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Preconcentration and Determination of Some Lanthanide Elements With Immobilized Bacteria by Flow Injection Inductively Coupled Plasma Atomic Emission Spectrometry

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The on-line combination of flow injection column preconcentration with sequential inductively coupled plasma atomic emission spectrometry is shown to provide an enrichment factor of 36 for La^{3+}, 34 for Ce^{3+} and 51 for Nd^{3+}, at 1 μg ml^{-1} for 5 ml of sample. The significant improvement in the (3 *s*) detection limit by preconcentration compared with that without preconcentration demonstrates the efficiency of preconcentration with the bacterium *Spirulina platensis* immobilized on controlled-pore glass. Recovery of all the lanthanide cations is quantitative and the capacity was calculated. Practical application of the method was demonstrated by the determination of La^{3+} and Nd^{3+} impurities in a cerium(III) nitrate reagent. Adsorption isotherm studies were used to describe the mechanisms and the nature of the column binding sites. Isotherm plots showed a Langmuirian shape for La^{3+} and an anti-Langmuirian shape for Ce^{3+} and Nd^{3+}. Equilibrium constants were also calculated.

Keywords: *Preconcentration; Spirulina platensis; rare earth elements; immobilization; flow injection; inductively coupled plasma atomic emission spectrometry*

Rare earth elements (REEs) occur at extremely low concentrations in nature.[1,2] Accurate and convenient methods of determining REEs are important for the development of geochemical knowledge and industrial applications.[3,4]

Many methods and techniques are available for the determination of REEs. Neutron activation analysis[5] provides suitable sensitivity, but has some drawbacks because of the complicated and sometimes prolonged experimental procedure. It requires chemical group separation of the elements of interest from interfering elements.[6] Isotope dilution mass spectrometry[7] usually provides precise results, but it has a major disadvantage for four lanthanides (Pr, Tb, Ho and Tm): they cannot be determined because they have only one isotope. Atomic absorption spectrometry (AAS) allows their accurate and precise determination.[8,9] On the other hand, AAS[10] can readily be applied for determining all REEs, but with poor sensitivity. Flame atomic emission spectrometry (AES) is more sensitive than AAS, and has been used for the determination of lanthanum in the presence of other REEs, but suffers some serious interferences from background emission.[11]

Inductively coupled plasma atomic emission spectrometry (ICP-AES) has been applied to the determination of REEs in ores and minerals,[12] rare earth concentrates,[13] and various geochemical materials.[14] It has several advantages such as wide dynamic ranges, freedom from chemical and ionization interferences and capability of simultaneous or sequential multi-element determinations. McLeod[15] has outlined the merits of flow injection (FI) interfaced to ICP and related the resulting substantial improvement in sensitivity to several factors associated with FI, namely: (i) a relatively constant solution flow rate, (ii) an improved plasma stability and (iii) a reduced build-up of carbon in the torch injector tip.

Several groups of workers have demonstrated the effectiveness of chelating resins for on-line preconcentration with multi-element detection,[15–17] because chelating resins have the advantages of having greater sensitivity and selectivity than ordinary ion exchangers. Ion-exchange or chelating resins are by far the most popular preconcentration materials.[18] The selectivity of these columns depends on the particular chelating agent used.

In general, micro-organisms have the ability to adsorb selectively a specific element in the presence of other elements or species.[18] Darnall *et al.*[19] have shown that trace metals can be quantitatively separated from the solution by means of a pure alga strain. Mahan *et al.*[20] have demonstrated trace metal uptake by several algae strains in a multi-component matrix. Other studies have used free algae or algae immobilized by physical adsorption using a batch method;[21] an alternative approach is to use a mini-column of covalently bound micro-organisms for on-line sample pre-treatment. This was prompted first by Elmahadi and Greenway[22] using immobilized algae in an AAS flow system for on-line trace metal ion preconcentration and determination. Other types of micro-organisms have also been immobilized for the assessment of preconcentration capabilities and trace determinations.[23,24] Micro-organisms have multi-binding sites; hence sensitivity and selectivity could easily be achieved[22,24] by judicious solution conditions[25] (pH) as well as elution conditions. For instance, chromate/dichromate weakly bound at pH values around neutrality can be bound completely at pH 2.0. This diversity gives the algae or micro-organisms a wide applicability.

Also, Darnall *et al.*[25] concluded that, in contrast to many conventional resins, the cells have relatively little affinity for alkaline earth metals, and thus in hard-water treatment applications the algae will be less prone to saturation by non-toxic ions.

In previous work,[22] we have observed that the diversity of these binding sites is a major advantage, because if one element has an affinity to coordinate with one functional group, *i.e.*, COO^-, and, if this element is present in a mixture of many other elements, each of them can form a stable complex with any of the other functional groups present in the cell wall of the micro-organism. Therefore, by changing pH or elution conditions, selectivity is acceptable and this is well demonstrated in the interference effects where the element under investigation is present with other elements in relatively high concentrations. There are many binding sites, in such a way that, by suitably choosing the working conditions (pH, elution, buffers, *etc.*), the recovery of these elements from the column is approximately quantitative. In conclusion, different elements have different affinities for certain binding sites.

The aim of this work was to demonstrate the applicability of covalently immobilized bacteria for the determination of trace amounts of La^{3+}, Ce^{3+} and Nd^{3+}, as these elements have different chemistries and they are also frequently present at very low concentrations. This work was carried out using FI and on-line sample treatment, and ICP-AES for multi-element detection. The isotherm for the different lanthanides was studied to describe the adsorption behaviour and the equilibrium constants were evaluated so as to obtain some information on the binding mechanism.

EXPERIMENTAL

Instrumentation

A SpectroflameD ICP-AES sequential spectrometer was used, with argon as plasmogenic gas and a cross-flow nebulizer working at 3.2 bar. The argon auxiliary gas flow rate was 30 ml min^{-1} and the cooling gas flow rate was 40 ml min^{-1}. A four-channel Gilson Minipuls-3 peristaltic pump was used. The FI manifold was as described previously[23,24] except that in this instance ICP-AES was used for detection.

Reagents

Doubly distilled de-ionized water (DDW) was used unless otherwise indicated. Buffer solutions (0.01 mol l^{-1}) were prepared by dissolving the appropriate amount of sodium dihydrogenphosphate dihydrate, sodium citrate, sodium oxalate, sodium borate decahydrate or anhydrous sodium carbonate in water. A stock solution of 1000 μg ml^{-1} La^{3+} was prepared from lanthanum nitrate hexahydrate purchased from Panreac (Barcelona, Spain; Reference 132669), by dissolving the appropriate amount of the salt in water. A 1000 μg ml^{-1} Ce^{3+} solution was made by dissolving the appropriate amount of cerium(IV) sulfate decahydrate (Panreac, Reference 1311248) in 20 ml of 2 mol l^{-1} HCl; 50 ml of 30% (m/m) H_2O_2 were added to reduce Ce^{4+} to Ce^{3+}, and finally dilution to the appropriate volume was made. A 1000 μg ml^{-1} Nd^{3+} standard (Specpure) was obtained from Alfa Products (Karlsruhe, Germany). Controlled-pore glass (CPG), diameter 240 Å and 120–200 mesh size, 3-aminopropyltriethoxysilane, lyophilized *Spirulina platensis* (Reference S-9134) and glutaraldehyde were all from Sigma (St. Louis, MO, USA). Nitric acid (60% m/m) and HCl (37.5% m/m) were obtained from Panreac. All manipulations were carried out in a fume cupboard, and in the cases of corrosive and toxic materials gloves were worn to avoid contact with the skin.

Immobilization Procedure for *Spirulina platensis*

A 0.025 g portion of bacteria was weighed into a 25 ml beaker and 0.05 g of NaOH dissolved in 10 ml of water was added; this procedure is the only one that gives a clear bacterial solution. It dissolves the whole sample and hydrolysis of proteins can occur. As the proteins contain residual cysteine, amines or polysaccharides and many functional groups, they enable a good preconcentration factor to be achieved and thus this treatment for dissolution is satisfactory, as it gives a very stable preparation and good capacity.

The mixture was placed on a steam-bath at 65 °C for 5 min with continuous stirring. The cooled solution was adjusted to pH 7.0 with 2 mol l^{-1} HCl and diluted to 25 ml with phosphate buffer of pH 7.0. The immobilization procedure on CPG was the same as previously described.[23] The immobilized bacteria (40 mg) were packed in a mini-column (inner diameter 2 mm; length 2.5 cm) of methyl methacrylate.

Binding Isotherms Evaluation

Binding isotherms are frequently used to describe adsorption processes. The batch procedure used to obtain binding data on the immobilized bacteria was performed in triplicate. According to the procedure, 8 ml of the test solution were passed through the column at a flow rate of 2 ml min^{-1}; the effluent was then collected in a 10 ml calibrated flask and diluted with buffer. This solution was analysed, and the metal concentration was evaluated from a calibration graph. The element adsorbed on the column was eluted with 100 μl of 1 mol l^{-1} HCl to waste, and the column was washed with water and buffer for use in the preconcentration of the next sample. The concentration of the adsorbed cations (La^{3+}, Nd^{3+}, Ce^{3+}) was calculated as the difference between the total element added and that determined in the effluent.

Capacity Studies

The batch method was used and the breakthrough was determined from the concentration of metal eluted as well as its concentration in the effluent. The procedure has been described previously.[23,24]

Preparation of Real Samples

For the direct aspiration method, 1.5 g of $Ce(NO_3)_3 \cdot 6H_2O$ (Merck, reference product 5326) were weighed and dissolved in 25 ml of DDW. The solution was spiked with standards of lanthanum and neodymium. For the preconcentration method, 0.05 g of $Ce(NO_3)_3 \cdot 6H_2O$ was weighed and dissolved in 1 l of DDW. Serial dilution was made with carbonate buffer (0.05 mol l^{-1}) for the determination of lanthanum and with phosphate buffer (0.01 mol l^{-1}) for the determination of neodymium.

Each sample was spiked with a known amount of the standard solution (La^{3+} or Nd^{3+}). The volume was made up to 25 ml with the appropriate buffer. Some metals present in the sample solution caused precipitation; this problem was solved by adding 0.05 g of Na_2EDTA and one drop of 0.2 mol l^{-1} NaOH to each 25 ml of sample. The resulting solution has a pH of 9.0 for La^{3+} in carbonate buffer and 7.0 for Nd^{3+} in phosphate buffer.

RESULTS AND DISCUSSION

Development of the Preconcentration Procedure. Selection of Instrumental Parameters

The wavelengths selected for analysis were 394.91, 408.672 and 401.225 nm for La^{3+}, Ce^{3+} and Nd^{3+}, respectively.

In order to assess the optimum integration time, values ranging between 0.5 and 3 s were tested. It was observed that the signal intensity increases with the integration time. When work is carried out with sequential spectrometers, the integration time of the detector must keep pace with the eluent flow rate, since with on-line measurements, samples do not stay in the detector for as long as desired. Therefore, an integration time as long as possible must be used in order to maximize the sensitivity and as short as possible to be able to establish when the sample passes through the detector. In this instance good results were obtained with an integration time of 2 s.

The effect of generator power on the intensity for known concentrations of lanthanides was investigated. The results are shown in Fig. 1. Between values of 1100 and 2100 W, the signal of Ce^{3+} increases with increasing power up to a certain value and then begins to decrease; this was not observed for lanthanum. This could be explained by the fact that La^{3+} needs more energy for atomization and excitation. The maximum

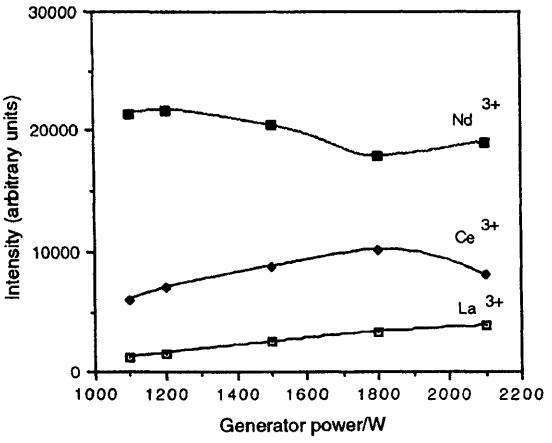

Fig. 1 Effect of generator power on the signal intensity of 1 μg ml^{-1} for La^{3+}, Ce^{3+} and Nd^{3+}. Lanthanum signal × 10^{-2}

intensity for Nd^{3+} was obtained at 1200 W, and this value was selected. Although the maximum intensity for Ce^{3+} and La^{3+} was obtained at 1800 and 2100 W, respectively, a generator power of 1500 and 1800 W was selected for Ce^{3+} and La^{3+} to save energy and decrease the noise level of the ICP at high generator power.

Effect of Flow Rate of Sample and Eluent

It can be observed that the preconcentration depends only slightly on the working flow rate. On the other hand, the elution of the sample retained on the column is more efficient with increasing flow rate, *viz.*, $\geqslant$2 ml min^{-1}. Under these conditions the whole sample is completely eluted from the column. Lower flow rates gave a lower intensity because of incomplete elution as the transient signal was taken before the elution was complete. Higher flow rates (2–3 ml min^{-1}) investigated showed that the flow rate has a negligible effect on the preconcentration procedures. Flow injection is used only to transport the sample *via* the column reactor. The lower intensity obtained below 2 ml min^{-1} was attributed to dispersion; by decreasing the elution flow rate, the dispersion in the nebulizer increases. Flow rates of 2 ml min^{-1} or higher produce no significant intensity changes. Indeed, this result was confirmed by other workers in similar studies,[22,26] who reported that the flow rate has a negligible effect on the preconcentration process and that dispersion in the nebulizer dominates all other types of dispersion.[27]

These optimized variables for La^{3+} are presented in Fig. 2. The optimum value for the sample flow rate was 2.0 ml min^{-1}; above a value of 3 ml min^{-1}, it was observed that there was

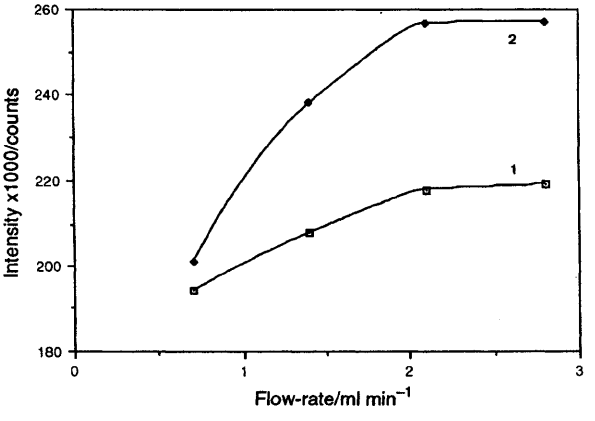

Fig. 2 Effect of flow rate of sample (1), and eluent (2) on the signal intensity of lanthanum (0.2 μg ml^{-1})

an increase in pressure and disconnection of tubes occurred. The eluent flow rate was also optimized and the result was found to be similar to that of the sample flow rate. As regards Ce^{3+} and Nd^{3+}, the behaviour was nearly identical.

Effect of pH on Preconcentration

Different buffer solutions were tested to assess the most appropriate buffer for each element that gives the maximum sensitivity in order to achieve a high preconcentration factor. These buffers were phosphate, carbonate, oxalate and borate (citrate buffer and NaOH–HCl medium were used only for lanthanum) at various pH ranges. In the preconcentration procedure, a volume of 5 ml of sample solution was initially made up in some or all of the above-mentioned buffers (at pH values of 5–10), and passed through the column. Then, the column was washed with water and eluted with acid. The washing process is a necessary step[26] to remove interstitial solution that otherwise would give a higher, erroneous capacity. Furthermore, the atomic emission signal is read in a much 'cleaner' medium than in the presence of large amounts of phosphate and sodium. In general, it improves the precision of the measurements. It should be noted that water does not elute any element in this study, only the acidic solution does; hence no loss of sample solution occurs as demonstrated in the recovery figures (Table 1).

Complete elution of the three lanthanides studied was carried out by injection of 100 μl of 1 mol l^{-1} HCl. No optimization of the acid concentration above this level was carried out. The change in sensitivity was taken as a criterion for the selection of the appropriate pH of the buffer tested. Fig. 3 shows the effect of pH for different buffers on the preconcentration capability or sensitivity. Fig. 3(*a*) shows the effect of pH and type of buffer on the preconcentration of Nd^{3+}. It was found that phosphate buffer of pH 7.0 was the appropriate buffer as a high signal intensity was obtained. Oxalate buffer was not appropriate as a very low signal intensity was obtained. This reveals that Nd^{3+} forms the most stable complexes with phosphate; Nd^{3+} competes for the functional groups on the bacterial resin, because this element has a much higher affinity to form stronger and more stable complexes with a particular functional group on the bacterial cell wall at pH 7.0 compared with the phosphate group. At lower pH values, protons will compete for the sites on the resin; hence, a lower intensity is obtained.[28]

Fig. 3(*b*) and (*c*) shows the effect of pH and buffer on the La^{3+} and Ce^{3+} uptake. Carbonate buffer of pH 9.0 was a good buffer for these elements compared with the other buffers tested as high uptake occurred. Also, oxalate buffer was not the buffer of choice as a weak intensity was obtained. NaOH–HCl, used here as a medium to adjust the pH, was a good complexing medium for lanthanum but carbonate was found to be better. Lanthanum and cerium coordinate with carbonate buffer at higher pH values (pH 9.0), which could be attributed[25] to the fact that at higher pH values the over-all micro-organism (alga) surface charge is negative; hence, both of these elements form the most stable complexes with carbonate compared with the other buffers. Therefore, the interaction with the functional groups of the bacteria as well as with the carbonate buffer is then possible, since the latter also forms complexes, although they are weaker. This shows the competition for the strong negative sites on the bacterial cell wall. After the maximum uptake under a certain pH had taken place, it was observed that this uptake levelled off; this phenomenon could be attributed to the formation of some hydroxides at higher pH values that compete for the metal, which results in lower uptake.

In conclusion, it was found that pH 9.0 (carbonate buffer) gave the maximum intensity for La^{3+} and Ce^{3+}. Nd^{3+} behaved

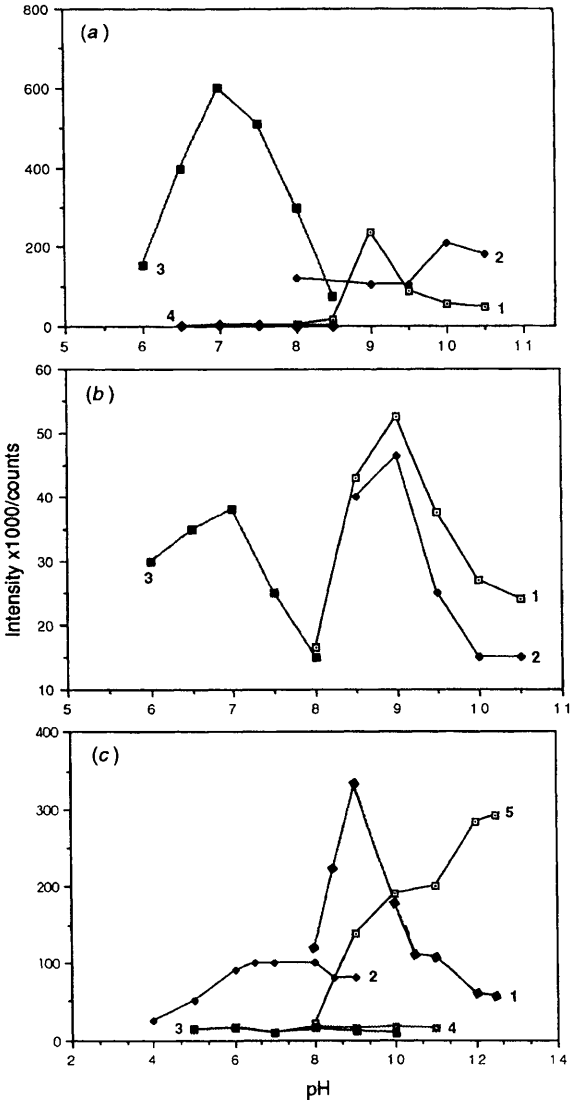

Fig. 3 Effect of pH and type of buffer on the signal intensity. (*a*) Neodymium, 1.5 μg ml^{-1}; (*b*) cerium, 0.25 μg ml^{-1}; and (*c*) lanthanum, 0.40 μg ml^{-1}. 1, Carbonate buffer; 2, borate buffer; 3, phosphate buffer; 4, oxalate buffer; and 5, NaOH–HCl medium

differently, as the maximum uptake occurred from phosphate buffer (pH 7.0). Oxalate buffer was not appropriate at any of the pH values tested for the three elements, as very poor uptake occurred.

Moreover, a higher sensitivity is obtained in phosphate buffer for Nd^{3+} (at pH 7.0) compared with the other buffers used. This can be rationalized in terms of the capability of the other buffers (oxalate, citrate, borate, carbonate) to form stronger complexes with Nd^{3+} compared with the complex formed with phosphate buffer at pH 7.0; hence Nd^{3+} competes more effectively for the stronger negative functional groups on the bacteria. On the other hand, La^{3+} and Ce^{3+} form weaker complexes in carbonate buffer compared with other buffers. Therefore, in this medium they compete for the negative functional groups on the bacteria.

At much higher pH values the uptakes levelled off and the intensity decreased; this could be because hydroxides compete with the functional group sites for the elements.[23,24] At lower pH values, protons will compete for the negative sites on the bacteria[26] and protonation of weakly basic coordinating groups will occur.[28] At the optimized pH values (7.0 for Nd and 9.0 for La and Ce) the over-all micro-organism charge is negative since these are high pH values. Thus, coordination or electrostatic attraction to these sites is easy and likely to occur.

Adsorption Isotherm and Equilibrium Constant Studies

Langmuirian adsorption assumes that the adsorption sites are equivalent and that the binding of a particular species is independent of the occupied binding sites.[29] Consequently, the binding reaction may be represented as a single equilibrium expression with the transposed Langmuir isotherm expressed as follows:

$$\frac{1}{a} = \left(\frac{k}{a_m}\right)\left(\frac{1}{c}\right) + \frac{1}{a_m} \qquad (1)$$

where a is the milligrams of metal adsorbed per gram of immobilized bacteria; k the equilibrium constant; c the equilibrium concentration (mg l^{-1}); and a_m the milligrams of metal adsorbed at saturation. If a single adsorption site was responsible for binding, a plot of $1/a$ *versus* $1/c$ should yield a straight line with a slope of (k/a_m) and an intercept of $1/a_m$. From this equation we can calculate the K values.

The adsorption of La^{3+}, Ce^{3+} or Nd^{3+} by *Spirulina platensis* is shown in Fig. 4. The lowest equilibrium concentration in the liquid phase of the three elements (La^{3+}) was below the ICP detection limits because the quantitative adsorption of this element was taken by the bacteria. Bacteria displayed a similar adsorption behaviour for Ce^{3+} and Nd^{3+}, indicating similar cell wall binding sites.[30] For these elements the isotherm diminished with the decrease in concentrations, while for La^{3+} the reverse behaviour occurred. These adsorption profiles are indicative of the particular metal solution environment and could be different in other matrices.[20] The isotherm for Ce^{3+}

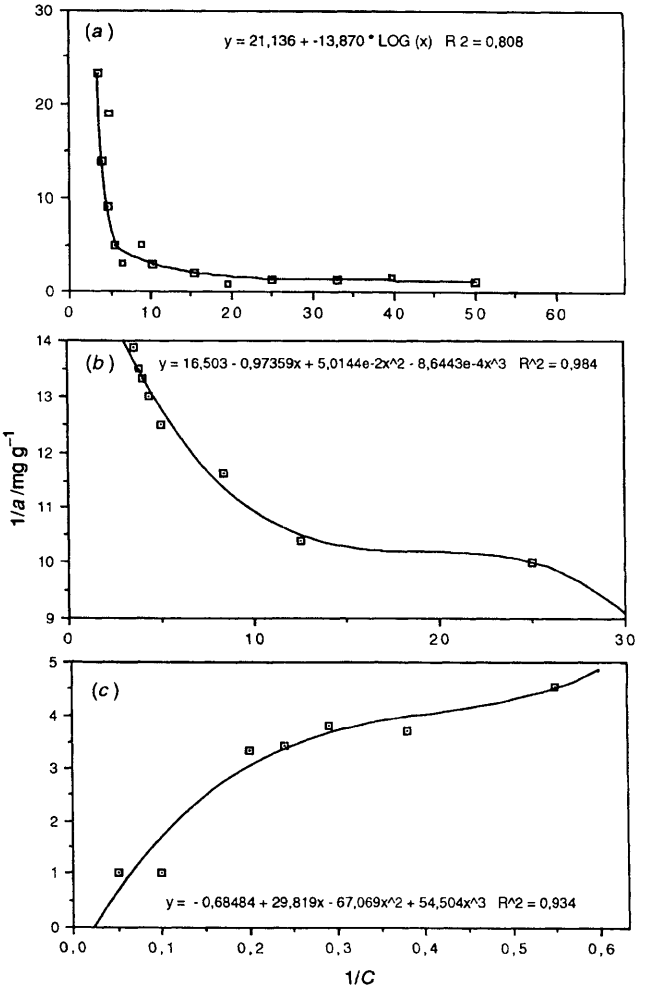

Fig. 4 Langmuirian isotherm plots for (*a*) cerium, (*b*) neodymium and (*c*) lanthanum

and Nd^{3+} [Fig. 4(*a*) and (*b*)] shows an anti-Langmuirian shape, indicating that the species that are first adsorbed facilitate the adsorption of additional species.[20] It was also observed that the uptake of neodymium at lower concentration levels is much higher than that of cerium. The situation with lanthanum is not comparable because it behaves in a Langmuirian fashion [Fig. 4(*c*)] but it was observed that the adsorption of La^{3+} generally took place at comparatively high concentrations. The latter argument verified that the uptake at lower concentrations increases in the order $La^{3+} < Ce^{3+} < Nd^{3+}$. This could be explained by the fact that lanthanides differ from each other in the number of electrons in the 4f orbitals, which are effectively shielded from interaction with the ligand orbitals by the electrons in the 5s and 5p orbitals. If hybridization is to occur, it must normally involve unoccupied higher energy-level orbitals (*e.g.*, 5d, 6s, 6p), and hybridization of this type can be expected only with the most strongly coordinating ligands.[31] Hence, we can conclude that the decrease in the radius of the cation from La^{3+} to Nd^{3+} is accompanied by an increase in the adsorption of metal ions in the order $La^{3+} < Ce^{3+} < Nd^{3+}$ at lower concentrations and the highest value of the enrichment factor is for neodymium, at 1 μg ml^{-1}, as will be discussed further.

Mahan and Holcombe[21] reported that for Ni^{2+}, Sr^{2+} and Mn^{2+}, the adsorption decreased with increasing concentration of the alga strain, while Fe^{3+} uptake by all algae strains shows a general increase as the concentration is increased. In our view, the apparent increase in extraction or adsorption efficiencies at low concentrations of Ce^{3+} and Nd^{3+} is an important factor, because it suggests an increase in the possible efficient adsorption at ultratrace levels. In previous studies,[18,20] quantitative uptake of Cd^{2+} occurred for solution concentrations under 1 μg l^{-1}. We believe that the latter achievement could be disadvantageous, because a breakthrough occurred at that very low concentration, and hence the value of the breakthrough capacity will be very low; this could affect the sensitivity of the method, as other elements which are present at high concentrations and with high formation constants could interfere in the determination.

From the Langmuir plot it is evident that there are at least two slopes, which is an indication of at least two adsorption mechanisms. As reported by Mahan and Holcombe,[21] a single formation constant cannot describe the micro-organism–metal binding over the concentration range of these studies.

Table 1 shows the stepwise formation constants evaluated from eqn. (1). These values throw some light on the stability or strength of metal binding with bacteria. For La^{3+} and Nd^{3+}, all values are less than one, indicating weak binding and a decreasing trend for K was evident. These low values have the advantage that they could facilitate the elution of these metals from the column. The K_1 value for Ce^{3+} is higher than that of La^{3+} and Nd^{3+}, which indicates that the strength of binding and the stability of the complex formed with the functional groups in bacteria increases with the increase in concentration. This shows that binding at lower concentrations facilitates additional binding sites. This behaviour confirms the complexity of the metal sorption mechanisms and has been exhibited by other micro-organisms.[29,32] Gardea-Torresdey *et al.*[33] have reported that cell wall modifications of functional groups such as thiol and amino groups show a concomitant decrease in gold adsorption, implicating both functionalities

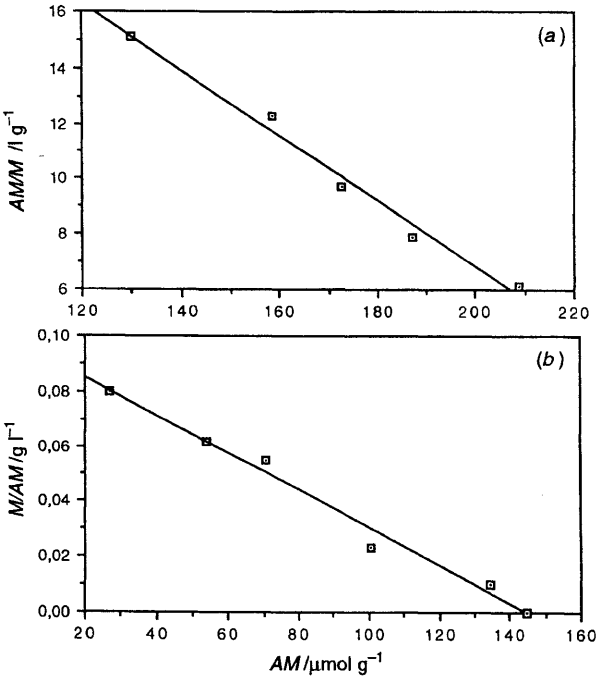

Fig. 5 Scatchard plot for (*a*) lanthanum and (*b*) cerium. *AM*, Lanthanum or cerium bound as μmol g^{-1} of immobilized bacteria; *M*, supernatant concentration of the free element as μmol g^{-1}

in binding. The first equilibrium constant calculated in this work for the three elements is higher than the reported values for Ni^{2+}, Sr^{2+} and Mn^{2+}, although it is difficult to make comparisons with the latter work, as the element and the method of calculation are different, but at least it gives some information on the different binding behaviour. Additional binding information was obtained by using the same procedure reported by Mahan and Holcombe.[21] Results for neodymium were ambiguous and more experimental work is needed. Fig. 5 shows the results for La^{3+} and Ce^{3+}. The values in Fig. 5(*a*) show the relationship between *AM*/*M versus AM*, *i.e.*, the amount of metal adsorbed for each separate concentration used divided by the supernatant concentration of the free metal in the corresponding experiment. It was plotted against the amount of metal adsorbed, which logically yields a straight line. As reported by Mahan and Holcombe, this shape is an indication of positive co-operative binding, in which the affinity of the receptor population increases with increasing metal binding. An estimation of the concentration of the adsorption sites for La^{3+} and Ce^{3+} by extrapolation to the *x*-axis and the calculated values were found to be 0.21 and 0.14 mmol g^{-1}, respectively. These values are much higher than those obtained for breakthrough capacity, which may reveal that additional binding sites are still available.

On-line Preconcentration and Direct Injection Assessment

Using the above established conditions, a 5 ml sample solution initially made up in the appropriate buffer for La^{3+}, Ce^{3+} and Nd^{3+} in the concentration range 0.1–1 μg ml^{-1} was preconcentrated[23] at a flow rate of 2 ml min^{-1}; the column was then washed with water and the metal ion accumulated on the

Table 1 Calculated stepwise equilibrium constants

Element	K_1	K_2	K_3	K_4	K_5
La^{3+}	0.84±0.02	55±0.03	0.38±0.04	0.29±0.01	0.24±0.01
Ce^{3+}	2.35±0.03	1.30±0.02	0.40±0.02	0.05±0.02	0.03±0.02
Nd^{3+}	0.68±0.02	0.64±0.01	0.66±0.01	0.35±0.01	0.21±0.01

column was eluted by injection of 100 µl of 1 mol l^{-1} HCl. The calibration graphs yielded straight lines. The relative standard deviation (s_r) for ten replicate injections of 1 µg ml^{-1} of each of the three elements was 1.3–1.5%. Calibration equations for direct aspiration over the range 1–30 µg ml^{-1} lanthanide, using the same manifold, without the column, had a good linearity. The mid-range s_r was 1.4–1.6%. Table 2 gives the analytical figures of merit for both methods. From the results obtained it is evident that much lower detection limits were obtained by preconcentration for all the elements investigated compared with direct aspiration. The improvement in detection limit was very high, the maximum improvement being obtained for Ce^{3+}. In our view this is related to the fact that in the blank signal, there must be some depression in the noise or signal caused as a result of treatment of the blank with EDTA. This reagent may complex some metals present in the buffer and as a result of this effect, signal depression occurred which affected the calculation of detection limits. The value of the detection limit obtained by preconcentration can be compared with that obtained by direct aspiration, where the blank is only de-ionized water and has no matrix effect. This agrees with the findings of Malamas *et al.*,[34] who reported that the addition of any complexing agent to the buffer components will change the range of quantitative uptake.

The detection limits reported by other workers[29] using a solvent extraction system for some REEs and inductively coupled plasma mass spectrometry (ICP-MS) as a detector were very low, being 0.29, 0.28 and 1.2 pg g^{-1} for La, Ce and Nd, respectively. The improvement in detection limit was only 10–11 times for these elements. Other workers,[35] who have used preconcentration by dialysis, reported a detection limit for Nd^{3+} of 0.84 ng ml^{-1}. The work reported by Esser *et al.*[36] also involved the preconcentration of some lanthanides by 8-hydroxyquinoline immobilized on silica and a chromatographic resin and their extraction in nitric acid from a sample volume of 1 l prior to their determination by ICP-MS. The detection limits achieved in the latter work for La, Ce and Nd were found to be 0.056, 0.033 and 0.049 ng ml^{-1}, respectively. In the present work, the detection limits achieved for these three elements are relatively high; however, compared with the work of Esser *et al.*, the present method is much better, it having the advantage of using only a 5 ml sample volume, a short preconcentration procedure and a high sample throughput. Moreover, in the present method the ICP-AES detector used is much less sensitive than the mass spectrometer used by Esser *et al.*; therefore, a much lower detection limit could have been achieved in the present work if these factors had been taken into consideration. Another factor presented in Table 2 is the increase in the sensitivity of the present method. The factor was calculated by comparing the slope obtained by the calibration graph with and without preconcentration. The increase in sensitivity was found to be 35–50-fold for the three lanthanides studied.

Calculation of Enrichment Factors

The enrichment factor (EF) has been defined as the ratio of the concentration of the species of interest after preconcentration compared with the initial concentration in the sample and thus describes the exact degree of preconcentration. Table 3 shows the EF results obtained for La^{3+}, Ce^{3+} and Nd^{3+}. The EF for these elements was high and Nd^{3+} gave rise to the highest value. A similar value of EF was obtained in a previous work on Nd.[35] EF values achieved in this work for La^{3+} and Ce^{3+} were higher than those in another work,[30] in which a value of only 10 was achieved.

The EF value depends on the concentration of the sample solution only until saturation of the column is achieved. Fig. 6 presents the effect of lanthanide concentrations on EF values. The EF value increases linearly with increasing concentration up to at least 1 µg l^{-1}, the highest concentration tested. These values of EF are similar to the increase in sensitivity values, thus showing that the effects of matrix, dispersion and spectral interferences in the ICP-AES determination were insignificant. These results coincide with those obtained by Tyson *et al.*[27] with AAS, who demonstrated that the contribution of the FI manifold (including the column) is minimal compared with the detector. The same results have been obtained by Israel and Barnes,[37] who measured the dispersion in ICP-AES, and reported that the splitting of sample in the nebulizer and spray chamber before its introduction into the plasma may also involve a secondary mixing effect that modifies dispersion behaviour; this phenomenon depends on the type of nebulizer.

Recovery

In order to determine the recovery of the preconcentration procedure, standard solutions of 0.5 µg ml^{-1} of La^{3+}, Ce^{3+} or Nd^{3+} were spiked with known concentrations (0.1–0.5 µg ml^{-1}) of these elements. Comparison of the evaluated concentration from a calibration graph by the preconcentration method with the concentrations added was used to calculate the recovery. As indicated in Table 3, the recovery values for all the elements are quantitative. Other workers[28] reported a quantitative recovery for some REEs including La^{3+}, Ce^{3+} and Nd^{3+} at concentrations of 1 µg l^{-1}, using a preconcentration procedure with solvent extraction and ICP-MS detection.

Capacity

The capacity was determined by the batch method described above using a 20 µg ml^{-1} concentration of the lanthanide elements; the values obtained are shown in Table 3. Approximately similar values were also evaluated from the breakthrough curve shown in Fig. 7. In this figure, metal uptake was plotted against the investigated concentrations; it was observed that the saturation level was achieved when the uptake of the metals remained constant on increasing the concentration level of the metal solution.[32] The average of these approximately similar values was taken for capacity measurement.

Generally, the breakthrough capacity is calculated from the amount of metal found in the effluent solution (after saturation) and the amount of metal eluted as described in this section. The breakthrough capacity calculated from the breakthrough

Table 2 Figures of merit obtained by the preconcentration method for 5 ml of sample (24 h^{-1} sampling frequency)

Element	La^{3+}	Ce^{3+}	Nd^{3+}
Detection limit (3*s*) by direct aspiration/µg ml^{-1}	0.50	0.06	0.02
Detection limit (3*s*) for preconcentration/µg ml^{-1}	0.009	0.21×10^{-3}	0.54×10^{-3}
Slope for direct aspiration/ml µg^{-1}	30.3	4.4	7.5
Regression coefficient (*r*), $n=5$	0.9999	0.9999	0.9980
Slope for preconcentration/ml µg^{-1}	1285	154	374
Regression coefficient (r), n = 5	0.9999	0.9990	0.9990
Increase in sensitivity	42.4	35.5	50.5
Improvement in detection limit	55	286	55

Table 3 Evaluation of EF and values of capacities and recoveries

Element	Concentration/ μg ml^{-1}	Direct aspiration*	Preconcentration*	EF	Capacity/ μmol g^{-1}	Recovery (%)
La^{3+}	1.0	13.9	498.0	36.0	17.0	100.2±0.04
Ce^{3+}	1.0	4.8	163.4	34.0	11.0	105.2±0.18
Nd^{3+}	1.0	7.3	370.0	51.0	13.0	100.1±0.20

* Signal intensity.

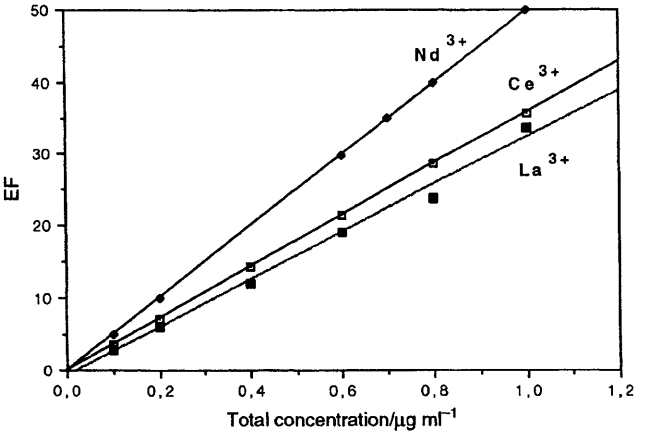

Fig. 6 Effect of metal ion concentrations on EFs

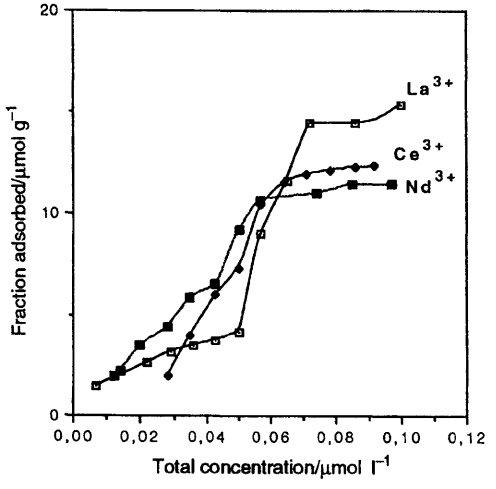

Fig. 7 Breakthrough curve for La^{3+}, Ce^{3+} and Nd^{3+}. Fraction of metal adsorbed *versus* total metal concentration

curve was only obtained for comparison with the batch methods (elution of metal from the column and metal found in the effluent). In this procedure 5 ml of the sample (20 μg ml^{-1}) were passed through the column at a flow rate of 2 ml min^{-1}; the effluent was then collected in a 10 ml calibrated flask and diluted to the mark with distilled water. The concentration of the metal in the effluent was found from the standard calibration graph. The capacity was evaluated from the following equation:

$$C = \frac{(C_i \cdot V_i - C_f \cdot V_f)}{W} \quad (2)$$

where C is the capacity in mmol g^{-1}, C_i is the initial concentration of the sample solution, C_f is the final concentration of the sample solution, V_i and V_f are the initial and final volumes used, respectively, and W is the mass in grams contained in the column.

The accumulated metal ions were eluted by injection of acid. The eluate was either sent directly to the nebulizer of the ICP or collected in a small calibrated flask as described above. The concentration of the metal eluted was found from the standard calibration graph by a preconcentration method. The two procedures were compared and were found to be similar, the average being taken.

The breakthrough capacity curve calculation was only carried out for comparison with the two batch methods. If a 40 ppm metal concentration were used, the same values and not higher capacity values would be obtained, because saturation levels were reached below 20 ppm, as the effluent from the column for each element contained an appreciable amount of that element. No similar work has been reported in the literature for breakthrough capacity for comparison, but, in our view, these values are not unfavourable considering the low levels of the REEs in general.[1]

Application to Real Sample Analysis

The concentrations of La^{3+} and Nd^{3+} present in a high-purity cerium(III) sample were determined by using the standard additions method. A solution of 1.50 g of $Ce(NO_3)_3 \cdot 6H_2O$ in 25 ml of water was spiked with different concentrations (0.5–1.0 μg ml^{-1}) of standards. Each solution was aspirated into the ICP-AES system for direct determination of both Nd^{3+} and La^{3+} using the established conditions for each element. The calibration equation for neodymium was $62.39x+162.65$ ($r=0.9996$) and for lanthanum $22.54x+421.9$ ($r=0.9942$).

Using the preconcentration method the concentrations of Nd^{3+} and La^{3+} in the sample were also calculated by using the standard additions method. A 10 ml volume of the previously prepared stock solution for the preconcentration method was diluted to 50 ml with carbonate or phosphate buffer for the determination of La^{3+} and Nd^{3+}, respectively. Then, 15 ml of either solution were spiked with different concentrations of the lanthanide standards (0.1–0.5 μg ml^{-1}) and diluted to 25 ml. A 10 ml portion of each of the latter solutions was preconcentrated and eluted as described previously. The equation of the calibration graph for neodymium was $497.37x+832.2$ ($r=0.9910$) and for lanthanum $363.6x+716.9$ ($r=0.9993$). The concentrations of Nd^{3+} and La^{3+} obtained by direct aspiration were found to be 0.022 and 0.016%, respectively, and those obtained by the preconcentration method were found to be 0.017 and 0.17%, respectively. These results agreed with the levels of concentration reported for lanthanum and neodymium in the analysed reagent. The preconcentration procedure was suitable for the determination of trace amounts of Nd^{3+} and La^{3+} in very dilute solutions of highly pure cerium(III) nitrate; the sample matrix had no significant influence on the method.

CONCLUSIONS

This work is the first investigation of the behaviour of some lanthanides on bacteria immobilized on CPG. The application of immobilized *Spirulina platensis* has been shown to provide a method for the preconcentration and determination of La^{3+}, Ce^{3+} and Nd^{3+} in aqueous systems. It has also been demonstrated that the method is capable of the efficient separation of La^{3+} and Nd^{3+} from the matrix. This automated method is simple and effective, with a high sampling frequency and

high EFs. Another advantage is its economy in use, since the bacteria are abundant and can be re-used in the immobilized form; there is also no need for solvent extraction. The precision of the method is between 1.3 and 1.5% expressed as s_r (n= 10). The capacity obtained in this work for all the elements tested was satisfactory considering the very low concentrations of the REEs in general.

The adsorption isotherm showed that lanthanum behaves differently from cerium and neodymium, thus indicating particular binding mechanisms; the results also suggest that the metal sorption processes are complex. The high values of the calculated adsorption site concentrations for lanthanum and cerium compared with the values of their breakthrough capacities indicate that further unoccupied absorption sites are available for binding; this finding might prove to be very useful in achieving greater binding, possibly by applying different conditions.

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