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1 **Vanadium(IV) and vanadium(V) complexation by succinic**  
2 **acid studied by affinity capillary electrophoresis.**  
3 **Simultaneous injection of two analytes in equilibrium**  
4 **studies.**  
5

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12  
13 **Abstract.** The interaction of V(IV) and V(V) with succinic acid was investigated by affinity  
14 capillary electrophoresis (ACE) in aqueous acid solutions at pH values 1.5, 2.0 and 2.4, and  
15 different ligand concentrations. V(IV) and V(V) form protonated complexes with succinic acid  
16 ligand at this pH range. The logarithms of the stability constants, measured at 0.1 mol L<sup>-1</sup>  
17 (NaClO<sub>4</sub>/HClO<sub>4</sub>) ionic strength and 25°C, are  $\log\beta_{111}=7.4\pm 0.2$  and  $\log\beta_{122}=14.1\pm 0.5$  for V(IV),  
18 and  $\log\beta_{111}=7.3\pm 0.1$  for V(V), respectively. The stability constant values, extrapolated to zero  
19 ionic strength with the Davies equation, are  $\log\beta^{\circ}_{111}=8.3\pm 0.2$  and  $\log\beta^{\circ}_{122}=15.6\pm 0.5$  for V(IV)  
20 and  $\log\beta^{\circ}_{111}=7.9\pm 0.1$  for V(V). The application of ACE to the simultaneous equilibria of V(IV)  
21 and V(V) (injection of two analytes) was also attempted. When the results were compared with  
22 those obtained when introducing only one analyte in the capillary, using the traditional version  
23 of the method, similar stability constants and precision are obtained. The possibility of studying  
24 two analytes simultaneously decreases the time needed for the determination of the constants;  
25 this feature is especially valuable when working with hazardous materials or when only small  
26 quantities of ligand are available.

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28 **Keywords:** Affinity capillary electrophoresis / Complexation / Stability constants/ Vanadium  
29 / Succinic acid

## 30 **1 Introduction**

31 Thermodynamic data on complex equilibria of metal ions are very important, not only  
32 for fundamental knowledge reasons, but also in many diverse fields, such as toxicology, clinical  
33 and biological chemistry, geochemistry and environmental chemistry, and analytical and  
34 industrial processes [1]. Affinity capillary electrophoresis (ACE) is gaining an increasingly  
35 prominent place in metal ion equilibrium studies [2-9]. This technique has been successfully  
36 used, not only for the determination of complex stability constant with a 1 to 1 metal/ligand  
37 ratio, but also in the study of more complicated systems [10,11]. ACE is useful for establishing  
38 the stoichiometries of the complexes formed because the changes in the mobility of the  
39 complexes enables deduction of their degree of protonation [9,10]. In this respect, ACE offers  
40 undeniable advantages over other classical methods.

41 A further interesting advantage of ACE is that it requires only a small quantity of analyte  
42 for thermodynamic equilibrium studies [9]. This is especially important when working either  
43 with highly toxic or radiotoxic substances or with precious ligands.

44 The separation efficiency of the ACE also allows the study of simultaneous  
45 complexation equilibria including two or more metal ions with the same ligand. Although the  
46 possibility of using ACE for simultaneous determination of binding constants of several  
47 proteins with a ligand was pointed out in a pioneer work [12], to our knowledge, this feature  
48 has not been explored in equilibrium studies of complexation of metal ions yet.

49 The differences between aqueous V(IV) and V(V) species offer the possibility of  
50 investigating whether the simultaneous equilibria of these metal ions. Since V(IV) and V(V)

51 are present as  $\text{VO}(\text{H}_2\text{O})_5^{2+}$  [13-15] and  $\text{VO}_2(\text{H}_2\text{O})_4^+$  [14-16] ions, respectively, in aqueous  
52 solution, their different charges allow their easy separation by capillary electrophoresis.

53 There is a growing interest in the study of vanadium because of the potential  
54 environmental hazards linked to its increasing use [17]. Vanadium is becoming important in  
55 energy storage because of its use as a valuable microalloying component. Such applications  
56 provide important opportunities in carbon dioxide reduction initiatives [18,19]. Although large  
57 vanadium concentrations can cause harmful effects on ecosystems [20,21], the element seems  
58 to play a dual role in environmental systems since it is also considered, at micro concentration  
59 levels, as an element of beneficial character with a range of application for human welfare [22].  
60 Disentangling these apparently contradictory facts requires that vanadium is studied “to  
61 understand properly and contextually apply the chemistry of vanadium to its toxicological  
62 properties, environmental chemical forms and the development of new regulations and  
63 standards in order to maximize the benefits and mitigate any potential risks of utilizing  
64 vanadium”[18].

65 Vanadium is present in oxidation degrees IV and V in most natural waters and soils.  
66 V(IV) is stable in moderately reducing conditions, especially under acidic pH values [23].  
67 Among a variety of possible geochemical processes, complex formation with natural organic  
68 matter (NOM) present in the aqueous phase may play an important role in the eventual  
69 dispersion of vanadium [23]. The formation of such complexes typically causes significant  
70 changes in migration properties of chemical elements in the environment. Succinic acid is  
71 chosen here for complexation studies of V(IV) and V(V) as a simple model of the structurally  
72 more complicated NOM [24,25]. Alongside, the complex equilibria of V(IV) and V(V) with  
73 succinic acid are poorly investigated. To our knowledge, only a few papers have been published  
74 containing stability constants for complexes of V(IV) with succinic acid [26-30] Existing data  
75 are gathered in table 1. Poor agreement is noted amongst the authors. Potentiometric studies

76 [26,27,29] led to different sets of constants and values being proposed. Even when a larger pH  
77 range (2.0-6.7) was used, Costa Pessoa et al. [29] could not differentiate 111 and 121  
78 complexes. Only one paper [31] has been found where the interaction of V(V) with succinic  
79 acid had been studied by  $^{51}\text{V}$  NMR spectroscopy, but no thermodynamic constants were  
80 provided.

81 The capabilities of ACE provide a suitable tool for the study of the complexation of  
82 V(IV) and V(V) with succinic acid. In this study, the possibility of studying V(IV) and V(V)  
83 simultaneously is explored. In this approach, two analytes are simultaneously injected and  
84 results compared with those obtained with the traditionally ACE methodology using single  
85 analyte injection. Finally, the obtained stability constant values are discussed in the light of  
86 available data from the literature for complexation of metal cations with succinic acid.

## 87 **2 Materials and methods**

### 88 **2.1 Chemicals and solutions**

89 All chemicals and reagents used were of analytical grade. 1 M perchloric acid solution  
90 was obtained from Fisher Scientific. Sodium perchlorate monohydrate ( $\geq 99\%$ ) was supplied  
91 by Merck. Ammonium vanadate solution ( $\geq 99\%$ ), vanadyl sulfate hydrate ( $\geq 99\%$ ), succinic  
92 acid ( $\geq 99\%$ ) and dimethyl sulfoxide (99.9%) were supplied by Sigma Aldrich. All solutions  
93 were prepared with fresh, high-purity deionized water ( $R = 18.2\text{ M}\Omega\text{ cm}$ ) produced by a  
94 Millipore direct Q cartridge system.

### 95 **2.2 Apparatus, procedure and data treatment**

96 *Capillary Electrophoresis measurements.* A P/ACE system MDQ capillary  
97 electrophoresis instrument (Beckman Coulter, France) was used. The system included a 0–30  
98 kV high-voltage built in power supply equipped with a UV-vis spectrophotometric diode array

99 detector. UV direct detection at 200 nm was used in this work. A capillary (50  $\mu\text{m}$  I.D., 363  $\mu\text{m}$   
100 O.D.) made from fused silica was obtained from Beckman Instruments. It had a total length ( $L_t$ )  
101 of 31.2 cm and an effective separation length ( $L_d$ ) of 21 cm. The capillary was housed in an  
102 interchangeable cartridge with circulating liquid coolant (temperature 25°C). Every  
103 measurement was repeated at least three times. Data acquisition and processing were carried  
104 out with Karat 32 software (Beckman Coulter, France).

105 The capillary was conditioned prior to use by successive washes with 0.1 M sodium  
106 hydroxide, deionised water and with the buffer solution used in the study. It was rinsed for 2  
107 minutes (at a pressure of 103.4 kPa) with the buffer between two runs, and kept filled with  
108 deionised water overnight.

109 The procedure used in this study was similar to that previously described in [25]. Series  
110 of solutions containing different concentrations of succinic acid were prepared at the desired  
111 pH at an ionic strength of 0.1 M, from stock solutions of  $\text{HClO}_4$  (0.1M),  $\text{NaClO}_4$  (1 M) and  
112 succinic acid. The dissociation of succinic acid was taken into account in the solution  
113 preparation. These solutions were used as the background electrolytes (BGE). V(IV) and/or  
114 V(V) solutions at fixed concentrations of  $5 \times 10^{-4}$  and  $1 \times 10^{-3}$  M, respectively were injected in  
115 the capillary. In order to avoid hydrolysis and/or polymerisation of the V(IV) and V(V) ions,  
116 which could potentially lead to the formation of additional species and/or to the modification  
117 of the vanadium species mobility values, the pH value of solutions containing the BGE was <  
118 2.5. At such pH values and V(IV) and V(V) concentrations, the contribution of hydrolysed and  
119 polymeric species is not noticeable (Fig. S1).

120 The normal polarity mode was applied (the injection was performed at the positive  
121 capillary end). The sample, containing the analyte (or analytes) and the neutral marker, was  
122 injected into the capillary. Dimethyl sulfoxide (0.05% DMSO) was used as a neutral marker for  
123 electroosmotic flow mobility determination. The capillary contained BGE with fixed

124 concentrations of succinic acid. Sets of runs were then made for different concentrations of  
125 succinic acid (from 0 to 0.4 M) in BGE. The separation voltage applied was 5 kV and the  
126 injection time (by a pressure of 3.45 kPa) was 4 s. The value of the applied voltage was chosen  
127 to respect the Ohm's law linearity. The current was about 45  $\mu\text{A}$  and the input power was about  
128 0.2 W. In these conditions we can estimate that the effect of Joule heating is insignificant in the  
129 thermostated part of the capillary and the deviation from the desired temperature 25°C is at  
130 most 1°C [32,33]. The non-thermostated capillary inlet has no influence on stability constant  
131 values obtained under such conditions [34-36]. The separation was performed at constant  
132 forward pressure of 1.4 kPa.

133 The electrophoretic mobility  $\mu$  ( $\text{m}^2 \text{s}^{-1} \text{V}^{-1}$ ) was calculated by using the following  
134 expression:

$$135 \quad \mu = L_t L_d (1/t - 1/t_{\text{eof}}) / U \quad (1)$$

136 where  $L_t$  (m) was the total capillary length,  $L_d$  (m) the length between the capillary inlet and  
137 the detection window,  $U$  the applied voltage in V,  $t$  (s) the migration time of the studied species,  
138 and  $t_{\text{eof}}$  (s) the migration time of a neutral marker.

139 All concentration and constant calculations were performed with the EXCEL® and  
140 ORIGIN® software programs. The experimental points were fitted by a least squares curve  
141 method.

142 *pH measurements.* The pH was controlled with a pH-meter. pH-meter GLP-21 (Crison,  
143 France) and a combination electrode was used for pH measurements after calibration against  
144 NIST standards (4.01 and 7.00). The pH values of the solutions were in an interval of  $\pm 0.05$   
145 units from desired values.

146 *Perchlorate measurements.* Combined ion specific electrode (ISE) "Fisher brand" for  
147 perchlorate from Fisher Scientific was used to check perchlorate concentrations of stock  
148 solutions and to control ionic strength. The RSD was less than 2%.

### 149 2.3 *Ionic strength corrections*

150 The Davies equation [37] was used to calculate activity coefficients of ions at low ionic  
151 strengths. The equation works fairly well up to ionic strengths ( $I_m$ ) of  $0.1 \text{ mol kg}^{-1}$ .

152 The Davies equation for the activity coefficient ( $\gamma$ ) of an ion  $i$  of charge  $z_i$  is at  $25^\circ\text{C}$ :

$$153 \log_{10}\gamma_i = -0.5102 z_i^2(I_m^{1/2}/(1 + I_m^{1/2}) - 0.3I_m) \quad (2)$$

## 154 3 Results and discussion

### 155 3.1 Monitoring of V(IV) and V(V) electrophoretic mobility: two analytes vs one.

156 The electropherograms obtained with an injection from the sample, containing the  
157 mixture of V(IV) and V(V) species, in the presence of different concentrations of succinic acid  
158 in BGE at pH=2 and an ionic strength  $0.1 \text{ mol L}^{-1}$  ( $\text{HClO}_4\text{-NaClO}_4$ ) are presented in Figure 1A.  
159 For comparison, the electropherograms obtained in the case of separate injections of V(IV) and  
160 V(V) are also shown in Figure 1B and 1C, respectively. As expected, the number of analytes  
161 injected does not have any influence on their mobilities. Only one peak is observed for each  
162 vanadium species, whose mobility is decreased with the increase of succinic acid concentration  
163 in BGE. This indicates that we deal with kinetically labile complexes [7,38]. The equilibrium  
164 is sufficiently fast and the time required for equilibrium negligible compared with the separation  
165 time. The kinetic behaviour of the aqua complexes of metal ions can serve as a reference point  
166 for complex formation reactions. The loss of the first water molecule from the first coordination  
167 sphere is an important step in complex formation of metal cations [39], and is often the rate  
168 determining step. For V(IV) aqua-complex ( $\text{VO}(\text{H}_2\text{O})_5^{2+}$ ), the half life for four equatorial water  
169 ligand exchange is  $\sim 1 \text{ ms}$ . The half time for a water ligand located opposite to the double-  
170 bonded oxygen is about  $10^{-11} \text{ s}$  [14,40]. For V(V) ( $\text{VO}_2(\text{H}_2\text{O})_4^+$ ), the water rate exchange  
171 constant is estimated at  $10^{8.2} \text{ s}^{-1}$  [41]. These values confirm our ACE observation of fast ligand  
172 exchange (only one peak is observed). V(V) peaks are narrower than V(IV) examples (Figure

173 1). This may indicate, that the exchange kinetic is faster for V(IV) than for V(V). This  
 174 observation also agrees with the kinetic data given above. V(IV) ( $\text{VO}^{2+}$ ) has a double charge in  
 175 comparison with V(V) ( $\text{VO}_2^+$ ). Thus, as expected, V(IV) mobility is greater (migration time is  
 176 lower) than for V(V). The mobility observed corresponds to the sum of mobilities of coexisting  
 177 charged species formed with the ligand under study (eq.3)

$$\mu_{\text{obs}} = \sum_i \alpha_i \mu_i \quad (3)$$

178 In Eq.3,  $\mu_i$  stands for the intrinsic electrophoretic mobility and  $\alpha_i$  for the molar fraction of  
 179 individual species ( $i$ ) present in the migration band.

180 The decrease in the global charge of the species when increasing the concentration of  
 181 succinic acid (migration time increases) (fig.1) suggests the formation of a less charged  
 182 complex species with increasing succinic acid concentration.

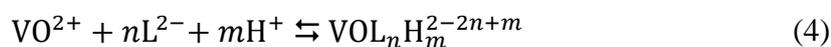
183

### 184 3.2 Chemical equilibria and stability constants

185 The equilibria of the vanadium species with the succinate ligand (denoted as  $\text{L}^{2-}$  further)  
 186 can be expressed as:

187

188 V(IV)



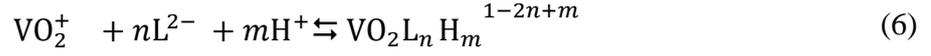
189

190 with the corresponding stability constant  $\beta_{1nm}$

$$\beta_{1nm} = \frac{[\text{VOL}_n\text{H}_m^{2-2n+m}]}{[\text{VO}^{2+}][\text{L}^{2-}]^n[\text{H}^+]^m} \quad (5)$$

191

192 V(V)



193

194 with  $\beta_{1nm}$ 

$$\beta_{1nm} = \frac{[\text{VO}_2\text{L}_n\text{H}_m^{1-2n+m}]}{[\text{VO}_2^+][\text{L}^{2-}]^n[\text{H}^+]^m} \quad (7)$$

195

196 Equilibrium constant values can be obtained by fitting experimental mobilities (Tables  
197 S2-S6) using the general equation (3). A non-linear fitting (NNLS) method is preferred over a  
198 linear regression method [42-44].

199 In this case, the molar fraction of species to be introduced in equation (3) can be written  
200 as

201 V(IV)

$$\alpha_{1nm} = \frac{[\text{VOH}_n\text{L}_m^{2+n+2m}]}{C(\text{V(IV)})} = \frac{\beta_{1nm} 10^{-n\text{pH}} \alpha_{dis} [\text{L}^-]^m}{1 + \sum m \beta_{1nm} 10^{-n\text{pH}} \alpha_{dis} [\text{L}^-]^m} \quad (8)$$

202

203 V(V)

$$\alpha_{1nm} = \frac{[\text{VO}_2\text{H}_n\text{L}_m^{1+n+2m}]}{C(\text{V(V)})} = \frac{\beta_{1nm} 10^{-n\text{pH}} \alpha_{dis} [\text{L}^-]^m}{1 + \sum m \beta_{1nm} 10^{-n\text{pH}} \alpha_{dis} [\text{L}^-]^m} \quad (9),$$

204 where  $\alpha_{dis}$  is a molar fraction of succinate.

205 Succinic acid protonation equilibria in aqueous solution are [40]:



208 Thus, the total succinic acid concentration is:

$$\text{C(L)} = [\text{H}_2\text{L}] + [\text{HL}^-] + [\text{L}^{2-}] \quad (12),$$

209

210 where  $C(L)$  is the total succinic acid concentration, and  $[H_2L]$ ,  $[HL^-]$  and  $[L^{2-}]$  the  
211 concentrations of the different forms of succinic acid at equilibrium. At  $pH \leq 2.5$  succinic acid  
212 is mainly present in its completely protonated form, as  $H_2L$  (Fig. S2).

213 Protonated complexes with succinic acid for the transition metal ions are known  
214 [25,45], but mostly deprotonated complexes of V(IV) with succinic acid have been described  
215 in the literature (Table 1). Only Costa-Pessoa et al. [29] suggest the formation of a protonated  
216 species with V(IV). Owing to the complex charge change linked to protonation or  
217 deprotonation, it is possible to distinguish protonated from deprotonated complexes by ACE  
218 [7,9,25]. The identification of the interacting species requires the variation of the concentration  
219 of each succinic acid species in order to know which influences the vanadium species mobility  
220 [10,46]. As all succinic species are present in the dynamic equilibria, it is not possible to vary  
221 the concentration of only one species without modifying the concentrations of the rest.

222 Thus, it is necessary to plot the V(IV) and V(V) mobilities, measured at different acidic  
223 pH values (Figure 2), as a function of the total succinic acid (A),  $[H_2L]$  (B),  $[HL^-]$  (C) and  $[L^{2-}]$   
224 (D) concentrations. We can state unambiguously the similarity of the curves in the case of  $HL^-$   
225 for V(IV) and V(V). However, in the case of V(V), a slightly lower mobility is observed at pH  
226 2.4, because of about 15% hydrolysis of V(V) and the corresponding formation of a less charged  
227 species (Fig. S1A). It can be concluded that only  $HL^-$  affects the vanadium species mobility.  
228 Thus, most probably, we have a formation of mainly protonated complex species of V(IV) and  
229 V(V) with succinic ligand in these conditions. The graphs presented in fig. 2 were obtained in  
230 the case of simultaneous injection of V(IV) and V(V). As expected, similar graphs are obtained  
231 when analytes are introduced separately (Fig. S3).

232 Considering only the equilibria with  $HL^-$  as the ligand, different models were explored:  
233 formation of one 1:1:1 complex, simultaneous formation of two complexes (1:1:1 and 1:2:2),  
234 formation of one 1:2:2 complex, simultaneous formation of two complexes (1:1:1 and 1:2:1).

235 We note that the system suffers from a mathematical indetermination, meaning that the product  
236  $\alpha_{111}\mu_{111}$  (eq. 3) is well defined but each individual term,  $\alpha_{111}$  and  $\mu_{111}$ , cannot be precisely  
237 determined. A similar case has been already described in [47]. In this case, to reach convergence  
238 it was necessary to make a reasonable guess of the  $\mu_{111}$  value and to keep this value constant.  
239 The same approach is used in this study.

240 In the case of V(IV), the best fits were found for the model with two complexes (1:1:1)  
241 and (1:2:2). The constants obtained at different pH values are given in table 2 for the two cases:  
242 that of only one analyte injected and also of two analytes simultaneously injected. The values  
243 obtained are practically the same. It is worthwhile to note that our value for  $\log\beta_{111}$  is very close  
244 to that proposed by [29]. For the second constant (1:2:2) the error is much larger, because the  
245 concentration of this species is low in our conditions.

246 In the case of V(V), only one complex (1:1:1) was found. Again, no significant difference  
247 was observed between the values obtained with only one analyte injection or the simultaneous  
248 injection of two analytes.

249 The average values for V(IV) and V(V) complexes are also shown in Table 2. The  
250 stability of protonated complexes (1:1:1) for V(IV) and V(V) is practically the same. We  
251 extrapolated the stability constant values to zero ionic strength with the use of the Davies  
252 equation (2). Thermodynamic stability constants are also presented in table 2.

### 253 3.3 Comparison with literature data

254 A Linear Free Energy Relationship (LFER) is applied to compare our values with  
255 literature data for complexes formed by different metal ions with succinate. This approach is  
256 commonly used in the literature to compare the affinity of a given ligand for a series of metal  
257 cations ( $M^{z+}$ ). The effect of the negatively charged oxygen-donor group of the ligand on  
258 complex stability appears to depend on the acidity of the metal ion considered, i.e., the affinity  
259 of the metal ion for the archetypal negative O-donor ligand, the  $\text{OH}^-$  ion [48]. Thus, the stability

260 constant of metal ion complexes with a ligand, containing an oxygen-donor group, correlates  
261 with the formation constant  $K_{\text{MOH}}$  on a log-log scale, indicating that the stability depends  
262 primarily on the Lewis acidity of the metal ion, similar to that expected for an electrostatic  
263 interaction. Moreover, a similar binding mode can also be inferred from such linear plots.

264 Figure 3 shows a LFER where  $\log\beta_{111}$  is plotted as a function of  $\log K_{\text{MOH}}$  for different  
265 metal ions, including the  $\log\beta_{111}$  values for V(IV) and V(V) measured by ACE in this study.  
266 Other stability constant values at 0.1 M ionic strength and 25 °C were taken from the literature  
267 (Table S7). A good linear trend is observed for all cations, except for V(V). The  $\log\beta_{111}$  for  
268 V(V) is lower than predicted from the LFER correlation. This suggests that V(V) complexation  
269 is not only governed by electrostatic interactions, but that there might be a significant  
270 contribution of a steric factor. Indeed, the  $\text{VO}_2(\text{H}_2\text{O})_4^+$  displays a very distorted octahedral  
271 configuration with a O=V=O bond angle of ca. 105° [15]. This can hamper the electrostatic  
272 interaction with the ligand and weaken complex stability. Most probably, for the same reason,  
273 the oxalate complexes of V(IV) are more stable than those of V(V)[49].

274

## 275 4 Conclusions

276 We demonstrate in this study the possibility of using the simultaneous injection in a  
277 capillary of two metal cations in equilibria studies by ACE. Certainly, this is a very attractive  
278 feature of this method since it can drastically decrease the time needed for equilibria studies  
279 and, moreover, allow the fast screening of interactions. This is especially important when  
280 manipulating hazardous and/or precious materials. It is expected that three or more analytes can  
281 be used simultaneously in equilibria studies provided they have different enough mobilities.

282 This study also describes a significant contribution to the knowledge of V(IV) and V(V)  
283 chemistry. For the first time, to our knowledge, the existence of a protonated V(V) complex

284 with succinic acid is illustrated and the corresponding stability constant measured. A significant  
285 insight is also provided on V(IV)-succinic acid complexation.

286

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291

### 292 **CRedit author statement:**

293 **Lucija Knežević:** Investigation, Formal analysis, Methodology, Data curation,  
294 Visualization.

295 **Emanuele Zanda:** Formal analysis, Methodology.

296 **Elvira Bura-Nakić:** Supervision, Funding acquisition, Project administration.

297 **Montserrat Filella:** Writing - Review & Editing, Validation.

298 **Vladimir Sladkov:** Conceptualization, Methodology, Validation, Resources, Writing-  
299 original draft.

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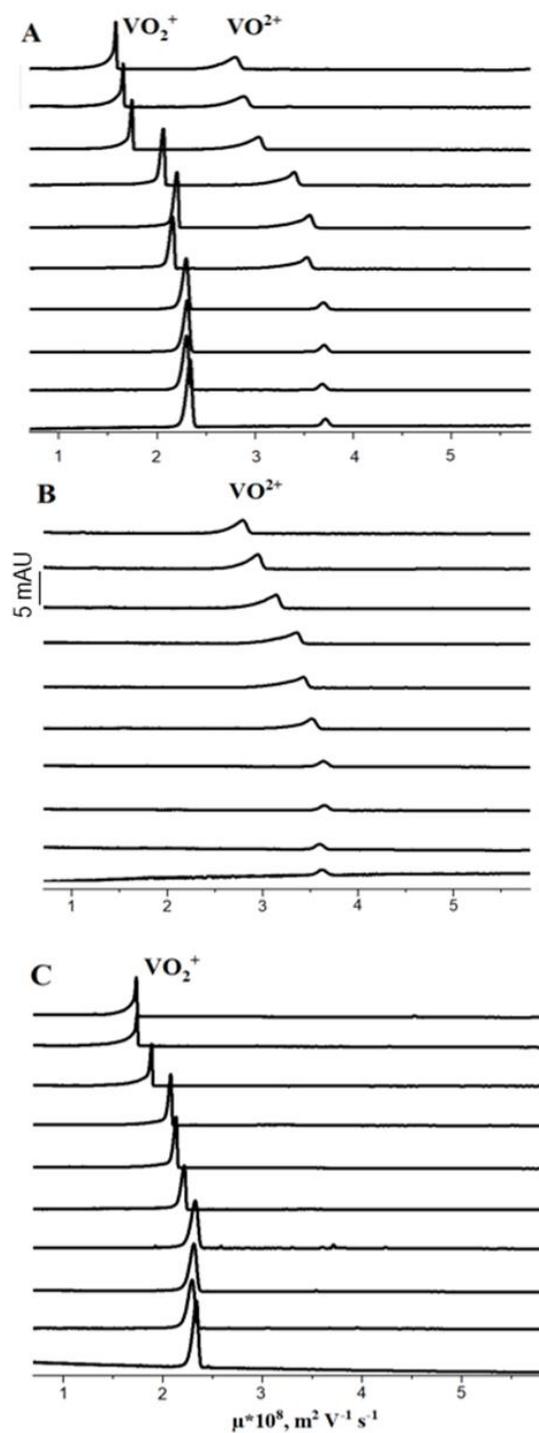
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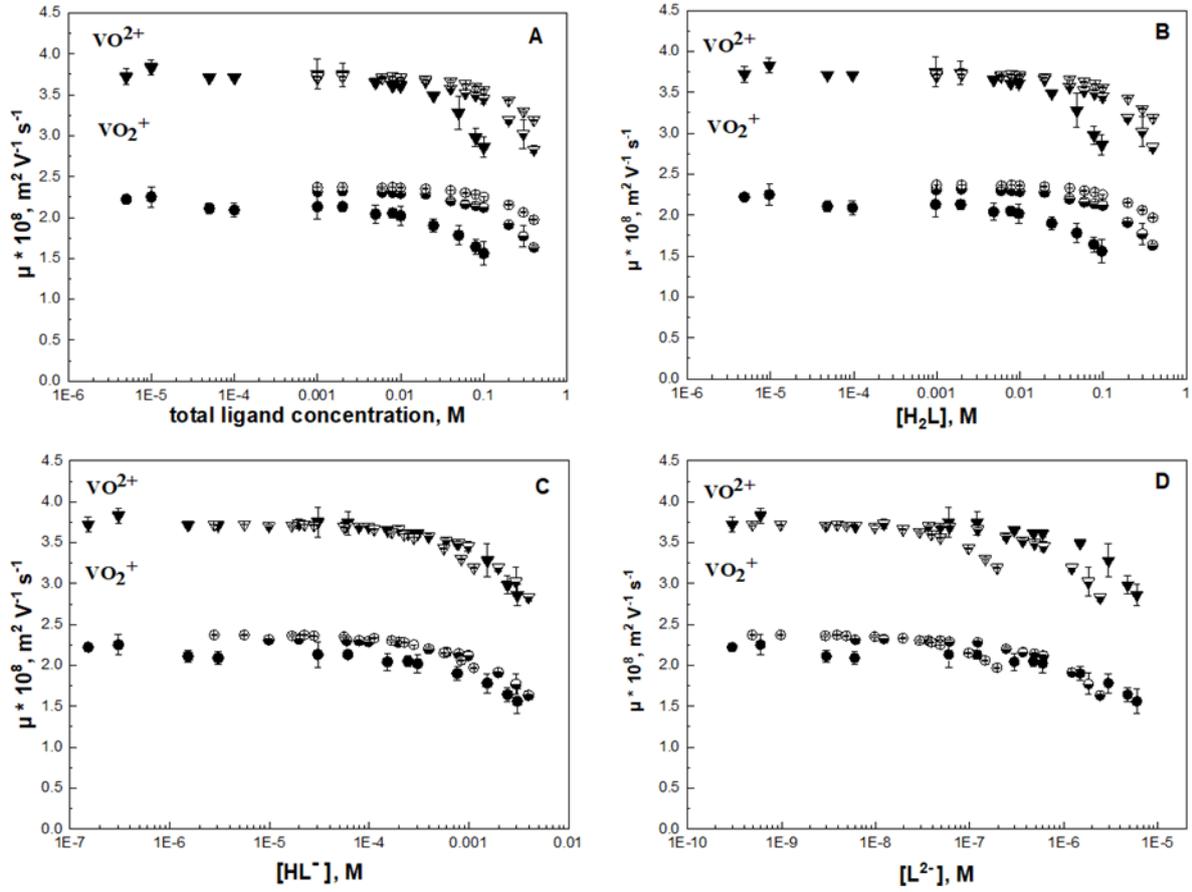
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308

309 **Figure 1.** Electropherograms of  $5 \times 10^{-4}$  M V(IV) and  $1 \times 10^{-3}$  M V(V), in the presence of  
 310 different amounts of succinic acid in BGE. Ligand concentrations in BGE from bottom to the  
 311 top: 0,  $1 \times 10^{-3}$ ,  $6 \times 10^{-3}$ ,  $8 \times 10^{-3}$ , 0.04, 0.08, 0.1, 0.2, 0.3 and 0.4 M. **A** – simultaneous injection of  
 312 two analytes. **B** – injection only V(IV). **C** – injection only V(V). pH=2, I=0.1 M ( $\text{NaClO}_4 -$   
 313  $\text{HClO}_4$ ). Detection wavelength: 200 nm, T=25°C.

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316 **Figure 2.** Mobilities of V(IV) (triangles) and V(V) (circles) at pH=1.5 (empty markers) pH=2  
 317 (half-full markers) pH=2.4 (full markers) as a function of total ligand concentration,  $[H_2L]$ ,  
 318  $[HL^-]$  and  $[L^{2-}]$ . Simultaneous injection of two analytes. All other conditions as in figure 1.

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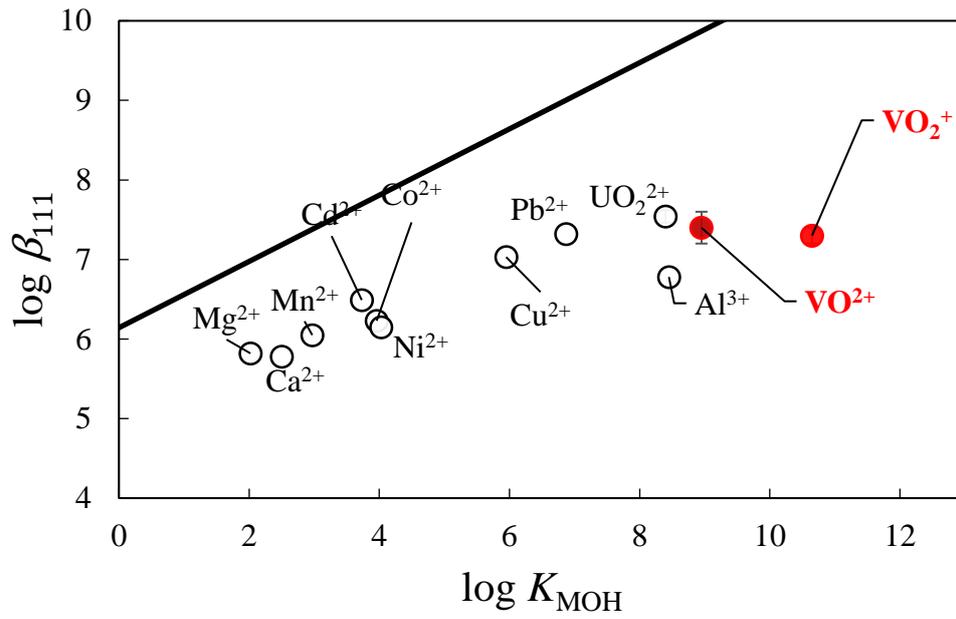
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331 **Figure 3.** Plot of the stability constant ( $\log \beta_{111}$ ) of different metal ions with succinic acid as a  
 332 function of the first metal hydrolysis ( $\log K_{\text{MOH}}$ ).

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**Table 1.** Published stability constants for the V(IV)-succinic acid system. The constants are given for the equilibrium:  $\text{VO}^{2+} + n\text{L}^{2-} + m\text{H}^+ \rightleftharpoons \text{VOL}_n\text{H}_m^{2-2n+m}$ . If not mentioned otherwise, the values are presented in the form given in the original sources.

Ionic strength, M (electrolyte used)	pH range studied	$T, ^\circ\text{C}$	$\log\beta_{110}$	$\log\beta_{111}$	$\log\beta_{11-2}$	$\log\beta_{11-3}$	$\log\beta_{121}$	$\log\beta_{120}$	Method	Ref.
~0.1 (NaClO <sub>4</sub> )		30±0.1	3.98						pot.	[26]
0.1 (NaClO <sub>4</sub> )	1.5-3.5	30±0.1	3.65±0.05						pot.	[27]
0.1 (KNO <sub>3</sub> )		25	3.43±0.08					6.5±0.6*	ESEEM sp.	[28]
0.2 (KCl)	2.0-6.7	25.0±0.1	3.20(4)**	7.2(2)		-7.25(3)	10.76(4)**	5.6(1)	pot.	[29]
0.1 (NaClO <sub>4</sub> )	3.5-5.0	20±2			-2.30±0.03***				sp.	[30]

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\* recalculated by the authors; in the original article stepwise constants are given:  $\log K_1=3.43\pm 0.08$  and  $\log K_2=3.11\pm 0.48$ .

\*\* it was not possible to refine them simultaneously.

\*\*\* recalculated by the authors from  $\log\beta_{112}(25.7)$ .

Abbreviations: pot. – potentiometry, ESEEM sp. – electron spin echo envelope modulation spectroscopy, sp. - spectrophotometry.

340  
 341 **Table 2.** Stability constant values obtained for V(IV)-succinic acid and V(V)-succinic acid  
 342 equilibria. Values at I=0.1 M (NaClO<sub>4</sub>-HClO<sub>4</sub>), except the last row where I=0 M. T=25°C.

343

N pH	V(IV)				V(V)	
	logβ <sub>111</sub>		logβ <sub>122</sub>		logβ <sub>111</sub>	
	1	2	1	2	1	2
2.5	7.2 ± 0.2	7.5 ± 0.2	14.6 ± 0.1	13.7 ± 0.6		
2.0	7.3 ± 0.2	7.4 ± 0.1	14.2 ± 0.2	13.6 ± 0.5	7.3 ± 0.4	7.3 ± 0.4
1.5		7.6 ± 0.1		14.4 ± 0.5		7.4 ± 0.4
<b>Average*</b>	7.3 ± 0.6	7.5 ± 0.2	14 ± 2	14 ± 1	7.3 ± 0.4	7.4 ± 0.6
<b>Average**</b>	7.4 ± 0.2		14.1 ± 0.5		7.3 ± 0.1	
<b>Average (I=0)</b>	8.3 ± 0.2		15.6 ± 0.5		7.9 ± 0.1	

344 N- analyte injection index

345 \* only for the same number of analytes injected and different pH values

346 \*\* for all measurements

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349 **6 References:**

- 350 [1] W. Hummel, M. Filella, D. Rowland, Where to find equilibrium constants?, *Sci. Total*  
351 *Environ.* 692 (2019) 49. <https://doi.org/10.1016/j.scitotenv.2019.07.161>.
- 352 [2] A. Timerbaev, R. Timerbaev, Recent progress of capillary electrophoresis in studying  
353 the speciation of actinides, *TrAC, Trends Anal. Chem.* 51 (2013) 44.  
354 <https://doi.org/10.1016/j.trac.2013.05.015>.
- 355 [3] F. Varenne, M. Bourdillon, M. Meyer, Y. Lin, M. Brellier, R. Baati, L.J.  
356 Charbonnière, A. Wagner, E. Doris, F. Taran, A. Hagège, Capillary electrophoresis–  
357 inductively coupled plasma-mass spectrometry hyphenation for the determination at  
358 the nanogram scale of metal affinities and binding constants of phosphorylated  
359 ligands, *J. Chromatogr., A* 1229 (2012) 280.  
360 <https://doi.org/10.1016/j.chroma.2012.01.066>.
- 361 [4] V.V. Sursyakova, G.V. Burmakina, A.I. Rubaylo, Composition and stability constants  
362 of copper(II) complexes with succinic acid determined by capillary electrophoresis, *J.*  
363 *Coord. Chem.* 70 (2017) 431. <https://doi.org/10.1080/00958972.2016.1270450>.
- 364 [5] S. Huclier-Markai, C. Alliot, J. Sebti, B. Brunel, J. Aupiais, A comparative  
365 thermodynamic study of the formation of scandium complexes with DTPA and  
366 DOTA, *RSC Adv.* 5 (2015) 99606. <https://doi.org/10.1039/C5RA16736A>.
- 367 [6] R. Konášová, J.J. Dyrťová, V. Kašička, Study of solvent effects on the stability  
368 constant and ionic mobility of the dibenzo-18-crown-6 complex with potassium ion by  
369 affinity capillary electrophoresis, *J. Sep. Sci.* 39 (2016) 4429.  
370 <https://doi.org/10.1002/jssc.201600830>.
- 371 [7] V. Sladkov, Affinity capillary electrophoresis in studying the complex formation  
372 equilibria of radionuclides in aqueous solutions, *Electrophoresis* 37 (2016) 2558.  
373 <https://doi.org/10.1002/elps.201600093>.
- 374 [8] A. Sornosa-Ten, P. Jewula, T. Fodor, S. Brandès, V. Sladkov, Y. Rousselin, C. Stern,  
375 J.-C. Chambron, M. Meyer, Effects of preorganization in the chelation of UO<sub>2</sub><sup>2+</sup> by  
376 hydroxamate ligands: cyclic PIPO–vs. linear NMA–, *New J. Chem.* 42 (2018) 7765.  
377 <https://doi.org/10.1039/C8NJ00166A>.
- 378 [9] V. Sladkov, J. Roques, M. Meyer, Assignment of complex species by affinity capillary  
379 electrophoresis: The case of Th(IV)-desferrioxamine B, *Electrophoresis* 41 (2020)  
380 1870. <https://doi.org/10.1002/elps.202000114>.
- 381 [10] V. Sladkov, Interaction of uranyl with Se(IV) and Se(VI) in aqueous acid solutions by  
382 means of capillary electrophoresis, *Electrophoresis* 31 (2010) 3482.  
383 <https://doi.org/10.1002/elps.201000247>.
- 384 [11] V. Sladkov, Uranyl complexation with acetate studied by means of affinity capillary  
385 electrophoresis, *J. Chromatogr., A* 1289 (2013) 133.  
386 <https://doi.org/10.1016/j.chroma.2013.03.017>.
- 387 [12] Y.H. Chu, L.Z. Avila, H.A. Biebuyck, G.M. Whitesides, Use of affinity capillary  
388 electrophoresis to measure binding constants of ligands to proteins, *J. Med. Chem.* 35  
389 (1992) 2915. <https://doi.org/10.1021/jm00093a027>.
- 390 [13] M.W. Makinen, M. Salehitazangi, The structural basis of action of vanadyl (VO<sub>2</sub><sup>+</sup>)  
391 chelates in cells, *Coord. Chem. Rev.* 279 (2014) 1.  
392 <https://doi.org/10.1016/j.ccr.2014.07.003>.
- 393 [14] K.A. Rubinson, I.M. Glynn, Concerning the form of biochemically active vanadium,  
394 *Proc. R. Soc. London, Ser. B* 212 (1981) 65.  
395 <https://doi.org/doi:10.1098/rspb.1981.0025>.

- 396 [15] J. Krakowiak, D. Lundberg, I. Persson, A Coordination Chemistry Study of Hydrated  
397 and Solvated Cationic Vanadium Ions in Oxidation States +III, +IV, and +V in  
398 Solution and Solid State, *Inorg. Chem.* 51 (2012) 9598.  
399 <https://doi.org/10.1021/ic300202f>.
- 400 [16] N. Mehio, J.C. Johnson, S. Dai, V.S. Bryantsev, Theoretical study of the coordination  
401 behavior of formate and formamidoximate with dioxovanadium(v) cation:  
402 implications for selectivity towards uranyl, *Phys. Chem. Chem. Phys.* 17 (2015)  
403 31715. <https://doi.org/10.1039/C5CP06165B>.
- 404 [17] J.A.J. Watt, I.T. Burke, R.A. Edwards, H.M. Malcolm, W.M. Mayes, J.P. Olszewska,  
405 G. Pan, M.C. Graham, K.V. Heal, N.L. Rose, S.D. Turner, B.M. Spears, Vanadium: A  
406 Re-Emerging Environmental Hazard, *Environ. Sci. Technol.* 52 (2018) 11973.  
407 <https://doi.org/10.1021/acs.est.8b05560>.
- 408 [18] D.J. White, L.S. Levy, Vanadium: environmental hazard or environmental  
409 opportunity? A perspective on some key research needs, *Environ. Sci. Process Impacts*  
410 23 (2021) 527. <https://doi.org/10.1039/D0EM00470G>.
- 411 [19] D.A. Santos, M.K. Dixit, P. Pradeep Kumar, S. Banerjee, Assessing the role of  
412 vanadium technologies in decarbonizing hard-to-abate sectors and enabling the energy  
413 transition, *iScience* 24 (2021). <https://doi.org/10.1016/j.isci.2021.103277>.
- 414 [20] Y. Teng, S. Ni, C. Zhang, J. Wang, X. Lin, Y. Huang, Environmental geochemistry  
415 and ecological risk of vanadium pollution in Panzhihua mining and smelting area,  
416 Sichuan, China, *Chin. J. Geochem.* 25 (2006) 379. [https://doi.org/10.1007/s11631-  
417 006-0378-3](https://doi.org/10.1007/s11631-006-0378-3).
- 418 [21] A.U. Nnama, F.N. Ekeh, I.O. Aguzie, S.O. Udegbumam, C.D. Nwani, Vanadium  
419 pentoxide induces hematological, oxidative stress and histological changes in  
420 *Oryctolagus cuniculus*, *J. Hazard. Mater. Adv.* 5 (2022) 100048.  
421 <https://doi.org/10.1016/j.hazadv.2022.100048>.
- 422 [22] E. Hanus-Fajerska, A. Wiszniewska, I. Kamińska, A Dual Role of Vanadium in  
423 Environmental Systems-Beneficial and Detrimental Effects on Terrestrial Plants and  
424 Humans, *Plants (Basel, Switzerland)* 10 (2021) 1110.  
425 <https://doi.org/10.3390/plants10061110>.
- 426 [23] J.P. Gustafsson, Vanadium geochemistry in the biogeosphere –speciation, solid-  
427 solution interactions, and ecotoxicity, *App. Geochem.* 102 (2019) 1.  
428 <https://doi.org/10.1016/j.apgeochem.2018.12.027>.
- 429 [24] C. Lucks, A. Rossberg, S. Tsushima, H. Foerstendorf, A.C. Scheinost, G. Bernhard,  
430 Aqueous Uranium(VI) Complexes with Acetic and Succinic Acid: Speciation and  
431 Structure Revisited, *Inorg. Chem.* 51 (2012) 12288.  
432 <https://doi.org/10.1021/ic301565p>.
- 433 [25] V. Sladkov, A.A. Bessonov, J. Roques, I.A. Charushnikova, A.M. Fedosseev,  
434 Complexation of An(VI) with succinic acid in aqueous acid solutions: uranyl vs.  
435 plutonyl, *New Journ. Chem.* 42 (2018) 7780. <https://doi.org/10.1039/C7NJ04061J>.
- 436 [26] D.N. Shelke, D.V. Jahagirdar, Ternary complexes: Equilibrium studies of mixed  
437 ligand complexes of vanadyl ion with some carboxylic and phenolic acids, *J. Inorg.*  
438 *Nucl. Chem.* 39 (1977) 2223. [https://doi.org/https://doi.org/10.1016/0022-  
439 1902\(77\)80400-4](https://doi.org/https://doi.org/10.1016/0022-1902(77)80400-4).
- 440 [27] K. Narasimhulu, U. Seshaiiah, Formation Constants of Oxovanadium(IV) & Cu(II)  
441 Chelates of Dicarboxylic Acids, *Indian J. Chem., Sect A* 19A (1980) 1027.
- 442 [28] H. Sakurai, K. Tsuchiya, A biomimetic model for vanadium-containing  
443 bromoperoxidase, *FEBS Lett.* 260 (1990) 109.  
444 [https://doi.org/https://doi.org/10.1016/0014-5793\(90\)80079-X](https://doi.org/https://doi.org/10.1016/0014-5793(90)80079-X).

- 445 [29] J. Costa Pessoa, T. Gajda, R. D. Gillard, T. Kiss, S. M. Luz, J. J. G. Moura, I. Tomaz,  
446 J. P. Telo, I. Török, Oxovanadium(IV) complexes of the dipeptides glycyl-L-aspartic  
447 acid, L-aspartylglycine and related ligands; a spectroscopic and potentiometric study,  
448 J. Chem. Soc., Dalton Trans. (1998) 3587. <https://doi.org/10.1039/A801888J>.
- 449 [30] V.I. Kornev, D.A. Merkulov, Homo- and heteroligand Vanadium(IV) complexes with  
450  $\beta$ -Hydroxyethyliminodiacetic and dicarboxylic acids, Russ. J. Coord. Chem. 23 (1997)  
451 488.
- 452 [31] A.S. Tracey, H. Li, M.J. Gresser, Interactions of vanadate with mono- and  
453 dicarboxylic acids, Inorg. Chem. 29 (1990) 2267.  
454 <https://doi.org/10.1021/ic00337a018>.
- 455 [32] S. Topin, J. Aupiais, N. Baglan, T. Vercouter, P. Vitorge, P. Moisy, Trace Metal  
456 Speciation by Capillary Electrophoresis Hyphenated to Inductively Coupled Plasma  
457 Mass Spectrometry: Sulfate and Chloride Complexes of Np(V) and Pu(V), Anal.  
458 Chem. 81 (2009) 5354. <https://doi.org/10.1021/ac900275d>.
- 459 [33] C.J. Evenhuis, M.U. Musheev, S.N. Krylov, Universal Method for Determining  
460 Electrolyte Temperatures in Capillary Electrophoresis, Anal. Chem. 83 (2011) 1808.  
461 <https://doi.org/10.1021/ac103216s>.
- 462 [34] V. Sladkov, Effect of non-thermostated capillary inlet in affinity capillary  
463 electrophoresis: Uranyl-selenate system at variable temperatures, J.Chromatogr. A  
464 1263 (2012) 189. <https://doi.org/10.1016/j.chroma.2012.09.041>.
- 465 [35] V. Sladkov, Uranyl complexation with selenate at variable temperatures studied by  
466 affinity capillary electrophoresis, J. Chromatogr. A 1276 (2013) 120.  
467 <https://doi.org/10.1016/j.chroma.2012.12.040>.
- 468 [36] V. Sladkov, Interaction of uranyl with acetate in aqueous solutions at variable  
469 temperatures, J. Chem. Thermodyn. 71 (2014) 148. [https://doi.org/](https://doi.org/10.1016/j.jct.2013.12.011)  
470 [10.1016/j.jct.2013.12.011](https://doi.org/10.1016/j.jct.2013.12.011).
- 471 [37] I. Grenthe, H. Wanner, E. Ostholts, in, OECD Nuclear Energy Agency, 2000, p. 17.
- 472 [38] S.N. Krylov, Kinetic CE: Foundation for homogeneous kinetic affinity methods,  
473 Electrophoresis 28 (2007) 69. <https://doi.org/https://doi.org/10.1002/elps.200600577>.
- 474 [39] L. Helm, A.E. Merbach, Water exchange on metal ions: experiments and simulations,  
475 Coord. Chem. Rev. 187 (1999) 151. [https://doi.org/https://doi.org/10.1016/S0010-](https://doi.org/https://doi.org/10.1016/S0010-8545(99)90232-1)  
476 [8545\(99\)90232-1](https://doi.org/https://doi.org/10.1016/S0010-8545(99)90232-1).
- 477 [40] J. Reuben, D. Fiat, Magnetic resonance studies of ion solvation. The hydration of the  
478 vanadyl(IV) ion, Inorg. Chem. 6 (1967) 579. <https://doi.org/10.1021/ic50049a034>.
- 479 [41] S. Yamada, Y. Ukei, M. Tanaka, Kinetics and mechanism of the complexation  
480 reactions of pervanadyl ion with some aminopolycarboxylates, Inorg. Chem. 15  
481 (1976) 964. <https://doi.org/10.1021/ic50158a048>.
- 482 [42] M.T. Bowser, D.D.Y. Chen, Monte Carlo Simulation of Error Propagation in the  
483 Determination of Binding Constants from Rectangular Hyperbolae. 1. Ligand  
484 Concentration Range and Binding Constant J. Phys. Chem. A 102 (1998) 8063.  
485 <https://doi.org/10.1021/jp9819947>
- 486 [43] M.T. Bowser, D.D.Y. Chen, Monte Carlo Simulation of Error Propagation in the  
487 Determination of Binding Constants from Rectangular Hyperbolae. 2. Effect of the  
488 Maximum-Response Range, J. Phys. Chem. A 103 (1999) 197.  
489 <https://doi.org/10.1021/jp982917e>.
- 490 [44] V. Šolínová, H. Mikysková, M.M. Kaiser, Z. Janeba, A. Holý, V. Kašička, Estimation  
491 of apparent binding constant of complexes of selected acyclic nucleoside  
492 phosphonates with  $\beta$ -cyclodextrin by affinity capillary electrophoresis, Electrophoresis  
493 37 (2016) 239. <https://doi.org/10.1002/elps.201500337>.

- 494 [45] J.R. Morphy, D. Parker, R. Katakya, M.A.W. Eaton, A.T. Millican, R. Alexander, A.  
495 Harrison, C. Walker, Towards tumour targeting with copper-radiolabelled  
496 macrocycle–antibody conjugates: synthesis, antibody linkage, and complexation  
497 behaviour, *J. Chem. Soc., Perkin Trans. 2* (1990) 573.  
498 <https://doi.org/10.1039/P29900000573>.
- 499 [46] V. Sladkov, B. Fourest, F. Mercier, Uranyl-Se(IV) interaction in aqueous acid  
500 solutions studied by time-resolved laser-induced fluorescence spectroscopy (TRLFS)  
501 and UV-Vis spectrophotometry, *Dalton Trans.* (2009) 7734.  
502 <https://doi.org/10.1039/B907235G>.
- 503 [47] V. Sladkov, M. He, P. Jewula, M.-J. Penouilh, S. Brandès, C. Stern, J.-C. Chambron,  
504 M. Meyer, A solution- and gas-phase study of uranyl hydroxamate complexes, *J.*  
505 *Radioanal. Nucl. Chem.* 318 (2018) 259. <https://doi.org/10.1007/s10967-018-6019-6>.
- 506 [48] R.D. Hancock, A.E. Martell, Ligand design for selective complexation of metal ions in  
507 aqueous solution, *Chem. Rev.* 89 (1989) 1875. <https://doi.org/10.1021/cr00098a011>.
- 508 [49] V.I.E. Bruyère, L.A. García Rodenas, P.J. Morando, M.A. Blesa, Reduction of  
509 vanadium(v) by oxalic acid in aqueous acid solutions, *J. Chem. Soc., Dalton Trans.*  
510 (2001) 3593. <https://doi.org/10.1039/B103320B>.  
511  
512