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A flow-injection renewable surface sensor for the fluorimetric determination of vanadium(V) with Alizarin Red S

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Abstract

Vanadium(V) is determined by a simple bead injection spectroscopy–flow-injection analysis (BIS–FIA) system with spectrofluorimetric detection using a commercially available flow cell (Hellma 176-QS). The 500 μl of a homogeneous bead suspension of an anionic resin (Sephadex QAE A-25) previously loaded with the fluorogenic reagent 1,2-dihydroxyanthraquinone-3-sulfonic acid (Alizarin Red S) was injected to fill the flow cell. Next, V(V) is injected into the carrier and reacts with the immobilized reagent on the active solid support placed in the flow cell to form a fluorescent chelate in the absence of surfactant agents. The complex is so strongly retained on the beads that the regeneration of the solid support becomes extraordinarily difficult, so needing the renovation of the sensing surface which is achieved by means of bead injection. At the end of the analysis, beads are automatically discarded from the flow cell and transported out of the system by reversing the flow.

The measurement of fluorescence is continuously performed at an excitation wavelength of 521 nm and an emission wavelength of 617 nm. Using a low sample volume of 800 μl, the analytical signal showed a very good linearity in the range 2–60 ng ml^{-1}, with a detection limit of 0.45 ng ml^{-1} and a R.S.D. (%) of 4.22 for 50 ng ml^{-1} of V(V) concentration ($n = 10$). The sensor showed both a good selectivity, which could also be increased by the addition of EDTA and F^- as masking agents, and applicability to the determination of V(V) in waters, physiological samples (serum and urine) and mussel tissues.

Keywords: Bead injection spectroscopy; Flow-injection analysis; Vanadium

1. Introduction

It is known that vanadium is an essential trace element due to its significant role in environment [1], industry and physiological systems, involving participation in various enzyme systems as an inhibitor and cofactor [2], catalysis of the oxidation of various amines and normalisation of sugar levels (alternative or adjunct therapeutic agent in diabetes) [3]. Moreover, laboratory and epidemiological evidence suggests that vanadium may also play a beneficial role in the prevention of heart-disease [4], no forgetting that it is toxic at ml^{-1} levels [5]. Nevertheless, at high concentration levels, vanadium is a potentially dangerous chemical pollutant that can play havoc with the entire agricultural system. This toxicity is due to excessive industrial exposure and from emission into the environment from refineries, steel and chemical industries, as a result of the combustion of petroleum derivatives [6].

A few of methods for the determination of vanadium(V) have been described. UV–visible spectrophotometry [7–10] and atomic spectrometry (ICP-MS [11], AA [12], etc.) are two of the principal tools that have been extensively used for its determination. Molecular fluorescence spectrometry is also an important analytical technique for quantitative determination of trace and ultratrace of V. This is usually preceded by a reaction of the metal with different reagents [13,14] or oxidation reactions of organic compounds such as anthraquinone derivatives [15–17] where the metal acts as catalyst. In other fluorimetric methods, the fluorescence intensity has been greatly enhanced by addition of effective

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activators such as cationic surfactants [18]. As an alternative to conventional fluorimetry, a new spectrofluorimetric method is proposed.

Nowadays, there is a demanding need for the development of automatic analytical methods capable of detecting a large variety of metals in different media. Flow-injection analysis (FIA) methods have become an important means in these analyses. The large diffusion and utilisation of FIA is mainly due to their good properties: stability, robustness, reliability, no expensive, etc. Perhaps, two of the inconveniences of these methods, in some occasions, are the lack of sensibility and selectivity at low concentration levels. In order to overcome this problem, flow-injection (FI) can be used in combination with solid phase spectrometry (SPS) using different supports to obtain high sensitivity combined with a relatively simple procedure and good selectivity. These systems are called flow-through sensors [19], and beads of different material nature are acting as surface media for retaining reagents/catalysts/analytes/reacting products. Beads act as solid phase extractor, as the analyte is absorbed or exchanged on their surface and can be separated from the sample, and also as solid reagent, as the absorbed species reacts with the bead surface promoting a physical change in it which can be monitored by using an appropriate detector.

In this FI-SPS methodology it is possible that: (1) the species of interest is strongly retained, so the regeneration of the support becomes extraordinarily difficult, or (2) there is lost in the retention efficiency. In these cases a new methodology based on the concept of bead injection spectroscopy (BIS) [20] can be used, being beads surface renewed after each cycle. This methodology, flow-injection renewable surface (FI-RS) sensing methodology [21], can be seen as the third generation of FI microanalytical techniques. Although this concept was first introduced to use with sequential injection analysis (SIA), demonstrating that this new concept is feasible, it also can work with FIA (low-cost instrumentation) using a commercial flow cell (Hellma 176 QS) [22] as alternative to a jet ring cell [20,23].

The aim of this work is the development of a new flow method for the determination of V(V) in different media based in the continuous measurement of the fluorescence complex formed between V(V) and 1,2-dihydroxyanthraquinone-3-sulfonic acid, which is sorbed on anionic resin beads. The reagent, also called Alizarin Red S (ARS), is one of the most useful photometric reagents for the determination of metals because it forms chelates with a multitude of metal cations [24], but it has only been used for the fluorimetric determination of few elements (boron [25], molybdenum [26], etc.). In the proposed sensor, based on a system previously proposed by García Campaña et al. [18], the immobilization of the reagent in the solid support makes possible the production of a more rigid environment in which the fluorescence of the binary complex is enhanced and, in addition, very favourable selectivity conditions are introduced. The most important contributions of this new system are both (1) the renovation of the sensing surface for each individual sample analysis due to the difficulty of regenerating the sensing surface, and (2) the high sensitivity, avoiding the use of activators. The procedure is very simple, inexpensive and fast, and allows the selective determination at trace levels of V(V) in waters, physiological samples (serum and urine) and mussel tissues, without the use of extraction or pre-concentration off-line steps.

2. Experimental

2.1. Chemicals

All experiments were performed with reagents of analytical-reagent grade, pure solvents and deionized water (used for the dilution of samples and reagents).

Standard vanadium(V) solution (100 μg ml^{-1}) was prepared by dissolution of the appropriate amount of NH_4VO_3 (Panreac, Barcelona, Spain) in deionized water. Working solutions were daily prepared by appropriate dilution of this one with deionized water. Sodium 1,2-dihydroxyanthraquinone-3-sulfonate (Carlo Erba, Milano, Italy) solution (1×10^{-3} M), prepared weekly, was used as fluorescent reagent. Adequate fresh solution of reagent was prepared every day by appropriate dilution with deionized water. Rest of solutions were stable more than one month when they were protected from sunlight and kept at about 5 °C in a refrigerator.

A 0.15 M KCl solution (Panreac, Barcelona, Spain) at pH 7.5 was used in the FIA experiments as carrier solution.

Sephadex QAE A-25 anion exchanger gel (Aldrich, Madrid, Spain) was used in the chloride form as beads (40–120 μm, capacity: 3.1 meq/g). Another tested solid supports were Sephadex DEAE A-25, Sephadex SP C-25 and C_{18} bonded silica.

2.2. Apparatus

A chromatography column (i.d.: 16 mm, $l = 30$ cm) was used in order to obtain a homogeneous aqueous suspension of ARS-loaded beads (Sephadex QAE A-25) by purging air gently through it.

Fluorescence emission measurements were obtained with a Varian Cary-Eclipse Fluorescence Spectrofluorimeter (Varian Iberica, Madrid, Spain). The spectrofluorimeter was equipped with a xenon discharge lamp (75 kV), Czerny-Turner monochromators, two detectors (sample and internal reference), an R-928 photomultiplier tube which is red-sensitive (even 900 nm) with manual or automatic voltage controlled using the Cary-Eclipse software for Windows 95/98/NT system. Instrument excitation and emission slits were set at 10 and 20 nm, respectively, and the scan rate of the monochromators was 120 nm min^{-1}.

Two four-channel Gilson Miniplus-3 peristaltic pumps with rate selector were used to generate the flow stream and

to perform bead discharge. A Hellma 176-QS quartz flow-through cell with a light pathlength of 1.5 mm (25 μm inner volume) was used to accommodate the resin beads. Some glass-wool was placed in the outlet to retain the resin gel beads. The amount of injected resin was the sufficient one to cover all the detection area.

Three variable-volume Rheodyne Model 5041 rotary valves with a single tube loop were used, two of them acting as injection valves (V1 for beads injection and V2 for sample injection) and the other one as selecting valve (V3 for bead discharge by flushing them out of the cell). Teflon tubing of 0.8 mm i.d. was also used in all cases.

Other apparatus consisted of a Crison Model 2002 pH-meter with a glass/saturated calomel combination electrode and a Selecta Ultrasons ultrasonic bath (Barcelona, Spain).

2.3. Procedure

Manifold is shown in Fig. 1.

Initially, in the chromatography column, 1 g of beads (Sephadex QAE A-25) were added to 30 ml of a solution of fluorescent reagent (ARS) 1×10^{-4} M. The mixture was maintained continuously as a homogenized suspension by purging air so getting the immobilization of the reagent. Flow rate was maintained at 1.40 ml min^{-1} (Pumps 1 and 2 working simultaneously).

The 500 μl of this bead suspension were aspirated, injected through the injection valve (V1), transported and loaded into the flow cell. So, beads were injected, retained and monitored, obtaining good reproducibility. Beads were trapped in the cell and perfused with the carrier stream, which was a 0.15 M KCl solution, pH 7.5. Sample solution containing V(V) was injected by pumping through the injection valve (V2) into the carrier stream so reaching the flow cell. For 800 μl of sample solution, concentrations were in the range 2–60 ng ml^{-1}. When the analyte reached the bead surface the reaction between V(V) and immobilized ARS was accomplished and the analytical signal developed. The RFI (relative fluorescence intensity) was recorded and measured continuously using an excitation wavelength of 521 nm and an emission wavelength of 617 nm. After the maximum RFI was reached, the sensing bead surface was not regenerated. At this moment, Pump 1 was switched off and Pump 2 used in flow reversal, so the cell was emptied, the beads being automatically discarded from the flow cell at the end of the assay cycle through valve (V3). The renewable surface fiagram is registered along all the process. All samples were analysed by triplicate.

2.4. Treatment of samples

A 0.5% (w/v) potassium permanganate solution was added dropwise to all real samples until the sample showed a definitive colour due to the permanganate, in order to assure that all vanadium was V(V).

2.4.1. Waters

Tap and well waters, kept at 5 °C, were filtered through a 0.45 μm Millipore membrane filter.

2.4.2. Urine and human serum

Urine and serum samples were kindly supplied by "Ciudad de Jaén" hospital. An equivalent volume of 0.6 M trichloroacetic acid solution was added to both samples in order to deproteinize and then the samples were centrifuged at 5000 rpm for 5 min and filtered through a 0.45 μm membrane filter (Millipore). Aliquots (1 ml) of these samples were spiked with appropriate amounts of V(V) and diluted to 10 ml to bring the metal concentrations within the linear range of the calibration graph.

2.4.3. Mussel tissues

Samples, placed in a quartz crucible, were dried in a forced-draft oven at 70 °C to constant mass and then ground

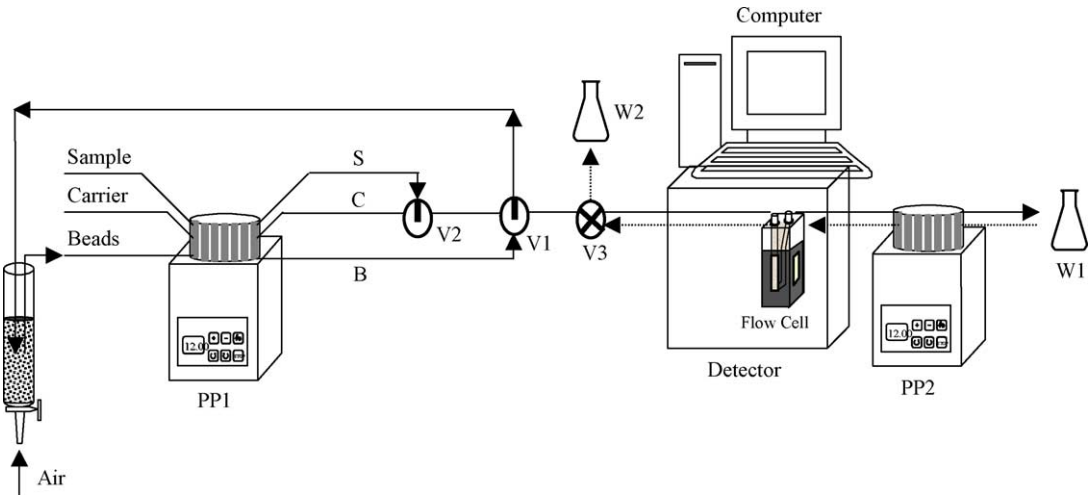

Fig. 1. Manifold. S: sample; C: carrier; B: beads; PP1, PP2: peristaltic pumps; V1, V2: injection valves; V3: selection valve; W1, W2: wastes.

into a fine powder. A suitable aliquot was weighed (50.13 g of dry mussel tissues) and its mineralization was carried out by heating slowly to 550 °C for 2 h and holding at this temperature for 12 h. The sample was removed and cooled and the ashes were wet carefully with deionized water, then added 2 ml of 4 M NaOH solution. The quartz crucible was covered with a watch glass, heated cautiously on a hot plate to boiling and finally let stand for a few minutes. Then, the solution was filtered through Whatman no. 42 paper over a 100 ml standard flask and the residues washed, diluting finally to volume with deionized water previous neutralization with concentrated HCl.

3. Results and discussion

3.1. Spectral characteristics and volume of bead suspension

Different types of ion-exchangers with different functional groups were tested in order to retain the fluorescent reagent: anion exchange beads on dextran (Sephadex QAE A-25, Sephadex DEAE A-25), cation exchange beads on dextran (Sephadex SP C-25). And non-polar sorbents (octadecyl silane C_{18}) was also tested. Due to the anionic nature of ARS in aqueous solution, the most suitable type of support was Sephadex QAE A-25 because of its high capacity to retain strongly the reagent and reproducibility in the bead suspension injection.

ARS reacts with V(V) to form a fluorescent complex in aqueous medium, but its low emission intensity is not appropriate to establish a sensitive fluorimetric determination of vanadium. The fluorescence spectra of the complex in aqueous solution showed maximum excitation and emission wavelengths at 517 and 610 nm, respectively. Sensitivity could be enhanced when a suitable activator was chosen. For example, the use of cationic surfactant agents [18] or ligands [10] increases the RFI of the V complex in aqueous solution without producing changes in the excitation or emission maxima.

As a result of the retention of the reagent on the solid sensing support, in absence of activators (surfactants agents or ligands), the obtained signal was greatly higher than that obtained in aqueous solution. The spectra of the complex fixed in Sephadex QAE A-25 beads showed the maximum excitation and emission wavelengths at 521 and 617 nm, respectively. Comparing spectra on beads and homogeneous solution, working in the same conditions and in the same flow cell, a bathochromic shift in both maxima emission and excitation wavelengths was found. This can be attributed to the modification of the surrounding environment of analyte on the solid phase with respect to the solution. The sorption of complex on the beads (sample volume of 800 μl) resulted in an incredible signal approximately 4000 times higher than that obtained in aqueous solution and in the absence of surfactants agents. This was the result of the pre-concentration of the analyte on the active solid support in the detection area itself of the spectrofluorimeter.

The influence of the slit widths of excitation and emission was studied, and it was observed that both an increase in the emission slit width and in the excitation slit width increased the fluorescence signal. The slit widths were tested in the range 1.5–20 nm in both cases. The effect of photomultiplier tube detector voltage on the fluorescence measurements was studied in the range 400–1000 V, in order to get the optimum detector voltage value. Increasing slit widths or voltage resulted in increasing fluorescence signal of both the complex and the baseline (reagent + beads). For these reasons, slit widths of 10 and 20 nm for excitation and emission, respectively, and a voltage value of 800 V were adopted as a compromise between sensitivity and low baseline, because higher values would not allow the determination of V(V) as the measurement range would be too narrow.

As beads are injected in the system by means of an injection valve, the uniformity and homogeneity of beads and its volume must be studied. Sephadex QAE A-25 beads show satisfactory uniformity, because these beads are similar in size and not packed in the cell in different way. When 1.0 g of Sephadex QAE A-25 beads were added to 30 ml of ARS solution in a chromatographic column, the homogeneity of the bead suspension could be easily obtained by purging air continuously through this suspension, and reproducible amounts were injected in the cell. The volume of beads suspension injected was optimised by varying between 100 and 800 μl. Below 500 μl, beads injected did not cover completely the whole flow-through irradiated zone of the detection unit (only a small zone of the beads was irradiated). Beyond 500 μl, the fluorescence emission started to decrease due to the dispersion of the analyte on the sensitive beads. Therefore, 500 μl of homogenized suspension of beads (Sephadex QAE A-25) with immobilized ARS was chosen as appropriate volume for the following experiments.

3.2. Nature and concentration of the carrier and pH of the sample

The influence of the pH of the carrier solution on the fluorescence intensity was investigated adjusting with NaOH or HCl in the range 1–12, and an electrolyte (KCl 0.10 M) was added in order to facilitate V(V) to reach the totally irradiated area quicker, because without this electrolyte, the complex was retained strongly on the previous zone of the beads which was not irradiated by the instrument (2 mm above the cell window, approximately). The optimum range pH for the formation of the complex was in the range 5–9. At pH values below 5 and above 9 fluorescence signals decreased significantly. For all measurements, pH 7.5 was chosen as optimum value.

Various electrolytes were tested at pH 7.5 using a concentration 0.1 M: NaCl, KCl, $NaNO_3$ and Na_2CO_3. KCl was selected because the analytical signals were slightly higher with respect to the other electrolytes. Various concentration levels of KCl were tested, between 0.01

and 0.3 M, obtaining the best result with a concentration 0.15 M. Higher concentrations caused a decreasing of analytical signal, and lower concentrations kept the analyte above the irradiated zone. At this value, 0.15 M, different buffer solutions ($H_2PO_4^-$/NaOH, $H_2PO_4^-$/HPO_4^{2-}) were also tested (pH 7.5), producing lower, less stable and less reproducible analytical signals than KCl. Therefore, KCl 0.15 M solution was used as carrier solution.

The influence of sample pH was examined in the range between 2 and 12. The sample pH values between 5 and 9 did not influence the analytical signal, so there is no need to adjust the pH sample in this range. Although most of the tested samples have a pH value between 5 and 9, it would be necessary to adjust this variable when its value is out of this range.

3.3. Concentration of reagents

ARS could be introduced in the system by means of an injection valve or adding it directly on the beads in the chromatography column. The second option is chosen due to different advantages respect of the first one: a better distribution of the reagent on the beads surface, a simplification of the manifold and a higher sampling frequency.

ARS concentration was tested in the range 10^{-5} to 10^{-3} mol l^{-1}, always using 1.0 g of beads and 30 ml of ARS solution, by injecting a sample solution containing 80 ng ml^{-1} of V(V). This concentration was enough for allowing the total reaction of the analyte. The analytical signal increased up to an ARS concentration of 1×10^{-4} mol l^{-1}; higher concentrations did not increase the signals significantly, therefore this concentration was used in all experiments.

3.4. FIA variables

The effect of the flow rate was investigated by injecting a sample solution of the analyte (40 ng ml^{-1}) at different flow rates. Increasing flow rate resulted in decreasing peak heights and caused over-pressure in the system but it was possible to obtain a higher sampling frequency. The flow rate was studied in the range 0.65–1.55 ml min^{-1}. A flow rate of 1.40 ml min^{-1} working with both peristaltic pumps, simultaneously, was adopted as a compromise between sensitivity and throughput. The reversed flow rate of P2 can be increased to 2.25 ml min^{-1} in the elimination of the beads from the flow cell.

The effect of the sample volume was studied. Using sample volumes between 40 and 1600 μl of 40 ng ml^{-1} vanadium standard solution, the increase of fluorescence was linear in the range 40–1000 μl (higher volumes did not increase significantly the signal). The use of a high sample volume involved an increase in the fluorescence intensity and in the fixation time, due to the fixation of a higher amount of analyte on the solid support. The volume chosen in order to calibrate the system for V(V) determination was 800 μl.

Table 1
Figures of merit for V(V) determination

Parameter	V(V)
Linear dynamic range (ng ml^{-1})	2–60
Calibration graph	
Intercept	14.12
Slope	10.73
Correlation coefficient	0.9997
Detection limit (ng ml^{-1})	0.45
Quantification limit (ng ml^{-1})	1.50
R.S.D. (%) ($n = 10$)[a]	4.22
Sampling frequency (h^{-1})	18

[a] Using a vanadium concentration of 50 ng ml^{-1}.

3.5. Analytical parameters

Table 1 contains the figures of merit of the proposed method using a sample volume of 800 μl. The calibration graph was constructed under the optimum working conditions, according to the procedure described above. The data were fitted by standard least-squares treatment and the calibration equations are shown. The proposed methodology was able to produce analytical fits with good linearity in the range 2–60 ng ml^{-1}. Fig. 2 shows the fiagram obtained with its corresponding calibration graph.

The reproducibility was established for ten independent analyses of sample solutions (800 μl) containing 50 ng ml^{-1} of V(V). The detection limit was estimated as the concentration of analyte which produce an analytical signal equal to three times the standard deviation of the background fluorescence [27]. The sensitivity obtained is high compared with other proposed methods [9,18]. The quantification [28] limit ($K = 10$) and the sampling frequency were also evaluated.

3.6. Effect of foreign ions

A study of the effect of foreign ions was made by adding different amounts of other ions to a solution containing

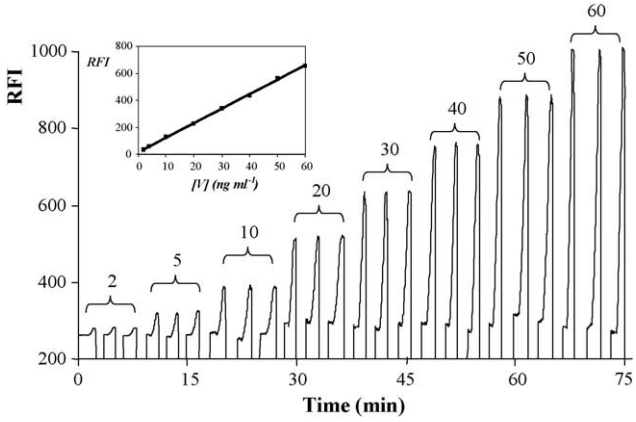

Fig. 2. Fiagram obtained in the calibration of the sensor for V(V). Concentrations: 2, 5, 10, 20, 30, 40, 50 and 60 ng ml^{-1}. Inset: corresponding calibration line.

Table 2
Interference study (determination of 40 ng ml^{-1} of V(V))

Foreign ions	Tolerated interferent/analyte (w/w) ratio
NO_3^-, SiO_3^{2-}, CO_3^{2-}, SO_4^{2-}, Cl^-, Na^+, K^+	>500
Ni^{2+}, Co^{2+}	100[a]
Mg^{2+}, Ca^{2+}, Mn^{2+}, Sn^{2+}, Zn^{2+} [b], Cu^{2+} [b], Cd^{2+} [b]	50
Be^{2+}, Al^{3+} [b]	10
Fe^{3+} [c]	2

[a] Maximum ratio tested.
[b] Tolerance ratio in the presence of 1 μg ml^{-1} EDTA.
[c] Tolerance ratio in the presence of 100 μg ml^{-1} F^-.

40 ng ml^{-1} of V(V). A foreign species was considered not to interfere if it produced an error smaller than ±5% in the analytical signal. If any interference was observed, the ratio interference:analyte (w:w) was reduced progressively until this interference ceased. Results are summarized in Table 2.

The most serious interferents were Al^{3+} and Fe^{3+} which react with ARS under these conditions. EDTA and F^- solutions were chosen to mask them, respectively, in order to increase the selectivity of the proposed system. The study of the appropriate concentration of EDTA and F^- in the sample solution was performed by varying from 0.1 to 200 μg ml^{-1} in both cases. An EDTA concentration of 1 μg ml^{-1} and a F^- concentration of 100 μg ml^{-1} were enough to produce an appropriate increase of tolerance levels to these foreign species in V(V) determination, so these values were selected for the proposed method. When EDTA concentration was higher than 1 μg ml^{-1} a competition of both reagents (EDTA and ARS) was produced, so decreasing the analytical signal of the complex. The tolerance level to Al^{3+} and Fe^{3+} without masking agents were 2 and 0.1, respectively, which makes impossible the determination of V in samples with these metals at these levels. The new tolerance levels achieved by adding EDTA or F^- are shown in Table 2, getting increases of 5 and 20 times, respectively. These tolerance levels are 500 (Al^{3+}) and 2000 (Fe^{3+}) times higher than those for homogeneous solution method. The tolerance levels to Zn^{2+}, Cu^{2+} and Cd^{2+} were also improved with an increasing of 10 times by adding EDTA solution.

From these results it can be said that the proposed method is highly selective in spite of using a poorly selective reagent, due to the strong retention of the V-ARS complex on the beads and its consequent separation from the matrix and from the rest of cations, and the possibility of dissociating others metal-ARS complexes on the beads surface with the help of masking agents.

4. Applications

In order to check the applicability of the proposed methodology for quantitative determination of vanadium, it was applied successfully to different types of waters (well and tap water), physiological samples (human serum and urine) and mussel tissues. In all instances, the applications were performed using 800 μl of sample volume, and after treatment and suitable dilution to fit the concentration of the analyte within the linear calibration range the samples were injected by triplicate.

Table 3
Analytical applications

Sample	Amount added (ng ml^{-1})	Amount found (ng ml^{-1})	Recovery ± R.S.D. (%)[a]
Tap water	10.0	9.6	97 ± 4
	20.0	19.3	96 ± 5
	30.0	29.7	99 ± 2
Well water	10.0	10.7	107 ± 5
	20.0	19.6	98 ± 5
	30.0	30.1	100 ± 2
Human urine	10.0	10.8	107 ± 3
	20.0	21.0	105 ± 2
	30.0	29.8	99 ± 4
Human serum	10.0	10.7	107 ± 4
	20.0	19.1	96 ± 3
	30.0	30.6	102 ± 3

[a] Relative standard deviation (average of three determinations).

Recovery studies were carried out to check the accuracy of the proposed method on different samples of water and physiological samples. All samples were found to be free from vanadium, so the samples were spiked with known amounts of V(V). For serum and urine samples a standard addition method was used owing to the matrix effect found. The recovery rates (Table 3) in all cases are good, between 96 and 107%, and showed relative standard deviations lower than 5% in all cases.

In the case of mussel tissue sample, it was necessary to adjust the sample pH to a neutral value due to strong basic medium used to dissolve these samples. The content of V(V) found in the sample was 2.14 ± 0.06 μg g^{-1} (medium value ± standard deviation), which was also evaluated using ICP-MS as reference method obtaining 1.99 ± 0.03 μg g^{-1}. The statistical study of precision and accuracy of both methods was performed from *F*-criterion and the *t*-test, respectively [29], at 95% confidence limits. The obtained results, t_{calc} = 0.073 (theoretical value 2.772) and F_{calc} = 3.325 (theoretical value 39.00), show that there is no significant difference between both methods with regard to accuracy and precision.

All these results indicate the utility of the proposed method for routine analytical control in all the tested samples.

5. Conclusions

The concept of BIS–FIA has been exploited to develop a renewable surface fluorimetric sensor for V(V). The fluorimetric reagent (ARS) is loaded on the anion exchange beads. After injecting the beads with ARS sorbed, the baseline is

established and the arrival of V(V) to the active surface originates a strong fluorescence signal. This signal is monitored on the solid phase beads trapped in the cell just in the irradiated zone. The sensor shows a sensitivity (4×10^3)-fold higher than the same system without using beads. Selectivity is also drastically improved as the analyte is separated from the sample plug when it reaches the renewable sensing support. In general, the proposed system shows better characteristics of sensitivity [9,18,30,31] and selectivity [8,32] compared with other spectroscopic methods.

Therefore, the procedure developed has demonstrated suitable analytical performances to determine V(V) at ng ml^{-1} levels in several kinds of samples: waters, urine and serum, as well as mussel tissues.

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