

## Sensitive Spectrofluorometric Determination of Vanadium with Sodium 1,2-Dihydroxyanthraquinone-3-sulfonate in Cationic Micellar Medium

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A spectrofluorometric method is described for the determination of vanadium(V) based on its complexation reaction with sodium 1,2-dihydroxyanthraquinone-3-sulfonate in presence of hexadecyltrimethylammonium bromide. The fluorescence is monitored at 607 nm with excitation at 505 nm. The experimental variables and interferences in this determination have been studied. The detection limit is 3.0 ng ml<sup>-1</sup> and the linear range is between 10 and 1000 ng ml<sup>-1</sup>. The method has been applied to the determination of vanadium in clams and seawater with satisfactory precision.

**Keywords** Spectrofluorometry, vanadium determination, sodium 1,2-dihydroxyanthraquinone-3-sulfonate, micellar medium

Vanadium is an essential trace element to man and animals, possessing specific physiological functions.<sup>1</sup> Generally, concentrations determined in land plants and animals are less than those found in marine forms.<sup>2</sup> However, numerous reports have warned of the toxic effects of vanadium resulting from excessive industrial exposure and from emissions into the environment from refineries, iron, steel and chemical industries as a result of the combustion of petroleum derivatives, which contain vanadium in the form of porphyrin complex.<sup>3</sup> The toxicity of vanadium compounds increases with valence, the pentoxide being the most toxic.

In the literature a few methods for the fluorometric determination of vanadium(V) are described. The oxidation reactions of anthraquinone derivatives have been used for the determination of vanadium(V). The anthraquinones used were 1,4-diamino-5-nitroanthraquinone<sup>4</sup>, 1-amino-4-hydroxyanthraquinone<sup>5,6</sup>, sodium 4,8-diamino-1,5-dihydroxyanthraquinone-2,6-disulfonate<sup>7-9</sup> and sodium 1,3-dihydroxy-4-aminoanthraquinone-2-sulfonate.<sup>10</sup> In all cases, the oxidation products are formed under certain conditions: as strongly acidic media and high temperatures (80–85°C).

The sodium salt of 1,2-dihydroxyanthraquinone-3-sulfonic acid (Alizarin Red S, ARS) has been widely used as a photometric reagent<sup>11</sup>, but it only has been used for the fluorometric determination of boron<sup>12,13</sup>, aluminum and gallium<sup>14</sup>, niobium<sup>15</sup> and molybdenum.<sup>13,16</sup> Of these,

the fluorescence intensity has been greatly enhanced for the determination of molybdenum and niobium by addition of a cationic surfactant.

These micellar media can improve the fluorescence determination of metals, presenting several advantages over conventional homogeneous solution techniques including increased sensitivity, reduced interferences and enhanced experimental convenience.<sup>17-19</sup>

In the present work, a spectrofluorometric method for determination of vanadium is proposed. The fluorescence of the complex formed between Alizarin Red S and vanadium(V) is developed in the presence of a cationic surfactant and the influence of the concentration is studied.

The method has been successfully applied to the determination of V in clams and seawater. Compared with other fluorometric methods, the proposed determination is quite sensitive, offers shorter analysis time and a more extended linear working range.<sup>10</sup>

### Experimental

#### *Reagents and chemicals*

All experiments were carried out with analytical-reagent grade chemicals using doubly distilled and demineralized water.

Sodium 1,2-dihydroxyanthraquinone-3-sulfonate solution (1.0×10<sup>-3</sup> mol dm<sup>-3</sup>): prepared by weighing exactly 0.171 g of the reagent (Carlo Erba, Milano, Italy)

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and diluting to 500 cm<sup>3</sup> with doubly distilled water.

Vanadium stock solution (0.100 g dm<sup>-3</sup>): prepared from NH<sub>4</sub>VO<sub>3</sub>, (Merck, Darmstadt, Germany). The concentration of vanadium(V) was gravimetrically standardized<sup>20</sup> as AgVO<sub>3</sub>. Working solutions were prepared by the appropriate dilution of the above solution.

Buffer solution (pH 7.7): prepared from 0.1 mol dm<sup>-3</sup> tris(hydroxymethyl)aminomethane and hydrochloric acid (Tris-HCl).

Surfactant solutions: stock solutions of the following surfactant agents were prepared: sodium dodecylsulfate (SDS) (Merck, 5% m/v), polyoxyethylene(9,5) *p*-*t*-octylphenol (Triton X-100) (Merck, 10% m/v), hexadecyltrimethylammonium bromide (HTAB) (Merck, 5% m/v) and cetylpyridinium chloride (CP) (Merck, 5% m/v).

8-Hydroxyquinoline solution (0.5% in chloroform): prepared from the reagent (Merck) by dissolving in chloroform (Merck).

#### Apparatus

Fluorescence was monitored with a Perkin-Elmer (Norwalk, CT, USA) Model MPF-66 spectrofluorometer, equipped with a 150-W xenon arc lamp and an R-928 photomultiplier. All measurements were performed in standard 10-mm path length quartz cells, thermostatically controlled at 25±0.5°C with a water-bath circulator (Frigiterm S-382, J.P. Selecta, Spain). The spectrophotometer was connected to a Perkin-Elmer Model 7300 Professional Computer provided with PETLS application software (C 646-0280). A 101 Rhodamine quantum counter sample (Perkin-Elmer) was used for source intensity adjustment. Fluorescence data are given without spectral corrections.

A Crison Digit-501 pH meter (Crison Instrument, Barcelona, Spain) was used for all pH measurements.

A mechanical shaker (Vibromatic-384, J.P. Selecta, Spain) and a centrifuge (J.P. Selecta, Spain) were used for extraction purposes.

#### Procedures

**Basic procedure.** Into a 10 cm<sup>3</sup> calibrated flask, a suitable aliquot containing between 0.1 and 10 µg of V was transferred. Subsequently, 2.0 cm<sup>3</sup> of the buffer solution (pH 7.7), 1.0 cm<sup>3</sup> of 5% (m/v) HTAB and 320 µl of 1.0×10<sup>-3</sup> mol dm<sup>-3</sup> ARS solution were added, diluting to the mark with doubly distilled water.

The fluorescence intensity was always measured at 607 nm with excitation at 505 nm, against a reagent blank prepared in a similar way, but without vanadium(V). A thermostat is used to maintain the temperature at 25°C.

**Procedure for clams (*Cititrea*).** The clam tissue sample was dried at 110°C until the weight was constant and it was then ground into a fine powder. Sub-samples of about 3 g were treated with 25 cm<sup>3</sup> of nitric acid in a sand bath and the solution was evaporated to dryness. Ten cubic centimeters of perchloric acid (70%, m/m) were then added to ensure the presence of vanadium(V)<sup>21</sup> and the solution was evaporated to incipient dryness, twice. The white residue was treated with 20 cm<sup>3</sup> of 0.1 mol

dm<sup>-3</sup> hydrochloric acid and heated to complete solution. The final solution containing the total vanadium as V<sup>V</sup>, was transferred to a 25 cm<sup>3</sup> calibrated flask and diluted to the mark with distilled water.<sup>22</sup> To assure a quantitative oxidation of the vanadium, add 0.3% potassium permanganate solution (w/v) dropwise until the solution shows a definitive color due to the permanganate.<sup>23</sup>

To eliminate the effect of other metals present in this sample, we have carried out the procedure recommended by Talvitie.<sup>24</sup> This consists in the formation of oxinates in a slightly acidic medium. For this purpose, aliquots of 3 cm<sup>3</sup> of the above solution were adjusted to pH 4 and transferred to a 10 cm<sup>3</sup> glass-stoppered centrifuge tube, extracting with 1.5 cm<sup>3</sup> of 0.5% oxine solution (mechanical shaking time 2 min). The extract was washed with water, then acidified with hydrochloric acid to pH approx. 3. The chloroform extract must contain V<sup>V</sup>, Fe<sup>3+</sup> and partly also Al<sup>3+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Mo<sup>VI</sup>, W<sup>VI</sup>, Cu<sup>2+</sup> and Bi<sup>3+</sup> oxinates if those elements are present in the solution sample. This portion is shaken with 1.5 cm<sup>3</sup> of an ammonia buffer solution (pH 9.4), thereby stripping vanadium into the aqueous phase and leaving behind iron together with the other metals in the chloroform solution. Aliquots of aqueous phase not more than 5.0 cm<sup>3</sup> in volume were used for the spectrofluorometric determination of V.

**Procedure for seawater.** After filtering the sample, take aliquots not more than 5.0 cm<sup>3</sup> in volume, add 0.6 cm<sup>3</sup> of 10 g dm<sup>-3</sup> sodium pyrophosphate solution to mask Fe<sup>3+</sup> and follow the procedure described above.

## Results and Discussion

#### Reaction with vanadium

ARS reacts with vanadium to form a complex which shows a slight fluorescence at 607 nm with excitation at 480 nm in aqueous medium and in the absence of surfactant agents at a pH between 4.2 and 6.0. This low emission intensity is not adequate to establish a sensitive fluorometric determination of vanadium(V).

#### Effect of micellar media

A study was carried out by the addition of different solutions of anionic (SDS), cationic (HTAB and CPC) and non-ionic (TX-100) surfactants to the V-ARS complex, with fixed concentrations of ARS and V (2×10<sup>-5</sup> mol dm<sup>-3</sup>) at a pH 5.5.

We have observed that with TX-100 and SDS, the fluorescent signals of the complex and the reagent blank exhibit a slight decrease. However, the use of cationic surfactant agents greatly affects the fluorescence intensity of the V-ARS complex. Comparing the two cationic surfactants used, HTAB produces the most important increase in the fluorescence intensity and in both cases, a decrease in the fluorescence of reagent blank can be observed. No changes in the excitation and emission maxima were seen (Fig. 1). From this study the HTAB medium was selected to achieve a sensitive

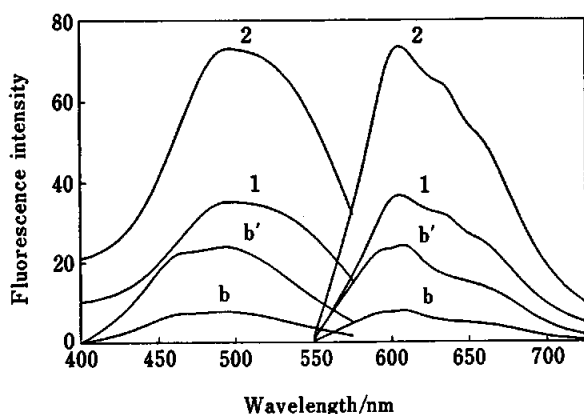


Fig. 1 Fluorescence excitation and emission spectra of the V-ARS complex in the absence (1) and presence (2) of HTAB, with respect to the reagent blank (b and b', with and without HTAB, respectively).  $[V]=[ARS]=2 \times 10^{-5} M$ ,  $[HTAB]=0.5\%$ ,  $pH=6.0$

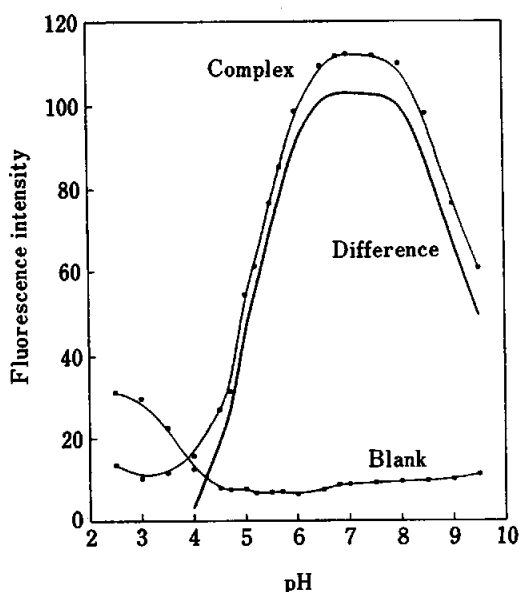


Fig. 2 Effect of pH on the fluorescence intensity of V-ARS complex:  $[V]=[ARS]=2 \times 10^{-5} M$ ;  $[HTAB]=0.5\%$ .

procedure for the determination of this ion.

#### Optimum experimental conditions

To determine the optimum pH value for the complex formation, excitation and emission scans were performed on a working solution using an HTAB micellar medium. In the presence of this surfactant, the optimum pH range is between 6.0–8.0 (Fig. 2). A shift in the maximum excitation peak can be observed in this region from 480 to 505 nm, together with an increment in the fluorescence intensity, compared with that in the absence of surfactant. No changes in the emission maximum were seen. For all measurements, a Tris-HCl buffer of pH

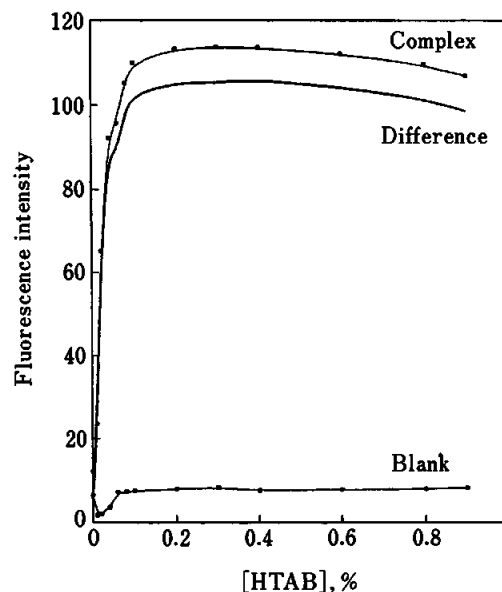


Fig. 3 Influence of the HTAB concentration on the fluorescence intensity of the V-ARS complex at  $pH=7.7$ .  $[V]=[ARS]=2 \times 10^{-5} M$ .

7.7 was used.

The effect of surfactant concentration on the fluorescence intensity of the complex is shown in Fig. 3. The enhancement starts only after the cmc has been reached ( $9.2 \times 10^{-4} \text{ mol dm}^{-3}$ , in aqueous solution). The plateau occurs well above the cmc, indicating clearly the micellar nature of the fluorescence enhancement.<sup>25</sup> The possible electrostatic and hydrophobic interactions occurred concurrently at the micellar level between the anionic complex with the reagent containing negative sulfonic groups and the positive quaternary ammonium group of the surfactant produces a maximum enhancement of the fluorescence due to achievement of a more rigid structure.<sup>26</sup> In further experiments, an HTAB concentration of 0.5%, has been used.

The fluorescence intensity of the complex reaches a maximum in a few seconds and remains constant for at least 5 h after sample preparation. The effect of temperature on the fluorescence measurements was studied by heating thermostatically the cell between 15 to 50°C. The fluorescence intensity remained constant until 30°C and decreases gradually above this value. A temperature of 25°C was used throughout.

The order in which reagent, surfactant, metal ion and buffer solution are mixed has no influence on the fluorescence intensity, so metal ion, buffer solution, HTAB and reagent, was chosen for the rest of the experimental work.

The effect of reagent concentration on the fluorescence intensity was studied for solutions containing  $2 \times 10^{-5} \text{ mol dm}^{-3}$  of vanadium and 0.5% HTAB. The fluorescence signal grows with the increase in ARS concentration until  $2.8 \times 10^{-5} \text{ mol dm}^{-3}$  then remains constant

between  $2.8 \times 10^{-5}$  and  $3.6 \times 10^{-5}$  mol dm<sup>-3</sup>. Above this range, the fluorescence intensity decreases slowly. A  $3.2 \times 10^{-5}$  mol dm<sup>-3</sup> ARS concentration was selected to ensure a sufficient excess of the reagent, throughout the experimental work.

The stoichiometry of the complex was studied under the conditions established by the Yoe and Jones<sup>27</sup> and the Job<sup>28</sup> methods. From this study, we concluded that the composition of the complex was 1:1 (V-ARS).

#### Analytical performance characteristics

Calibration graphs, with three replicates for each concentration value and including the blank as additional value to calculate the equation of regression line<sup>29</sup>, are established in the ranges 10–100 ng ml<sup>-1</sup> (excitation and emission slits set at 12 nm, lack-of-fit P-value<sup>30</sup>=39.2%) and 41–1000 ng ml<sup>-1</sup> (excitation and emission slits set at 7 nm, lack-of-fit P-value=51.4%). These two ranges have been selected to achieve the lowest reagent background signal. In both cases, the reagent concentration is adequate to ensure the required ARS/V molar ratio.

The performance characteristics, calculated from the calibration data set<sup>31</sup>, are summarized in Table 1. The IUPAC detection and quantitation limits<sup>32</sup> are 3.0 and 10.0 ng ml<sup>-1</sup>, respectively.

#### Effect of diverse ions

The effect of several ions on the fluorescence intensity of the V-ARS complex has been investigated using the methodology proposed by the authors.<sup>33</sup> In the determination of 60 ng ml<sup>-1</sup> of V, foreign ions can be tolerated at levels given in Table 2. The tolerance criterion used was a value of  $t_{\alpha,SR}$  ( $S_R$ , regression standard deviation of the mean value of three measurements of analytical signal and  $t_{\alpha}$ , student's  $t$  one-tail for  $\alpha=0.05$  significance level). An interferent to vanadium ratio of 1000 (m/m) was tested; and if interference occurred, the ratio was progressively reduced until interference ceased. Only Be<sup>2+</sup> and Sn<sup>2+</sup> were found to cause positive interference in the determination at a ratio greater than 10:1 m/m (foreign ion/V). Also, in Table 2 the tolerated concentrations of several interferents forming extractable oximates are shown. To check the effectiveness of the separation from extraneous metals, 60 ng cm<sup>-3</sup> of vanadium was analyzed in the presence of several metals at a concentration level superior to that the analyzed samples may encountered. The derivative concentrations of these metals can be considerably reduced in the final extract by virtue of only one transfer of the vanadium between aqueous and chloroform phases, achieving an improved tolerance level. If any interferents are encountered in the samples analyzed, these can be avoided by the procedures mentioned above.

#### Applications of the proposed method

The proposed spectrofluorometric method has been applied to the determination of vanadium(V) in clams, sampled at the Algeciras Bay (Cádiz, Spain) and seawater

Table 1 Performance characteristics calculated from the calibration data set (three replicates for each standard)

Feature (units)	Value
Intercept (a.u.)	12.6
Slope (a.u. ng cm <sup>-3</sup> V)	0.40
Linearity (%) <sup>a</sup>	98.7
Analytical sensitivity (ng cm <sup>-3</sup> V) <sup>b</sup>	2.0
Detection limit (ng cm <sup>-3</sup> V) <sup>c</sup>	4.2
Determination limit (ng cm <sup>-3</sup> V)	13.9
Precision (%) <sup>d</sup>	
400 ng cm <sup>-3</sup> V	1.5
60 ng cm <sup>-3</sup> V	2.1

a.  $1 - \text{RSD}(b)$ ;  $(\text{RSD})(b) = \text{Relative standard deviation of the slope}$ .

b. Quotient between standard deviation of residuals and slope.

c. Standard deviation of the blank, calculated from regression analysis equations.

d. Relative standard deviation of the concentration.

Table 2 Tolerated concentrations for the determination of 60 ng cm<sup>-3</sup> of vanadium

Foreign ion	Tolerated concentration/ mg dm <sup>-3</sup>
Be <sup>2+</sup>	0.06
Sn <sup>2+</sup>	0.6
Mo <sup>VI</sup>	1.2 <sup>a</sup>
W <sup>VI</sup>	2.4 <sup>a</sup>
Zr <sup>4+</sup>	1.6
Cr <sup>3+</sup>	3.1
Ba <sup>2+</sup>	7.0
Bi <sup>3+</sup>	12.0 <sup>a</sup>
BO <sub>3</sub> <sup>3-</sup>	12.4
Al <sup>3+</sup>	15.0 <sup>a</sup>
Fe <sup>3+</sup>	18.0 <sup>a</sup> , 15.2 <sup>b</sup>
Cu <sup>2+</sup>	30.0 <sup>a</sup>
Zn <sup>2+</sup>	60.0 <sup>a</sup>
Li <sup>+</sup> , Na <sup>+</sup> , K <sup>+</sup> , Rb <sup>+</sup> , Cs <sup>+</sup> , Mg <sup>2+</sup> , Ca <sup>2+</sup> , Sr <sup>2+</sup> , Mn <sup>2+</sup> , Co <sup>2+</sup> , Ni <sup>2+</sup> , Cd <sup>2+</sup> , Pb <sup>2+</sup> , SiO <sub>3</sub> <sup>2-</sup> , NO <sub>3</sub> <sup>-</sup> , NO <sub>2</sub> <sup>-</sup> , PO <sub>4</sub> <sup>3-</sup> , SO <sub>4</sub> <sup>2-</sup> , SCN <sup>-</sup> , Cl <sup>-</sup> , Br <sup>-</sup> , I <sup>-</sup> , P <sub>2</sub> O <sub>7</sub> <sup>4-</sup>	>60.0

a. Results obtained after oxine treatment.

b. In presence of sodium pyrophosphate as masking agent.

from Almería Bay (Spain) near a commercial training port. In both cases, the validation is tested using the statistic protocol, developed by the authors<sup>34</sup>, based on the standard addition methodology. This protocol implies the use of a data set of three calibrations, with standard solutions (SC), with standard additions to a constant portion of sample (AC) and with sample portions (YC, Youden calibration). The trueness of the analytical result, for each sample kind, is checked by statistical comparison with the analyte contents calculated from SC and AC. To detect any losses of VV,

Table 3 Determination of vanadium in clam and seawater

	Analyte content			Sample <sup>a</sup>
	Working solution (in $\mu\text{g dm}^{-3}$ V)			
	from SC	from AC	P-value	
Clam	20.49	20.66	87.8%	$2.13 \pm 0.2 \mu\text{g g}^{-1}$
Seawater	13.77	12.84	23.2%	$44.5 \pm 8.0 \text{ ng cm}^{-3}$

Validation of the method by standard addition methodology.

a. Mean from 3 determinations.

known amounts were added before the sample treatment.

The vanadium contents obtained in the different samples from SC and AC in the solutions, obtained by applying the procedure recommended and the P-value for the corresponding t-test, are given in Table 3. Also, in the same table, the final contents in the samples are shown. The present result for the clam sample ( $2.13 \pm 0.2 \mu\text{g g}^{-1}$ ) is in good agreement with that previously obtained by flame atomic absorption spectrometry<sup>22</sup> ( $2.26 \pm 0.16 \mu\text{g g}^{-1}$ ). Accuracy for the samples studied is satisfactory.

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