresponding to the second semicircle can be attributed to the charge transfer resistance, R_t, and the corresponding double-layer capacitance, C_{dl}, while R_e is ascribed to the electrolyte solution resistance (2800 and 525 Ω for sodium and lead solutions, respectively). The values of these elements can be obtained from the Nyquist plots and the diffusion coefficient, D, can be also calculated using the following equations proposed by Feliu *et al.*:[25]

$$(Z'_W)_{\omega \to 0} = \left(\frac{RT}{nF}\right)\frac{1}{i_L}$$

where $(Z'_W)_{\omega \to 0}$ is the limiting resistance, i_L the limiting current density, R the gas constant, T the absolute temperature, F the Faraday constant and n the number of electrons involved in the electrochemical process.

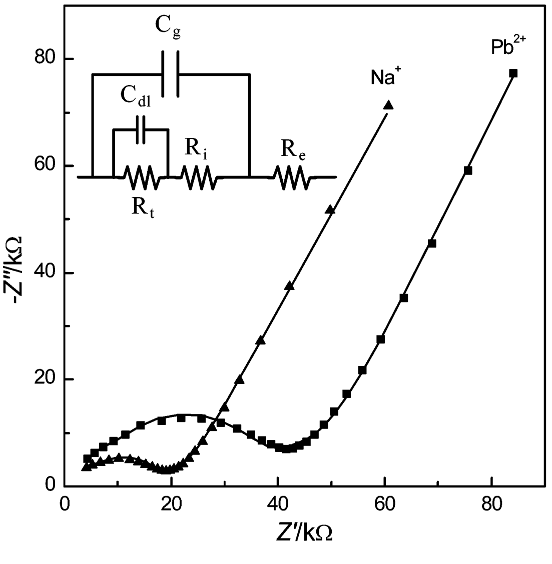

Fig. 2 Nyquist diagrams corresponding to the polysiloxane matrix based-sensor in contact with 10^{-2} M NaCl (▲) and 10^{-2} M $Pb(NO_3)_2$ (■) solutions. Inset: equivalent circuit proposed to explain the behaviour of the graphite electrode coated with the lichen-modified membrane.

$$i_L = \frac{nFDC}{\delta}$$

where C is the concentration of the electroactive element and δ is the diffusion layer thickness.

From an extrapolation of the linear zone in the Nyquist diagram (lowest frequency values) to the real axis, the value of $(Z'_W)_{\omega \to 0}$ is obtained. This parameter is used to determine i_L by means of eqn. (1). According to Fick's first law, the diffusion coefficient can be calculated using eqn. (2) and by assuming that δ agrees with the membrane thickness.[26] The most representative parameters obtained for both of the tested solutions are summarized in Table 1.

Prior to the measurements, the membrane was saturated with electrolyte until a steady state was attained and the values of R_i remained practically constant. The Pb^{2+} ion shows the largest R_i value, which can be attributed to its higher ionic charge. This R_i value is representative of the impediment of the ion to movement across the membrane, since the lichen active sites involved in the binding process exhibits a higher affinity to ions with more positive charge.[27] This fact agrees with the diffusion coefficient values obtained for Pb^{2+} and Na^+ of 1.5×10^{-8} and 1.8×10^{-9} $cm^2\ s^{-1}$, respectively. On the other hand, C_g is a parameter related to the chemical and physical nature of the membrane, and in particular is linked to the amount of water inside it. C_g values obtained for both lead and sodium cations are of the same order of magnitude (*ca.* 10^{-10} F), which is a typical value for a polysiloxane matrix itself, as described in literature for undoped and crown-ethers doped membranes.[17]

The R_i parameter obtained from the first semicircle can be employed to study the electrode sensibility towards changes in the electrolyte solution concentration. Therefore, concentrations of NaCl and $Pb(NO_3)_2$ in the range from 10^{-6} to 10^{-2} M were tested. The linear relationship between R_i and $\log[Pb^{2+}]$ is limited to solutions with Pb^{2+} concentrations higher than 10^{-4} M. In contrast, the plot of R_i as a function of $\log[Na^+]$ exhibits a linear relationship in a wider range of concentration (Fig. 3), indicating the ability of the modified electrode

Table 1 Electrochemical parameters obtained by means of EIS for the modified electrode in NaCl 10^{-2} M and $Pb(NO_3)_2$ 10^{-2} M solutions

	Na^+	Pb^{2+}
R_i/Ω	16796	35932
C_g/F	3.2×10^{-10}	1.2×10^{-10}
$D/cm^2\ s^{-1}$	1.5×10^{-8}	1.8×10^{-9}

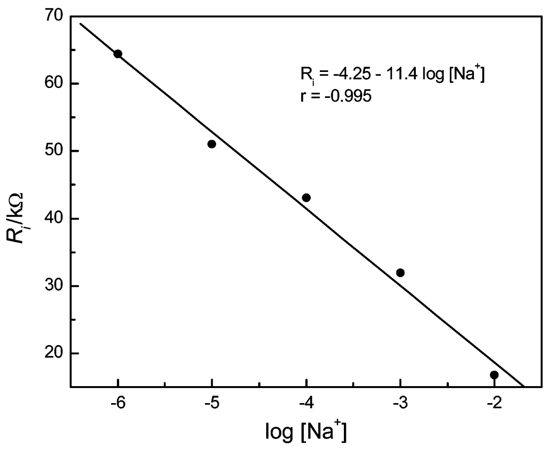

Fig. 3 Variation of the ionic resistance corresponding to the lichen–polysiloxane matrix sensor as a function of the NaCl concentration.

to act as an impedancimetric sensor for sodium ion. A similar behaviour concerning to these cations was described for the sensors based on crown-ether doped sol–gel membranes.[19] This fact may suggest that the polysiloxane matrix is involved in the impedancimetric sensing of Na^+ rather than the lichen particles.

It is assumed that the electroanalytical performance of the sensor in the amperometric determination of lead is based on the uptake of metal ions by the active sites of the lichen structure. For this purpose, the lead was first bioaccumulated in the lichen particles embedded in the silica matrix and subsequently measured by AS-LSV. The amperometric responses obtained for the developed sensor are illustrated in Fig. 4. It is observed that the anodic peak current ($i_{p,a}$) varies as a function of Pb^{II} concentration. The corresponding calibration plot in the studied range of concentration shows a linear behaviour that confirms the satisfactory performance of this sensor in the amperometric determination of lead in water samples. In these experimental conditions a preconcentration time, t_p, of 5 min has been used and the maximum uptake of lead ions is reached at Pb^{II} concentrations higher than 3 μg ml^{-1}, that is, the anodic peak current value remains practically constant. The contribution of the blank polysiloxane matrix in the uptake of lead ions has been examined using identical experimental conditions to those adopted for lichen membranes. The corresponding results indicate the lack of affinity of the polysiloxane matrix itself towards lead ions. The experimental parameter t_p plays an important role in the accumulation of metal ions and, thus, is related to the sensor sensibility and the detection limit. Larger preconcentration times would result in the determination of lead at trace levels, of the order of ng ml^{-1}, thereby improving the detection limit.

The relative standard deviation for four successive measurements of 2 μg ml^{-1} Pb^{II} under the aforementioned conditions was 2.1%. In addition to a suitable sensor response, the stability of the electrode coating was assessed regularly, proving that the same sensor can be used for several weeks with a negligible change in performance (loss of signal 2.9% in 10 days).

The ability of the sensor to be applied in multicomponent analysis has been also successfully tested. This fact is related to the ion exchange properties of the lichen active sites, exhibiting high affinity towards different metal ions. Fig. 5 shows the linear scan voltammogram obtained after the preconcentration step in a complex solution containing Cd^{II}, Pb^{II} and Cu^{II}. The anodic stripping peak potentials are −605, −322 and +6 mV for cadmium, lead and copper, respectively. The high resolution of the voltammetric response indicates that the sensor could be applicable to multicomponent determinations in complex samples. This preliminary study reveals that several metal ions in solution could be measured simultaneously. The sensor selectivity for different cations and other analytical parameters will be the objective of further work.

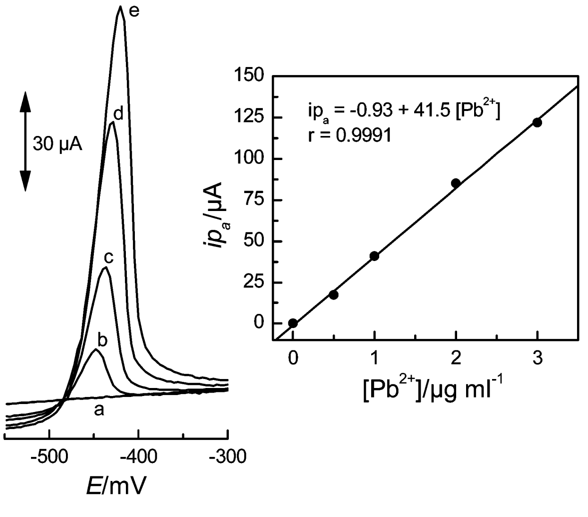

Fig. 4 AS-LSV responses obtained for the lichen–polysiloxane matrix sensor as a function of Pb^{II} concentration and the corresponding calibration plot. Preconcentration: 5 min at open circuit in Pb^{II} solutions of: (a) 0, (b) 0.5, (c) 1.0, (d) 2.0, and (e) 3.0 μg ml^{-1}, in 10 mM acetate buffer, pH 5.0. Measurement: −1.0 V applied for 10 s, followed by potential swept at a scan rate of 500 mV s^{-1} in the range from −1.0 to +1.0 V, in 0.1 M HCl.

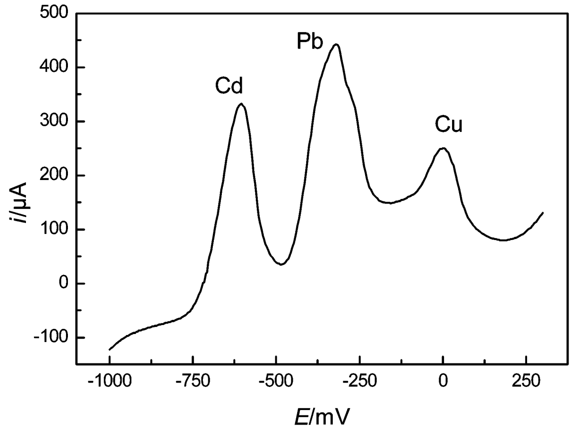

Fig. 5 AS-LSV response obtained for the lichen–polysiloxane matrix sensor in a multicomponent solution. Preconcentration: 20 min at open circuit in a Cd^{II}, Pb^{II} and Cu^{II} solution (5 μg ml^{-1} of each one), in 10 mM acetate buffer, pH 5.0. Measurement parameters indicated in Fig. 4.

Conclusions

The improved physical characteristics of the developed rigid polysiloxane material, obtained by means of an optimized sol–gel process, represent a great advantage compared with conventional modified carbon paste for the preparation of electrochemical sensors based on the entrapment of different sensing agents, including lichens. In addition, it has been assessed that this matrix avoids the leakage of the lichen particles, since they are completely embedded during the sol–gel process. The combination of such lichen-modified material with a solid graphite electrode provides a rugged device that shows a high long-term stability and is able to operate in solutions containing organic solvents. This fact, together with the suitable electroanalytical performance of the resulting device, proves the ability of such a modified electrode to be applied as an impedancimetric sensor for Na^+ as well as an amperometric sensor for the determination of heavy metal ions, for instance, lead. Since lichens are widely employed as bioindicators of environmental pollution, we propose the procedure here described as an alternative to conventional methods[2] in order to test and quantify the accumulating capacity of several lichens species. The fast response of the developed electrochemical sensors in the monitoring of toxic elements, results in a great advantage *versus* long-term pretreatment of lichen samples exposed to potential pollutants.

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