

Short Communication

Stabilization of the initial electrochemical potential for a metal-based potentiometric titration study of a biosorption process

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Abstract

An interactive metal-based potentiometric titration method has been developed using an ion selective electrode for studying the sorption of metal cations. The accuracy of this technique was verified by analyzing the metal sorption mechanism for the biomass of *Rhizopus arrhizus* fungus and diatomite, two dissimilar materials (organic and mineral, strong sorbent and weak sorbent) of a different order of cation exchange capacity. The problem of the initial electrochemical potential was addressed identifying the usefulness of a Na-sulfonic resin as a strong chelating agent applied before the beginning of sorption titration experiments so that the titration curves and the sorption uptake could be quantitatively compared. The resin stabilized the initial electrochemical potential to -405 ± 5 mV corresponding to $2 \mu\text{g l}^{-1}$ of lead concentration in solution. The amounts of lead sorbed by *R. arrhizus* biomass and diatomite were 0.9 mmol g^{-1} ($C_e = 5.16 \times 10^{-2} \text{ mM}$) and $0.052 \text{ mmol g}^{-1}$ ($C_e = 5.97 \times 10^{-2} \text{ mM}$), respectively. Lead sorption by the fungal biomass was pinpointed to at least two types of chemical active sites. The first type was distinguished by high reactivity and a low number of sites whereas the other was characterized by their higher number and lower reactivity. © 2005 Elsevier Ltd. All rights reserved.

Keywords: Potentiometric titration; Ion selective electrode; Lead sorption; *Rhizopus arrhizus*

1. Introduction

The important role of sorbent materials in controlling the mobility and bioavailability of metal ions in aqueous solutions has been extensively studied from different angles varying parameters of interest such as the

type of a metal (Guibal and Roulph, 1990; Apel and Torma, 1993; McBride et al., 1997; Christensen et al., 1999), the sorbent type (Mullen et al., 1989; Kratochvil and Volesky, 1998; Fein, 2000), experimental conditions (pH, temperature, reactor geometry) (Babich and Stozky, 1979; Piron and Domard, 1997), etc.

Metal sorption involves competition between protons and metals which is influenced by the physico-chemical properties of the sorbents and implies formation of electrostatic or covalent bonds with the functional groups of the mineral or organic solids and, consequently, a possible release of protons and other cations.

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Sorption onto geometrically and chemically heterogeneous “surface” such as biomass, well known for controlling the mobility and bioavailability of metal ions in aqueous systems, has been studied. Biosorption could occur also through interactions between metals ions and functional groups of the cell-wall biopolymers of living or dead organisms (Beveridge, 1986; Gadd, 1988; Volesky et al., 1993). The physico-chemical properties of these sorbents, such as their polyelectrolytic nature, the diversity of the functional groups and the variability of their composition, have suggested that the metal-based potentiometric titration method, previously used for studying the formation of soluble organo-metallic complexes (Schultz, 1971), could assist in estimation of their metal binding properties, differentiating the energetic sites capable of reacting with metallic cations at low concentrations and describing the behavior and surface affinity for a certain metallic element.

An interactive metallic potentiometric titration method has been developed for studying the sorption of metal ions by an organic or mineral surface and analyzing the proton and metal competition. This type of a titration method permitted following directly the sorption experiment and changes in the real time of its operating parameters using equilibrium criteria adapted to the kinetic characteristics of complex compounds. The accuracy of this technique was verified by studying the lead sorption mechanism of the *Rhizopus arrhizus* fungal biomass (biosorbent) and the diatomite (a siliceous non-active compound). The problem of a stable initial electrochemical potential was addressed using a sulfonic resin exchanging sodium as a strong chelating agent before the beginning of the sorption experiments.

2. Background and theory

The use of the potentiometric titration methods diversified with improved performance of ion selective electrodes (ISE) and with merging of new analytical methods and automatic instruments using electrochemical sensors for routine analyses. Several types of sensing electrodes are commercially available classified according to the membrane material type used for preparing the electrodes, making them selective for a particular ion. These electrochemical sensors could be an ISE with a homogeneous or heterogeneous solid-state membrane or the most familiar type of a sensing electrode is the glass membrane electrode routinely used for measuring pH. The acid–base titration is the most common titration method using the electrode selective for protons, whereas other types of ISE are generally used during precipitation or chelating reactions where the analyte is detected directly (Vesely et al., 1978) or indirectly (Schultz, 1971).

For obtaining satisfactory results during titrations, the following conditions are required and should be optimized when using a specific ISE:

- the electrode should detect specifically and precisely the analyte and not interact with other chemical species in solution (electrolyte);
- during potentiometric titration and depending on the system reactivity, measurements are conducted continuously for a long period of time. The electrode should be robust with a limited and controlled time drifts;
- the electrode response time should be fast over a broad concentration range, mainly in low concentration intervals.

Lead (II)-selective electrodes incorporating a solid state membrane composed of a mixture of silver sulfide and lead sulfide meet these requirements and are generally used for direct potentiometric determination of lead free ion activities or as indicating electrodes in potentiometric titrations during sorption reactions.

For diluted solutions, the concentration of free lead ions is equal to the activity. The divergence between activity and concentration increases with the solution concentration. This divergence should be controllable by choosing an adequate background electrolyte which, without interfering in the reaction, maintains a constant ionic strength and by estimating the activity coefficient $f_{\text{Pb}^{2+}}$ applying Debye–Hückel equation:

$$-\log f_{\text{Pb}^{2+}} = A * z^2 * \frac{\sqrt{\mu}}{1 + B * d * \sqrt{\mu}}$$

where μ is the solution ionic strength, A and B are specific constants equal to 0.5115 and 0.3291 at 25 °C, d is a parameter depending on the ion size (4.5 for lead) and z is the ion valence (2 for lead).

The electrochemical potential across the membrane of any electrode depends on the analyte concentration. The potential difference (E_{out}) for an ISE across the boundary between the membrane and the analyte solution is given by the following equation:

$$E_{\text{out}} = \frac{\Delta G_{\text{Solvation}}}{nF} - \frac{RT}{nF} \ln \left\{ \frac{|\text{Pb}^{2+}|_{\text{Mem}}}{|\text{Pb}^{2+}|_{\text{Out}}} \right\}$$

where $\Delta G_{\text{Solvation}}$ is the free enthalpy of solvation (J mol^{-1}), R is the universal gas constant ($8.3144 \text{ J mol}^{-1} \text{ K}^{-1}$), T is the absolute temperature (K), n is the number of the exchanged electrons, F is the Faraday constant (96487 C eq^{-1}) and $|\text{Pb}^{2+}|_{\text{Out}}$ and $|\text{Pb}^{2+}|_{\text{Mem}}$ are the lead activity in solution and in the membrane, respectively.

The electrochemical potential difference between the analyte solution (E_{out}) and the solution inside the ISE (E_{in}) is

$$E = E_{\text{out}} - E_{\text{In}} = \frac{\Delta G_{\text{Solvation}}}{nF} - \frac{RT}{nF} \ln \left\{ \frac{|\text{Pb}^{2+}|_{\text{Mern}}}{|\text{Pb}^{2+}|_{\text{Out}}} \right\} - E_{\text{In}}$$

Knowing that almost all the defined parameters of the above equation are constants, the potential difference between the analyte solution and the solution inside the ISE could be simplified significantly. The following Nernst equation is then obtained:

$$E = E^0 + \frac{RT}{nF} \ln \{ |\text{Pb}^{2+}|_{\text{Out}} \}$$

where E_0 is the standardisation potential of the electrode (V).

To take into account, semi-empirically, the electrolyte effect (Na^+ , NO_3^-) and the interference factors, a constant BI was introduced to express the experimental detection limit of the given electrode when the measured ion activity tends to zero. It includes the possible interferences of all the ions present in solution, especially those contributing to the ionic electrolyte strength. The Nernst equation can be modified as follows:

$$E = E^0 + \frac{RT}{nF} \ln \{ |\text{Pb}^{2+}|_{\text{Out}} + \text{BI} \}$$

Thus, the obtained calibrating curve of the response of the electrode E versus lead free ion activity $|\text{Pb}^{2+}|_{\text{Out}}$ could be divided into two parts:

- a linear response zone: the slope of the curve will be the Nernst factor (RT/nF);
- a non-linear response zone characterizing the low lead concentration domain. In this zone, the BI factor effect is dominant and the electrode response progressively reaches a limit value.

This progressive damping in the electrode response resulted in obtaining a non-reproducible and not significant electrochemical potential values in the low lead concentration domain, particularly in the presence of the sorbent material. A key for resolving this problem is presented in the following section.

3. Materials and methods

All high resolution titration experiments were conducted in a 500 ml jacketed glass vessel (Wheaton) sealed by a lid fitted with five ports for a titrant injection, a N_2 line, a pH electrode, ISE electrode and a temperature probe, respectively. The vessel temperature was maintained at 25 °C by recirculation of thermostated water from a bath through the vessel jacket to control the possible temperature effect on the reaction thermodynamics and kinetics. The headspace was purged with N_2 at low pressure during titrations to re-

move CO_2 and limit its dissolution in the electrolyte and the carbonates formation resulting in the solution pH changes.

Titration aliquots of a specified volume (down to $1 \mu\text{l} \pm 0.18\%$) of 1 mM $\text{Pb}(\text{NO}_3)_2$ solution, at pH 5 and of the same ionic strength as the solution in the vessel, were injected through a polypropylene line by an automatic and accurate burette (ABU901 Radiometer) controlled by a microprocessor. The solution in the vessel was agitated by a magnetic stirrer (500 rpm) until pH and $E(\text{Pb})$ became stable after each titrant aliquot injection. All experiments were carried out at pH 5 with 5 and 40 mg of samples of *R. arrhizus* biomass or diatomite, respectively, suspended in 300 ml of NaNO_3 as the background electrolyte to maintain an ionic strength of 0.01 M. The selected weight depended on the value of the cationic exchange capacity or the total organic acidity of the chosen materials. pH and $E(\text{Pb})$ were measured using a precise (± 0.1 mV) dual-function ionometer (PHM250 Radiometer) linked to (1) a Ross combined electrode (Ross 8102, NaCl saturated) after its calibration with IUPAC standards and to (2) an ion selective electrode (ISE 25 Pb-9, Orion, Radiometer). Before each sorbent titration, a calibration was performed (sodium nitrate blank or control) with the background electrolyte using the same procedure as for the titrations of solids.

The pH value was chosen such as to avoid lead precipitation and to optimize the sorption process. The ionic strength value chosen is the optimum for quantifying an analyte using ISE electrodes whereby $\mu = 0.01$ M is close to the natural system values.

The types of sorbent matter used to verify the titration accuracy and the electrochemical potential reproducibility were:

- *R. arrhizus* fungus (DSM 905) cultivated in a liquid medium at 24 °C in the dark. After 5–6 days of growth, fungal biomass was collected by filtration, ground, washed several times with distilled water to eliminate culture medium residues, lyophilized and stored under vacuum. The studied fungal biomass was characterized by the CEC (cation exchange capacity or organic total acidity determined by the acid base titration method) of 2.54 meq g^{-1} .
- Diatomite: a siliceous non-active compound used as a filter aid additive in the pharmaceutical process. The diatomite was washed, lyophilized and stored under vacuum. The diatomite used was characterized by a CEC (determined by the acid base titration method) of 0.27 meq g^{-1} .

The problem with stabilizing the initial electrochemical potential of lead was resolved by initially adding to the solution a known quantity of lead ($20.8 \mu\text{g l}^{-1}$) in the presence of a strong solid chelating agent (superior to

the sorbent materials) of the known and predetermined affinity to the metal. In this case, the dependency of the sorption equilibrium on the sorbent reactivity or on the ionic strength was diminished permitting a precise establishment of the same constant initial concentration and of a reproducible initial electrochemical potential (± 5 mV) for each series of experiments. The chosen chelating agent was 30 mg of a sulfonic resin-Na (Dowex 50WX8) suitably conditioned with the electrolyte and enclosed in a nylon-mesh double tea bag (0.2 μm). Before starting sorption titrations, the interior bag was removed from the solution.

3.1. Developed software

The newly developed automated high resolution titration control system (PC, TestPoint Software) was connected to an automatically controllable burette (ABU 901 Radiometer) and to a dual-function (pH, *E*) high precision ionometer (PHM 250 Radiometer). This system allowed the proportionally-controlled release of variable volumes into the potentiometric titration cell and the measurement of pH, lead potential and temperature. This multitask and interactive system permitted to change and specify the operating parameters during titrations such as the volume, rate and frequency of injections, and the stability factor considering the complex nature of each solid to be titrated. The waiting time between any two volume additions was fixed by the user and depended on the transitional signal (electrochemical potential fluctuations versus time between each two volumes added in order to reach equilibrium) and on the equilibrium condition (dE/dt variations less than 0.2 mV/10 min) by fixing the number of consecutive constant points with a desired precision. The chosen electrochemical potential stability criterion should take into account the stability of the electrical apparatus and of the electrode signal during the control titration.

The specially developed software allowed to appropriately handle the entire process and the titration apparatus, particularly to:

- choose the titration method (incremental or quasi-equilibrium) by selecting the automatic control or working at the equilibrium. Several stop conditions could be specified such as the maximum added volume, the final electrochemical potential or even the maximum data points;
- save data points in a computer file, with an excel extension, having a column format recording time, added volumes, electrochemical potential, rate of injections, pH and its minimum, mean and maximum potential values obtained for each point of titrant addition;
- save the transitional signals or the electrochemical potential fluctuations versus time between each added volume in order to reach equilibrium;
- analyze directly the first and second derivatives of pH and of the electrochemical potential, the Gran's function and the sorbed quantity (sorption uptake) instantaneously, the control titration file should be selected to perform these calculations. The obtained data file could be saved when the experiment was completed.

3.2. ISE maintenance

Various factors such as pH, temperature, ionic strength, presence of interfering ions could affect the measurements of an electrochemical potential using an ISE and their reproducibility. In the present study, the most influencing factors were the following:

- white light effect: the light catalyzes the corrosion of the membrane and lead to a decreasing of the sensitivity of the electrode (Young, 1990). The vessel should be completely covered by a light shield such as an aluminum foil;
- solid organic matter presence could partially deactivate the electrode surface and consequently lead to a decreasing ISE sensitivity and its increased response time.

When the corrosion of the electrode surface was reversible, a chemical regeneration was sufficient: the electrode was immersed in 10^{-3} M $\text{Pb}(\text{NO}_3)_2$ solution which desorbed the deposit on the surface.

When the corrosion of the electrode surface was irreversible, an abrasive paper was used to clean the electrode surface subsequently immersed in a concentrated lead solution (10^{-3} M);

- knowing the pH influence on the ISE lead measurements, pH was maintained constant or at least the pH value varied in a predetermined small interval (± 0.5 pH unities);
- ionic strength of solutions was maintained constant using a suitable electrolyte such as NaNO_3 (0.01 M) to control the divergence between activities and concentrations (verified by a conductivity meter);
- temperature of solutions was maintained constant at 25 °C (± 0.5);
- the electrode was immersed in the analyte solution at the same depth and the solutions were homogenized at the same constant agitation rate;
- titration of a control (0.01 M NaNO_3 solution) was performed directly before a titration to assure the reproducibility of the electrode signal.

3.3. Chemicals and equipment

All chemicals used were of analytical reagent grade. The water used was ultra purified. After titration, the obtained solutions were analyzed by (1) ICP-AES (inductively coupled plasma-atomic emission spectrometry, Jobin Yvon JY 238) to verify the obtained lead value by ISE and by (2) a CHN Analyser (1106-Carlo Erba) to make sure of the non-existence of amounts of dissolved organic compounds.

The ionic strength of all titration solutions was kept constant and measured by a conductivity meter (CDM 80 Radiometer Conductivity Meter).

4. Results and discussion

4.1. Titration equilibrium time

Fig. 1 represents the energy profile of the active sites as assessed by the *R. arrhizus* titration in relation with the equilibrium time after analyzing the transitional signals. Some active sites required 80 min to reach equilibrium (dE/dt variations less than 0.2 mV/10 min) whereas others needed 10 min. The average time required for the sorption reaction to reach equilibrium was approximately 28 min. This value can be compared to 11.2 min, the mean value obtained during titration of diatomite, and to the control mean value of 10 min.

The classification of the waiting time to reach equilibrium during *R. arrhizus* sorption titration (Fig. 2) indicated the existence of 3 types of chemically active sites. Some appeared to be kinetically slow (equilibrium time of 80 min), others had an average sorption rate (equilibrium time of 30 min) and the third type was distinguished by a fast sorption reaction rate (equilibrium

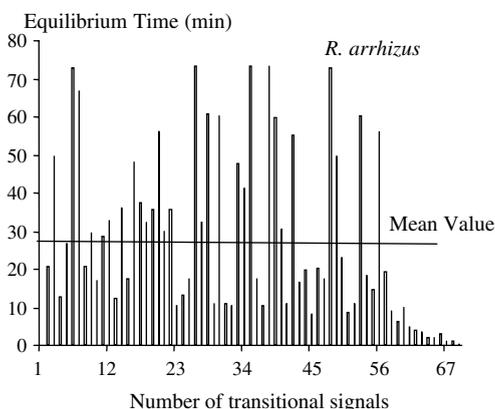


Fig. 1. Energy profile of the sites surface developed during *Rhizopus arrhizus* titration versus the equilibrium time.

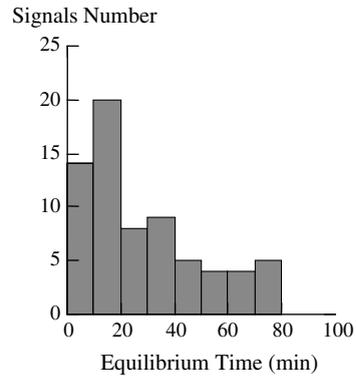


Fig. 2. Transitional signals classification versus the equilibrium sorption time.

time of 10 min). The first type of sites was characterized by a high reactivity and a low number whereas the others featured less reactivity but were more abundant.

Consequently, lead was sorbed by strong binding sites that became completely saturated at low metal concentrations and by a weaker type of sites, apparently dominant throughout cell walls of the biomass examined. One possible explanation of the sorption differences obtained as the result of the present work when compared to the literature seems to rest in the examination method used. Obviously, batch adsorption isotherm work is insufficient for distinguishing among the types of binding sites whereas the application of spectroscopic techniques is necessary and used in the literature to reveal the differences in sorption affinity (Fourest and Volesky, 1996). Sarret et al. (1998) reported an identical behavior after studying the structural binding sites of lead sorbed by *Penicillium chrysogenum* cell walls using extended X-ray absorption fine structure spectroscopy (EXAFS). Minor COOH (~5%) surface ligands have a high affinity for Pb as compared to PO₄ ligands (~95%) having a weaker affinity for lead.

4.2. Sorption processes

The titration curves of the control, fungus and diatomite (Fig. 3) showed the importance of the use of Na-sulfonic resin to stabilize the initial electrochemical potential. Indeed, the initial electrochemical potential of lead in solution is proportional to the initial concentration of lead which, in turn, depends on the reactivity and on the exchange cationic capacities of the used sorbents.

The diatomite and *R. arrhizus*, having different CEC values (0.27 meq g⁻¹ and 2.54 meq g⁻¹, respectively), caused different $E(\text{Pb})$ in solution (−429 mV (diatomite) and −458 mV (*R. arrhizus*)) (Fig. 3). It should be noticed that the $E(\text{Pb})$ value depended also on the initial lead concentration in distilled water, normally certified to

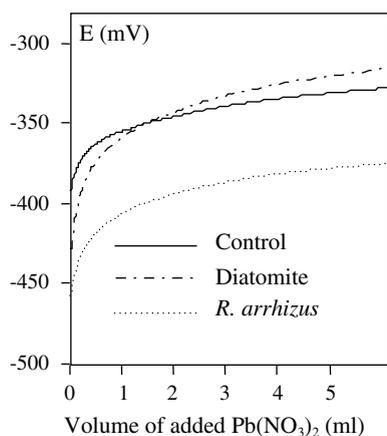


Fig. 3. Initial electrochemical potential during the control, diatomite and *Rhizopus arrhizus* titrations without using the Na-sulfonic resin.

be lower than 10^{-9} M ($0.207 \mu\text{g l}^{-1}$). There is virtually no apparatus that could deliver the required detection sensitivity. Consequently, not having the same initial electrochemical potential (*R. arrhizus* (-458 mV) and diatomite (-429 mV)) and not knowing the initial lead concentrations, the respective metal uptakes by *R. arrhizus* and diatomite could not normally be compared for the present sorption experiments.

The importance of using a strong solid chelating agent before initiating the titration as a technique for stabilizing the electrochemical potential was validated by observing the titration curves for the control and for the two materials (Fig. 4a–c). The identical initial electrochemical potential point (-405 ± 5 mV) was adjusted by using the Na-resin (a strong chelating agent) the presence of which resulted in the same lead concentration of $2 \mu\text{g l}^{-1}$ (value verified by ICP-AES) no matter what solid sorbent was examined (Fig. 4a–c).

The sulfonic resin in the tea bag, having the highest affinity and the highest ion exchange capacity, sorbed the cations present in the solution including protons. The obtained equilibrium did not depend on the reactivity of the sorbent (*R. arrhizus* or diatomite) or on the ionic strength and permitted establishing an initial constant concentration of lead and an initial reproducible electrochemical potential for each experimental series. Consequently, after stabilizing the initial electrochemical potential, the titration experiment could begin and the metal uptakes at various added volumes were quantified and compared among the materials tested as well as quantitative sorption isotherms determined.

The stabilization curves (E , pH) (Fig. 4a–c) of the control, *R. arrhizus* and diatomite showed that an ion exchange reaction occurred between cations in solution (lead) and the resin with a gradual increase of pH. Assuming that the solution ionic strength did not change

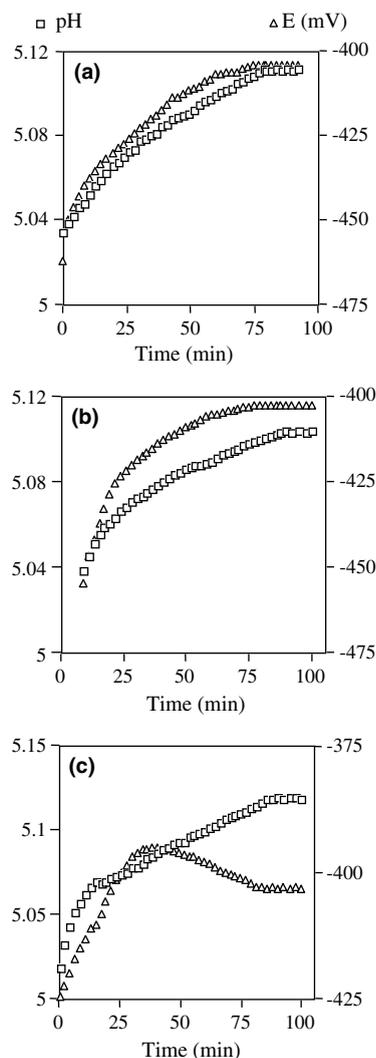


Fig. 4. Stabilization of the pH (□) and the initial electrochemical potential (Δ) before the control titration (a), diatomite titration (b) and *Rhizopus arrhizus* titration (c) using the Na-sulfonic resin.

before and after the resin addition (verified by a conductivity meter), the observed increase of pH (during the stabilization of the initial electrochemical potential for the control or the solids) was due to an ion exchange reaction between the Na-resin and protons.

The long time of stabilization was due to the diffusion mechanisms of lead cations inside the double nylon bags considerably slowing down the process. The same experiment conducted without the nylon bag (resins added directly into the vessel) would lead to an immediate stabilization of pH and E .

When the initial point was stabilized, the titration curves and the sorption uptake could be compared quantitatively (Fig. 5). The lead uptakes by *R. arrhizus*

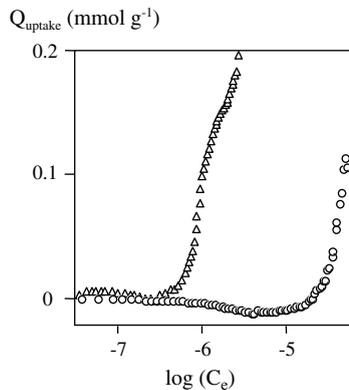


Fig. 5. Lead sorption onto diatomite (O) and *Rhizopus arrhizus* (Δ).

biomass and diatomite were of 0.9 mmol g^{-1} ($C_e = 5.16 \times 10^{-2} \text{ mM}$) and $0.052 \text{ mmol g}^{-1}$ ($C_e = 5.97 \times 10^{-2} \text{ mM}$), respectively, after adding 0.02 mmol of lead. The values obtained were in accord with values reported in the literature (Volesky, 2003) for other filamentous fungi: 0.589 , 0.801 and $0.439 \text{ mmol of Pb g}^{-1}$ for *P. chrysogenum*, *Rhizopus nigricans* and *R. arrhizus*, respectively.

The lead uptake curves for *R. arrhizus*, as compared to those for diatomite, were characterized by the appearance of several steps indicating most probably the presence of several types of sorption sites having particular reactivities, whereas the diatomite sorption curve during the titration experiment appeared quite regular. This observation was confirmed by the energy profile of active sites developed during *R. arrhizus* titration in relation with the equilibrium time after analyzing the transitional signals (Fig. 2) indicating the existence of various types of sites characterized by specific affinities.

The lead sorption by *R. arrhizus* biomass was characterized by a quantity of released protons (Fig. 6). The pH curve (Fig. 6) indicated also the existence of several kinds of sites reacting during the sorption process. The sudden change of the slopes in the pH could indicate the initialization of a particular process (Naja, 2001) or the sorption on a different type of sites. The weak pH variations during lead uptake onto diatomite corroborated the conclusions made from the sorption curve.

Consequently, the used biomass featured not only a chemical heterogeneity (various functional groups) but also a structural heterogeneity (different types and size of pores) and, therefore, the developed metal-based potentiometric titration can lead to elucidation of the chemical structure of the biomass and of its structural heterogeneity. However, spectroscopic methods are subsequently necessary to verify and complete the obtained information.

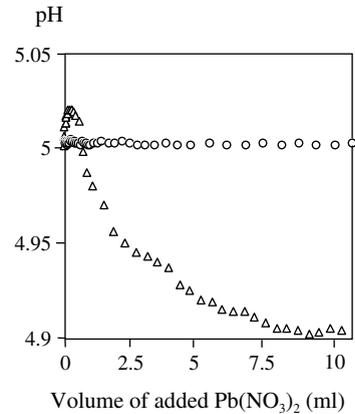


Fig. 6. pH changes during lead sorption onto diatomite (O) and *Rhizopus arrhizus* (Δ).

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