A 2:2:2 Complex of Vanadium(V) with 4-(2-Thiazolylazo)orcinol and 2,3,5-Triphenyl-2H-Tetrazolium Chloride

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Abstract

Abstract. The complex formation in the vanadium(V)/4-(2-thiazolylazo)orcinol (TAO)/2,3,5-triphenyl-2H-tetrazolium chloride (TTC) liquid-liquid extraction-chromogenic system was studied. The chloroform-extracted complex has a composition of 2:2:2 under the optimum conditions (pH 4.8–5.2, extraction time 3 min, concentration of TAO 3.4 × 10⁻⁴ mol dm⁻³, and concentration of TTC 9.4 × 10⁻⁴ mol dm⁻³) and could be regarded as a dimer (D) of two 1:1:1 species (S) presented by the formula (TT⁺)[VO₂(TAO)]. The constant of extraction was calculated by two methods and some analytical characteristics were determined. The wavelength of maximum absorption (λ_max), molar absorptivity (ε_λ), and fraction extracted (E) were found to be λ = 545 nm, ε_545 = 1.97 × 10⁴ dm³ mol⁻¹ cm⁻¹, and E = 97.9 %. The ground-state equilibrium geometries of the complexes S and D were optimized by quantum chemical Hartree-Fock calculations using 3-21G* basis functions. The bonding and interaction energies were calculated as well.

Keywords: liquid-liquid extraction; spectrophotometry; tetrazolium salt; 5-methyl-4-(2-thiazolylazo)resorcinol; 2:2:2 complex; HF calculations.

1. Introduction

Vanadium is a trace element with many industrial applications.¹ The recent interest in this element is also related to the observed beneficial role of its compounds for different aspects of human health.²⁻⁴ Complexes with various reagents have been proposed for determination of vanadium.⁵⁻⁷ However, the concentration of its species in environmental and biological samples is often lower than the corresponding limits of determination. A classical approach to solve this problem and improve the method’s characteristics is to combine the instrumental method (e.g. spectrophotometry) with liquid-liquid extraction (LLE) – a simple technique for separation and preconcentration which does not require expensive equipment.⁸

Among the complexes applied for vanadium LLE-spectrophotometric determination and speciation of particular interest are those with participation of azocompounds (AC) and tetrazolium cations (TZ⁺).⁵⁻⁶⁻¹⁰ The following ACs were investigated as components of ternary V(V)-AC-TZ complexes: 4-(2-pyridylazo)resorcinol ¹⁰⁻¹², 4-(2-thiazolylazo)resorcinol,¹³⁻¹⁴ and 4-(2-thiazolylazo)orcinol (TAO).¹⁵ The obtained results show that the V(V):AC molar ratio in these complexes differs,¹¹⁻¹⁵ as a rule, from the typical 1:1 ratio established in the presence of other ion-association reagents (Table S1). However, the reason for this peculiarity is unclear and quantum-chemical calculations on V-AC-TZ ternary complexes have never been conducted.

In the light of this, the purpose of the current work is experimental (LLE-spectrophotometric) and theoretical (calculations at the HF/3-21G* level) study on the complexes formed between V(V), 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) and TAO. Scarce information about the binary V(V)-TAO complex in water-ethanol medium has been provided by Shalamova.¹⁶ TTC was selected for the present study because of its high application potential¹⁷⁻²⁰ and recent interest to its ion-associates.²¹⁻²⁶

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2. Experimental

2.1. Reagents and Apparatus

- NH₄VO₃ (puriss. p.a., VEB Laborchemie Apolda) dissolved in doubly distilled water, 2 × 10⁻⁴ mol dm⁻³.
- TAO (95%, Sigma-Aldrich Chemie GmbH) dissolved in slightly alkalized (KOH) distilled water, 3 × 10⁻³ mol dm⁻³.
- TTC (p.a., Loba Feinchemie GMBH), 4.7 × 10⁻³ mol dm⁻³ aqueous solutions.
- Ethanol (96%).
- Chloroform (p.a.), additionally distilled.
- Acetate buffer solution prepared from 2 mol dm⁻³ aqueous solutions of CH₃COOH and NH₄OH. The resulting pH was checked by HI-83140 pH meter.
- A Camspec M508 spectrophotometer (United Kingdom), equipped with 10 mm path-length cells.

2.2. Procedure for Establishing the Optimum LLE-Spectrophotometric Conditions

Aliquots of V(V) solution (1 cm³), TAO solution, buffer solution (2 cm³) and TTC solution were pipetted into 100 cm³ separatory funnels. The resulting solutions were diluted with distilled water to a total volume of 10 cm³. Then 10 cm³ of chloroform were added. The funnels were closed with stoppers and shaken for extraction. After separation of the layers, portions of the organic extracts were transferred through filter papers into cells. The absorbances were read against respective blank samples.

2.3. Procedure for Determining the Complex Composition in Water-ethanol Medium

1 cm³ of V(V) solution, i cm³ of TAO solution (i varies from 0.2 to 4 cm³), 2 cm³ of buffer solution (pH 5.4) and 3 cm³ of ethanol were added into test tubes with ground stoppers. The volumes were made up to 10 cm³ with distilled water. Then the tubes were closed and shaken for homogenization. Portions of the obtained solutions were transferred into cells. The absorbances were read against respective blank samples.

2.4. Procedure for Determining the Distribution Coefficients

The distribution coefficients 

\[ D = \frac{\Sigma c(V(V)_{org})}{\Sigma c(V(V)_{aq})} \]

were found from the ratio 

\[ A_i = \frac{A_{i-1}}{A_{i-1}} \]

where \( A_i \) and \( A_{i-1} \) are the absorbances (measured against blanks), obtained after a single and triple extractions, respectively. The single extraction and the first stage of the triple extraction were performed with 10 cm³ of chloroform under the optimum extraction-spectrophotometric conditions (Table 1). The organic layers were transferred into 25 cm³ calibrated flasks and the flask for the single extraction was brought to volume with chloroform. The second stage of the triple extraction was performed by adding a 7 cm³ portion of chloroform to the aqueous phase, which remained after the first stage. The third stage was performed in the same manner. The two successive organic layers were transferred to the flask containing the organic layer obtained after the first stage. The volume was brought to the mark with chloroform and shaken for homogenization.¹⁵,²⁷,²⁸

3. Theoretical

The ground-state equilibrium geometries of the single and dimeric complexes were optimized at the HF level using 3-21G* basis functions. Their vibration spectra were calculated in order to check for imaginary frequencies (no such vibrational eigenvalues were calculated).

The stability of the complexes S and D was evaluated by the bonding and interaction energies found by the equations 1 and 2:²⁹–³¹

\[ \Delta E_b = E_{SS} - \sum_i E_{i} \]  

(1)

\[ \Delta E = E_{SS} - \sum_i E_{i}^{SP} \]  

(2)

where \( E_{SS} \) is the energy of the given complex, whereas \( E_{i} \) and \( E_{i}^{SP} \) are the energies of the fragments found with ‘ghost’ orbitals and single-point calculations respectively with geometries as obtained by the optimizations. Thus, the basis-set superposition error (BSSE) was estimated by the equation 3:

\[ BSSE = \sum_i \left( E_{i}^{SP} - E_{i} \right) \]  

(3)

The theoretical calculations were performed with the GAUSSIAN 03 program package. The results were visualized with the ChemCraft program.

Table 1. Extraction-spectrophotometric optimization of the V(V)-TAO-TTC-water-chloroform system

| Parameter                        | Optimization range     | Optimal value     | Figure |
|----------------------------------|------------------------|-------------------|--------|
| Wavelength, nm                   | Visible range          | 545               | Fig. 1 |
| pH of the aqueous phase          | 3.7–6.7                | 4.8–5.2           | Fig. 2 |
| Extraction time, min             | 0.25–6                 | 3                 | Fig. S1|
| Concentration of TAO, mol dm⁻³   | (0.15–6.0) × 10⁻⁴      | 3.4 × 10⁻⁴        | Fig. 3 |
| Concentration of TTC, mol dm⁻³   | (0.24–14.1) × 10⁻⁴     | 9.4 × 10⁻⁴        | Fig. 3 |
4. Results and Discussion

4.1. Optimum LLE-spectrophotometric Conditions

Absorption spectrum of the chloroform-extracted ternary complex is shown in Figure 1. The maximum is at \( \lambda = 545 \) nm. It is shifted to 5 nm as compared to the maximum of the binary V(V)-TAO complex in water-ethanol medium \( (\lambda = 550 \) nm) and practically coincides with that of other complexes of the type V(V)-TAO-TZ in chloroform studied in our previous paper. It should be mentioned that the position of this maximum is constant independently of changes in pH and concentrations of the reagents. The optimum LLE-spectrophotometric conditions are given in Table 1. The optimization experiments included varying the pH (Figure 2), time of the extraction, and concentration of the reagents (Figure 3). The concentration of V(V) in the aqueous phase was kept constant during the experiments \( (2 \times 10^{-5} \text{ mol dm}^{-3}) \); the temperature was ca. 22 °C.

4.2. Composition, Formula and Equation

The molar ratios of the components of the ternary complex, TAO:V(V) and TTC:V(V), were determined by the mobile equilibrium method (Figure 4) which is applicable for compounds of the type \( A_nB_m \), where \( n = m \ (n \geq 1) \). The slopes \( a \pm SD \) of the obtained straight lines for \( n = m = 2 \) (Figure 4) are close to 2:1.95 ± 0.05 (straight line 1: R = TAO) and 1.96 ± 0.09 (straight line 2: R = TTC). At the same time, the corresponding slopes for \( n = m = 1 \) \{1.21 ± 0.05 (R = TAO) and 1.20 ± 0.04 (R = TTC)\} and \( n = m = 3 \) \{2.69 ± 0.10 (R = TAO) and 2.72 ± 0.15 (R = TTC)\} are far from 1 and 3.

The TTC:V(V) molar ratio was determined by an independent method based on the effect of dilution on the

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Figure 1. Absorption spectra of the ternary complex (curve 1) and blank (curve 2) in chloroform. \( c_{V(V)} = 2 \times 10^{-5} \text{ mol dm}^{-3}, c_{TAO} = 4.2 \times 10^{-4} \text{ mol dm}^{-3}, c_{TTC} = 9.4 \times 10^{-4} \text{ mol dm}^{-3}, \text{pH} 5.0. \)

Figure 2. Absorbance of the complex (curve 1) and blank (curve 2) in chloroform vs pH of aqueous phase. \( c_{V(V)} = 2 \times 10^{-5} \text{ mol dm}^{-3}, c_{TAO} = 4.0 \times 10^{-4} \text{ mol dm}^{-3}, c_{TTC} = 8.0 \times 10^{-4} \text{ mol dm}^{-3}, \lambda = 545 \text{ nm}. \)

Figure 3. Absorbance of extracted complex vs concentration of the reagents (R): 1. R = TAO, \( c_{V(V)} = 2 \times 10^{-5} \text{ mol dm}^{-3}, c_{TAO} = 9.4 \times 10^{-4} \text{ mol dm}^{-3}, \text{pH} 5.0, \lambda = 545 \text{ nm}; 2. R = TTC, \( c_{V(V)} = 2 \times 10^{-5} \text{ mol dm}^{-3}, c_{TTC} = 4.0 \times 10^{-4} \text{ mol dm}^{-3}, \text{pH} 5.1, \lambda = 545 \text{ nm}. \)

Figure 4. Determination of R-to-V(V) molar ratios by the mobile equilibrium method. The data are derived from the experimental points shown in Fig. 3. Straight line equations: (1) \( y = 1.95x + 9.75 \) (R = TAO, \( r^2 = 0.9974 \)); (2) \( y = 1.96x + 8.98 \) (R = TTC, \( r^2 = 0.9824 \))
degree of dissociation. The experimental points (Figure 5) determine a straight line for TTC:V(V) = 2:2 ($y = -3433x + 2587; r^2 = 0.9978$) and a curve for TTC:V(V) = 1:1. Therefore, the results of both methods agree well. They show that the complex has a composition of 2:2:2. Its formation and extraction can be represented with equation 4, which is consistent with the state of V(V)$^7$ and TAO$^{34}$ at the working conditions ($p_{\text{H}_{\text{opt}}}$ and $c_{\text{V(V)}}$). We believe that V(V) does not change its oxidation state during the complex formation.$^{9–15}$

$$2\text{H}_2\text{VO}_4^{-}(\text{aq}) + 2\text{H}_2\text{TAO}(\text{aq}) + 2 \text{Tt}^+(\text{aq}) \rightleftharpoons (\text{Tt})_2[\text{VO}_2(\text{TAO})]_2(\text{o}) + 4\text{H}_2\text{O}(\text{aq})$$ (4)

4. 3. Constant of Extraction and Fraction Extracted

The constant of extraction was calculated by two methods: the dilution method$^{33}$ (Figure 5) and the Likussar-Boltz method$^{35}$ (Figure 6), extended by the equation 5, proposed in our previous paper$^{15}$ for this kind of complexes.

$$K_{\text{ex}} = 0.0625 \times (4/k) \times y_{\text{max}} \times (1-y_{\text{max}})^{-4} \quad (5)$$

The corresponding values agree very well: Log $K_{\text{ex}} = 13.47 \pm 0.02$ and Log $K_{\text{ex}} = 13.53 \pm 0.05$.

The fraction extracted was calculated by the formula

$$E\% = 100 \times \frac{D\text{V(V)}}{D\text{V(V)} + 1}$$

where $D\text{V(V)}$ is the distribution coefficient for the optimum extraction conditions. The following value was obtained: $E = (97.9 \pm 0.1)\%$.

4. 4. Analytical Characteristics

The dependence between the concentration of V(V) and the absorbance of the extracted complex was studied under the optimum conditions (Table 1). A very good linearity was obtained in the concentration range of 0.2–4.6 μg cm$^{-3}$ ($r^2 = 0.9998$, $N = 8$) (Figure S2). The linear regression equation was $A = 0.399 \gamma - 0.0076$, where $A$ is the absorbance and $\gamma$ is the concentration of V(V) (μg cm$^{-3}$). The standard deviations of the slope and intercept were 0.002 and 0.005, respectively. The limits of detection (LOD) and quantitation (LOQ), calculated as 3 and 10 times standard deviation of the intercept divided by the slope, were LOD = 0.04 μg cm$^{-3}$ and LOQ = 0.14 μg cm$^{-3}$. The molar absorptivity (ε) and Sandell’s sensitivity (SS) were $\varepsilon_{545} = 1.97 \times 10^4$ dm$^3$ mol$^{-1}$ cm$^{-1}$ and $SS_{545} = 2.6 \times 10^{-3}$ μg cm$^{-2}$, respectively.

4. 5. Composition of the Binary V-TAO Complex in Water-ethanol Medium

Shalamova$^{16}$ provides the following scarce information about the binary V(V)-TAO complex in water-ethanol medium: $p_{\text{H}_{\text{opt}}} = 5.0–5.5$, $\lambda_{\text{max}} = 540$ nm, and $\varepsilon_{\text{max}} = 1.3 \times 10^4$ dm$^3$ mol$^{-1}$ cm$^{-1}$. There is no information about the composition of the complex.

In order to fill this gap we used the method of Asmus$^{36}$ (Figure S3) and the mobile equilibrium method$^{32}$ (Figure S4). The results show that the molar ratio between the reacting TAO and V(V) is 1:1 (not 2:2).

4. 6. Optimized Ground-state Equilibrium Geometries

The ternary complex has a composition of 2:2:2 and can be regarded as obtained by dimerization of two 1:1:1 (V:PAR:TT) single complexes (see Table S1 and Refs. S10–S18).

Single complexes. The optimized ground-state equilibrium geometries of two possible single complexes S1...
and S2 are illustrated in Figure 7. For the optimization of the first structure (S1) we started from a T-shaped structure between the anionic and cationic parts, \([\text{VO}_2\text{(TAO)}]^+\) and \(\text{TT}^+\), respectively. The tetrazolium ring was initially located over the deprotonated O(8) atom. The T-shape was changed during the fully-relaxed optimization but the close distance between O(8) and the tetrazolium ring was kept: e.g. the distance O(8)···N(28) = 3.501 Å. Two close interactions are observed between the fragments 1 and 2 in the complex S1. They are two weak H-bonds between the benzene ring hydrogens and the oxygen atoms of the fragment 1: H(64)···O(7) = 2.460 Å and H(63)···O(18) = 2.189 Å. These bonds cause a interring twist between the tetrazolium and benzene residues: \(<\text{C}(45)\text{C}(33)\text{C}(30)\text{N}(29) = 30.8^\circ\) however \(<\text{C}(39)\text{C}(34)\text{N}(28)\text{N}(29) = -83.3^\circ\) and \(<\text{C}(35)\text{C}(34)\text{N}(28)\text{N}(29) = 94.8^\circ\).

In Fig. 7b is depicted the optimized ground-state structure of the single complex S2. In this structure the oxygen atom O(18) which is bound to vanadium is directed to the tetrazolium ring. As a result, this atom and the atoms from the tetrazolium ring form pentagonal pyramid, whose vertex is the O(18) atom. This kind of interaction between the complex anion and the TT+ part of the single complex leads to a slight reduction (0.054 Å) of the distance V(17)···N(9) as compared to the single complex S1. Moreover, in the complex S2 the bonds O(7)···V(17) and V(17)···N(12) are 0.034 Å and 0.015 Å shorter than these in the complex S1. All this indicates, from a structural point of view, that the complex anion in the S2 system should be a bit more stable than this in the ion-association system S1.

With respect to the cationic part of the complex S2 one can say that one of the benzene rings is almost conjugated with the tetrazolium ring (located in one plane): \(<\text{C}(45)\text{C}(33)\text{C}(30)\text{N}(29) = 2.5^\circ\). The remaining two benzene rings are located with respect to the tetrazolium ring almost like in the ion-association single complex S1: \(<\text{C}(39)\text{C}(34)\text{N}(28)\text{N}(29) = -132.2^\circ\) and \(<\text{C}(35)\text{C}(34)\text{N}(28)\text{N}(29) = 46.8^\circ\).

**Dimer.** In Figure 8 is depicted the optimized ground-state structure of the dimer D. We believe that in the non-polar media (chloroform) the most probable dimeric structure would be from the type \((\text{TT}^+\text{-}\text{(VO}_2\text{TAO})\text{···(VO}_2\text{TAO})\text{-TT}^+)\), forming some kind of a sandwich aggregate. The attempts to find a structure which is analogue to the single complex S2 failed. The optimization of such structure led to the dimer D, which seems to be the only stable sandwich aggregate.

The structure of the supersystem D shows two H-bonds between O(8) / O(72) and the hydrogens from the two neighbouring benzene rings: O(8)···H(50) = 2.011 Å, O(8)···H(55) = 1.997 Å, O(72)···H(119) = 2.014 Å, O(72)···H(114) = 1.991 Å. The two complex anions in the dimer D are almost parallel one to another. The average distance between the planes of the two complex anions is about 4 Å.

With respect to the coordination bonds around the vanadium atom, one can say that only minor changes are observed between the single complex S1 and the dimer D. For example, the distance N(9)···V(17) is almost the same (difference only 0.001 Å), the bond V(17)···O(7) is a bit shorter (0.014 Å) in the aggregate D than in the single complex S1, whereas the bond N(12)···V(17) is 0.008 Å longer in the system D.

Minor differences in the bond lengths were also found between the same distances from the two complex cations in the dimer D: the distance N(9)···V(17) in the fragment 1 (with a smaller labelling of the atoms) is 0.006 Å shorter than the distance N(73)···V(81) in the fragment 2 (with a larger labelling of the atoms); the bond lengths O(7)···V(17) and O(71)···V(81) are almost identical (difference only 0.001 Å); the bond length N(12)···V(17) in the fragment 1 is only 0.007 Å longer than the distance N(76)···V(81) in the fragment 2. These slighting differences are due probably to the different orientation of the anionic parts of the fragments with respect to the cationic ones.

It should be mentioned that the weak H-bonds between one of the oxygen atoms of the VO2 groups and hydrogen atoms from the CH3-groups of TAO probably significantly affect the overall stability of D.
O(19)···H(90) = 2.272 Å and O(71)···H(26) = 2.480 Å. The existence of these bonds is a good explanation of the observed difference in the composition of the complex studied in this paper (V:TAO:TTC = 2:2:2) and the similar complex with 4-(2-thiazolylazo)resorcinol (TAR) (V:TAR:TTC = 1:2:3). Such bonds do not exist in the V(V)-TAR-TTC complex since there is no CH₃-groups in the TAR molecule.

4. Bonding and Interaction Energies

The calculated bonding and interaction energies of the single complexes S1 and S2 and the dimer aggregate D are listed in Table 2. As seen, the bonding and the interaction energies of the single complexes have high negative values. They show that the single ion-association complexes are stable. Comparing the systems S1 and S2 one can see that the complex S1 is more stable than the complex S2. The bonding energy of the dimer system D is positive which means that the formation of this aggregate in the gas phase is not favoured despite the negative value of the interaction energy for it. The BSSE values are almost equal for all systems.

5. Conclusion

Vanadium(V) forms a well chloroform-extractable complex with TAO and TTC. It has a composition of 2:2:2 and can be regarded as a dimer of two 1:1:1 complex species. The structure, stability, and other characteristics of the dimer and its constituent parts were found. The constant of extraction, fraction extracted, molar absorptivity, Sendall’s sensitivity, limit of detection, and limit of determination were determined as well. The obtained results shed light on an insufficiently explored area of the chemistry of the ion-association complexes of vanadium.

6. Acknowledgements

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7. Supplementary Material

Supplementary Material (Figures S1-S4 and Table 1S) are available electronically on the Journal’s web site.

8. References

1. K. K. Chatterjee, Uses of Metals and Metallic Minerals, New Age International (P) Ltd. Publishers, New Delhi, India, 2007, pp. 272–275
2. K. Gruzewska, A. Michno, T. Pawelczyk, H. Bielarczyk, J. Physiol. Pharmacol. 2014, 65, 603–611.
3. D. Rehder, Met. Ions Life Sci. 2013, 13, 139–169. http://dx.doi.org/10.1007/978-94-007-7500-8_5
4. A. Srivastava, Mol. Cell. Biochem. 2000, 206, 177–182. http://dx.doi.org/10.1023/A:1007075204494
5. K. Pyrzyńska, T. Wierzbicki, Talanta 2004, 64, 823–829. http://dx.doi.org/10.1016/j.talanta.2004.05.007
6. K. Pyrzyńska, Microchim. Acta 2005, 149, 159–164. http://dx.doi.org/10.1007/s00604-004-0304-5

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Table 2. Energies (in a.u.) and bonding, and interaction energies of the single complexes S1 and S2 and of the dimeric aggregate D (in kJ mol⁻¹)

| Complex | E / a.u. | ΔE_b / kJ mol⁻¹ | ΔE / kJ mol⁻¹ | BSSE / kJ mol⁻¹ |
|---------|---------|----------------|--------------|----------------|
| S1      | −3115.471055 | −267.5 | −328.6 | 61.1 |
| S2      | −3115.468137 | −242.6 | −308.7 | 66.1 |
| D       | −6230.955679 | 19.7  | −50.0  | 69.8 |

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Povzetek

Tvorba kompleksa v vanadij(V) / 4-(2-tiazolilazo)orcinol (TAO) / 2,3,5-trifenil-2H-tetrazolijev klorid (TTC) tekočina–tekočina ekstrakcijskem sistemom je bila proučevana. S kloroformskim ekstrahiranim kompleks ima sestavo 2:2:2 pri optimalnih pogojeh (pH 4.8–5.2, ekstrakcijski čas 3 min, koncentracija TAO 3.4 × 10^{-4} mol dm^{-3} in koncentracija TTC 9.4 × 10^{-4} mol dm^{-3}) in ga lahko smatramo kot dimer (D) dveh 1:1:1 zvrsti (S) s formulo (TT^+)(VO_2(TAO))_. Konstanta vlogovala je bila izračunana z uporabo dveh metod in nekaj analitskih karakteristik je bila izračunana z pomočjo Hartree-Fock kvantomehanskih izračunov z uporabo 3-21G* baznih funkcij. Izračunane so bile tudi vezne in interakcijske energije.