Theoretical basis for the estimation of nitrite as a 4-nitroso-2,5-dimethylresorcinolate complex

Ille Johannes[*] and Laine Tiikma

Institute of Oil Shale, Tallinn Technical University, Akadeemia tee 15, 12618 Tallinn, Estonia

Abstract. A theoretical basis is presented for testing nitrite as cobalt and iron(II) 4-nitroso-2,5-dimethylresorcinolates. The joint effect of pH and concentration of the reagents on the absorbance is described by algorithms considering the stability of the complex formed, solubility product of the insoluble salt formed, and acidic dissociation of the iron and cobalt complexes and 4-nitroso-2,5-dimethylresorcinol. Acidic dissociation constants, molar absorptivities, stability constants, and solubility products of the species are estimated. The pH-dependences of the partial contributions to the total absorbance of the five coloured species formed are described.

Key words: nitrite, alkylresorcinols, analysis.

INTRODUCTION

Testing of nitrate and nitrite in water samples, soils, and food products is of considerable importance. An increased nitrate and nitrite concentration in water and agricultural products indicates their contamination. The potential health hazard from immoderate nitrate and nitrite content in food comes from the formation of carcinogenic *N*-nitrosoamines. Numerous analytical methods for nitrate testing involve reduction of nitrate to nitrite prior to spectrophotometric determination of the nitrite formed.

The colorimetric nitrite standard test method and its modifications [1–4] are based on the reaction of nitrite with an aromatic amine (sulphanilamide, sulpha-pyridine, sulphathiazole) to form a diazonium salt, and on the following reaction with a coupling reagent (1-naphtol-4-sulphonate, *N*-1-naphtylethylenediamine) to produce a highly coloured potentially carcinogenic azo dye.

---

[*] Corresponding author, johannes@netexpress.ee

We have created a simple and less toxic test method for colorimetric estimation of nitrite with 1,3-dihydroxyalkylderivatives (alkylresorcinols) [5, 6]. The new method is based on the formation of coloured nitrosoalkylresorcinolates. According to the simple procedure proposed the absorbance of nitrite samples should be measured at 420 nm, after V mL of the sample has been mixed with 0.25 V mL of the reagent solution containing pH buffer, alkylresorcinol, and cobalt or iron(II) sulphate.

Recently the theoretical basis of the method was described and the characteristic constants were found using 5-methylresorcinol, the main water soluble phenolic compound produced at the retorting of kukersite, as alkylresorcinol [7].

The aim of the present work was to create a theoretical basis for quantitative description of the nitrite test method with 2,5-dimethylresorcinol (2,5-DMR), another alkylresorcinol produced industrially from oil shale retort water.

THEORETICAL APPROACH
Reactions

When a nitrite test solution is sufficiently acidic for protonation of nitrite according to the reaction

$$NO_2^- + H^+ \rightleftarrows HNO_2, \tag{I}$$
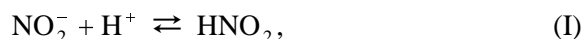
the nitrous acid formed can react with 2,5-DMR as follows:

$$HNO_2 + (CH_3)_2 C_6 H_2 (OH)_2 \rightarrow NO(CH_3)_2 C_6 H(OH)_2 + H_2O. \tag{II}$$

The specific structure of 1,3-dihydric phenols provides an inclined to complex formation *ortho*-position of the nitroso substituent against one of the hydroxyl groups of 2,5-DMR. Such position is not typical at nitrosation of most phenolic compounds yielding as a rule *para*-nitroso compounds.

The nitroso compound formed in Reaction II dissociates as a weak acid

$$NO(CH_3)_2 C_6 H(OH)_2 \rightleftarrows NO(CH_3)_2 C_6 H(OH)O^- + H^+. \tag{III}$$

The anion formed in Reaction III can give a coloured complex according to the reaction

$$Me^{n+} + nNO(CH_3)_2 C_6 H(OH)O^- \rightleftarrows Me[NO(CH_3)_2 C_6 H(OH)O]_n, \tag{IV}$$
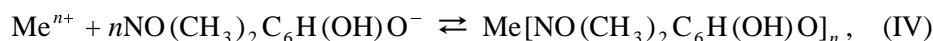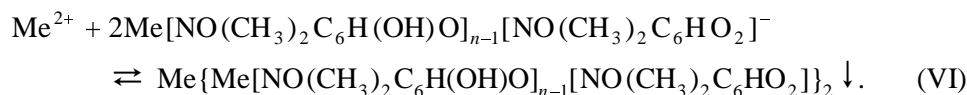
where n is 3 in the cobalt complex and 2 in the iron complex [6].

Analogically to the complex with 5-methylresorcinol [7], the complex formed is assumed to dissociate as a weak acid as follows:

$$Me[NO(CH_3)_2 C_6 H(OH)O]_n$$
$$\rightleftarrows Me[NO(CH_3)_2 C_6 H(OH)O]_{n-1}[NO(CH_3)_2 C_6 HO_2]^- + H^+. \tag{V}$$
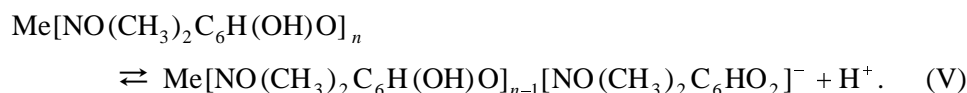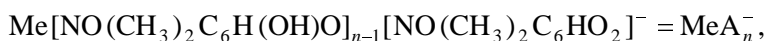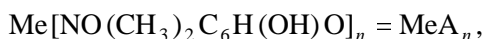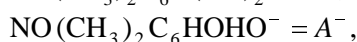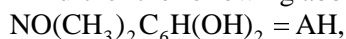
The cation added in excess and the anion of the complex can form an insoluble salt according to the reaction

$$Me^{2+} + 2Me[NO(CH_3)_2C_6H(OH)O]_{n-1}[NO(CH_3)_2C_6HO_2]^-$$

$$\rightleftarrows Me\{Me[NO(CH_3)_2C_6H(OH)O]_{n-1}[NO(CH_3)_2C_6HO_2]\}_2 \downarrow. \quad (VI)$$

Algorithms for the prediction of absorbance

Abbreviations

Further the following abbreviations will be used:

$$NO(CH_3)_2C_6H(OH)_2 = AH,$$
$$NO(CH_3)_2C_6HOHO^- = A^-,$$
$$Me[NO(CH_3)_2C_6H(OH)O]_n = MeA_n,$$
$$Me[NO(CH_3)_2C_6H(OH)O]_{n-1}[NO(CH_3)_2C_6HO_2]^- = MeA_n^-,$$
$$Me\{Me[NO(CH_3)_2C_6H(OH)O]_{n-1}[NO(CH_3)_2C_6HO_2]\}_2 = Me(MeA_n)_2.$$

Effects of the initial concentrations

For the test method of the nitrite studied an excess of alkylresorcinol is obligatory. Conversely, the nitrosation process is too slow for testing diluted nitrite solutions. Besides, due to the by-reactions, such as decomposition of nitrous acid and formation of dinitrosoalkylresorcinols, the quantity of the complex formed is not proportional to the nitrite concentration. The nitrosation reaction (II) is an irreversible process. Consequently, the alkylresorcinol concentration should affect mainly the nitrosation rate. When the steady state of absorbance (E) in time is attained with a sufficient excess of alkylresorcinol (0.01 M in this study), practically all nitrite should be converted into the respective nitrosoalkylresorcinol.

The simplest algorithm considers the total effect of all the coloured species formed and describes the relationship between E and the initial concentrations of nitrite (C_N) and the complexing cation (C_{Me}) at the optimum pH value.

The expression for stability constants of a complex MeA_n

$$\beta = [MeA_n]/([Me^{n+}][A^-]^n) \quad (1)$$

can be represented by absorbance measured as follows:

$$\beta_H = E\varepsilon_H^n/(C_{Me}\varepsilon_H - E)/(C_N\varepsilon_H - nE)^n, \quad (2)$$

where β_H is the conditional stability constant and ε_H is the conditional molar absorptivity of the complex valid at the pH value of the test series only.

When β_H and ε_H are known, the absorbance at any C_{Me} and C_N can be computed from Eq. 2. When β_H and ε_H are unknown, their values can be estimated from Eq. 2 using the least squares method for E values found at various C_{Me} and C_N.

202

Co-effects of pH, C_{Me}*, and* C_N

A more comprehensive algorithm considers the effect of pH on the partial contribution of the coloured species formed. The validity of the algorithm depends on whether the absorbance is estimated for a clear or a turbid solution.

In clear solutions the mass balance of nitrite and the total absorbance are compiled as follows:

$$C_N = x_1 + x_2 + nx_3 + nx_4, \tag{3}$$

$$E = \varepsilon_1 x_1 + \varepsilon_2 x_2 + \varepsilon_3 x_3 + \varepsilon_4 x_4, \tag{4}$$

where x_1, x_2, x_3, x_4 and ε_1, ε_2, ε_3, ε_4 are the equilibrium concentrations and the molar absorptivities of AH, A^-, MeA_n, and MeA_n^-, respectively.

The stability constant of the complex is expressed by the equation

$$\beta = (x_3 + x_4)\big/[C_{Me} - (x_3 + x_4)]\big/[C_N - n(x_3 + x_4)]^n. \tag{5}$$

When β and ε_1, ε_2, ε_3, and ε_4 are known, the E values can be predicted by Eq. 4. For this aim, first the total concentration of the complex $(x_3 + x_4)$ should be computed from the unrevealed relationship (Eq. 5). The concentrations of the other coloured species formed are related to the total concentration of the complex as follows:

$$x_4 = K_2 (x_3 + x_4)\big/(K_2 + [H^+]), \tag{6}$$

$$x_3 = x_4 [H^+]\big/K_2, \tag{7}$$

$$x_2 = K_1 [C_N - n(x_3 + x_4)]\big/(K_1 + [H^+]), \tag{8}$$

$$x_1 = [H^+][C_N - n(x_3 + x_4)]\big/(K_1 + [H^+]), \tag{9}$$

where K_1 and K_2 are the acidic dissociation constants of AH and MeA_n in Reactions III and V.

The unknown values of β and ε_3 can be estimated from Eq. 5. First, the total concentration of the complex should be calculated from the relationship deduced from Eqs. 3, 4, and 6–9

$$
\begin{aligned}
&x_3 + x_4 \\
&= (K_2 + [H^+])[E(K_1 + [H^+]) - C_N(\varepsilon_1[H^+] + \varepsilon_2 K_1)]\big/[\varepsilon_3(K_1 + [H^+])(qK_2 + [H^+]) \\
&\quad - n(\varepsilon_1[H^+] + \varepsilon_2 K_1)(K_2 + [H^+])],
\end{aligned}
\tag{10}
$$

where E and $[H^+]$ are found experimentally, but ε_1, ε_2, $q\,(\varepsilon_4/\varepsilon_3)$, K_1, and K_2 should be known or estimated by special series of experiments described below.

Turbidity can arise in a sample when the solubility product (*SP*) expressed as

$$SP = [Me^{2+}]\,x_4^2 \tag{11}$$

is attained for Reaction VI.

Like in Eqs. 3 and 4, the mass balance and the total absorbance of turbid solutions are compiled from the partial contributions of the coloured species formed

$$C_N = x_1 + x_2 + nx_3 + nx_4 + 2nx_5, \tag{12}$$

$$E\,(\text{turbid}) = \varepsilon_1 x_1 + \varepsilon_2 x_2 + \varepsilon_3 x_3 + \varepsilon_4 x_4 + \varepsilon_5 x_5, \tag{13}$$

where x_5 and ε_5 are the equilibrium concentration and the absorptivity of the insoluble species in the bulk of the solution, respectively.

When the cation initial concentration is significantly higher than the complex concentration, x_4 can be calculated from the approximated relationship

$$x_4 = (SP/C_{Me})^{1/2}, \tag{14}$$

suggesting that the concentration of the anionic species of the complex should be constant in turbid solutions and fixed by the cation concentration only.

When SP and ε_5 are known, the E value can be calculated by Eq. 13, computing first the value for x_4 from Eq. 14. Then x_3 is calculated using Eq. 7. Thereafter the equilibrium concentrations of the other coloured species are found as follows:

$$x_3 + x_4 = x_4 (K_2 + [H^+])/K_2, \tag{15}$$

$$x_2 = \{x_4 (K_2 + [H^+])/(K_2 C_{Me} \beta)\}^{1/n}, \tag{16}$$

$$x_1 = \{x_4 (K_2 + [H^+])/(K_2 C_{Me} \beta)\}^{1/n} [H^+]/K_1, \tag{17}$$

$$x_5 = (C_N - x_1 - x_2 - nx_3 - nx_4)/2n. \tag{18}$$

When SP and ε_5 are unknown, their values can be found from the following equation using the least squares method:

$$\varepsilon_5 = 2n (E - \varepsilon_1 x_1 - \varepsilon_2 x_2 - \varepsilon_3 x_3 - \varepsilon_4 x_4)/(C_N - x_1 - x_2 - nx_3 - nx_4), \tag{19}$$

where the concentrations of the species are replaced with their expressions 14–18.

Algorithms for the estimation of acidic dissociation characteristics

The equilibrium of an acidic dissociation reaction

$$AH^* \rightleftarrows H^+ + A^- \tag{VII}$$

is defined by the expression of the dissociation constant

---

* To distinguish the abbreviation of 4-nitroso-2,5-dimethylresorcinol, AH, from the general designation of a weak acid, the latter and its anion are written in italic.

$$K_a = [A^-][\text{H}^+]\big/[AH],\tag{20}$$

by the equations of mass balance

$$C = [A^-] + [AH],\tag{21}$$

and the total absorbance of the molecular and anionic species

$$E = \varepsilon_A[A^-] + \varepsilon_{AH}[AH].\tag{22}$$

Co-solution of Eqs. 20–22 enables to deduce two linear dependences on E for functions calculated from the experimental data as follows:

$$(E_A - E)\big/[\text{H}^+] = -\varepsilon_{AH}C\big/K_a + E\big/K_a\tag{23}$$

and

$$(E - E_{AH})[\text{H}^+] = \varepsilon_A C K_a - E K_a,\tag{24}$$

where E_A and E_{AH} are the practically constant optical densities of the alkalized and acidified solutions where the weak acid studied occurs totally in its anionic or molecular form.

Unfortunately, the exact total concentration (C) is unknown for the concentrates of the complexes obtained after reversible processes of complex formation, solid phase extraction, and re-extraction. So, when the complexes are studied, Eqs. 23 and 24 should be modified as

$$(E_A - E)\big/[\text{H}^+] = -E_{AH}\big/K_a + E\big/K_a\tag{25}$$

and

$$(E - E_{AH})[\text{H}^+] = E_A K_a - K_a E.\tag{26}$$

It is easy to find the dissociation characteristics from the coefficients of the linear regressions 23–26 expressed in general form as

$$y = a + bE.\tag{27}$$

When E_A is known (Eqs. 23 and 25)

$$K_a = 1\big/b\tag{28}$$

and when E_{AH} is known (Eqs. 24 and 26)

$$K_a \equiv b\tag{29}$$

independently of the total concentration of the reagent.

The unknown molar absorptivity for reagents with a known C value and the corresponding absorbance for complex solutions can be calculated as follows:

$$\varepsilon_{AH} \ (\text{or} \ \varepsilon_A) = /\,a\,/\,(bC)\,/ \qquad\qquad (30)$$

and

$$E_{AH} \ (\text{or} \ E_A) = /\,a\,/\,b\,/ \qquad\qquad (31)$$

independently of whether the molecular or anionic form is observed.

For complexes with an unknown total concentration the ratio

$$q = E_A \big/ E_{AH} = \varepsilon_4 \big/ \varepsilon_3 \qquad\qquad (32)$$

as an extinction characteristic independent of the initial concentration is suggested.

EXPERIMENTAL

Reagents and apparatus

All chemicals used were of analytical-reagent grade, except 2,5-DMR (98.4%) separated from oil shale phenols at the former Institute of Chemistry at Tallinn Technical University.

Nitrite stock standard solution, 0.01 M, was prepared by dissolving 0.6901 g of sodium nitrite, dried 24 h in a desiccator containing concentrated sulphuric acid, in 1000 cm^3 of distilled water.

Nitrite working standard solution was diluted from the appropriate volume of the *stock solution* on the day of sampling.

2,5-DMR solution, 0.05 M, was prepared by dissolving 3.455 g of 2,5-DMR in 500 cm^3 of distilled water. The solution was prepared freshly when it turned yellowish.

Me^{2+} solution, 0.05 M, was prepared by dissolving 3.88 g of $CoSO_4$ dried at 450 °C in 500 cm^3 of distilled water or 6.95 g of $FeSO_4 \times 7H_2O$ in 500 cm^3 of 0.05 M acetic acid preserved with 1 cm^3 of chloroform.

pH buffers were prepared by mixing an appropriate ratio of 0.1 M solutions of CH_3COOH, CH_3COONa, and alkali.

The absorbance of the samples was recorded by a spectrophotometer SPECOL 10. The pH values were measured by a METLER TOLEDO pH-meter 320.

Procedure

An aliquot of the 2.5×10^{-4} M of *Nitrite working standard solution* was transferred into a 25 cm^3 flask, and certain volumes of *Buffer solution*, *Me^{2+} solution*, and *2,5-DMR solution* were added. The sample volume was adjusted to 25 cm^3 with distilled water. Absorbance of the yellow samples formed was estimated in 1 cm cells at 420 nm. To guarantee equilibrium of the reactions the measurements were carried out the next day.

The nitrosation product, 4-nitroso-2,5-dimethylresorcinol, is an unpurchasable new reagent. Therefore, the reagent was synthesized according to the procedures described in [8]. For estimation of the dissociation characteristics of the nitroso-compound its 5×10^{-4} M solutions in 0.01 M H_2SO_4, CH_3COOH, CH_3COONa, and NaOH were mixed to obtain a series with pH 2–6.

When dissociation characteristics of CoA_3 and FeA_2 were studied, the complexes formed in the 2.5×10^{-4} M nitrite solution under the optimum conditions found were separated from the excess of the reagents by solid phase disk-extraction. For this aim 500 cm^3 of the complex solution was passed through the J. T. Baker Speedisk SDB. The complex on the disk was washed with water and eluted with 50 mL of ethanol. The aliquots of the concentrates obtained were diluted $1:20$ with various pH buffer solutions.

All the experiments were carried out at room temperature.

RESULTS AND DISCUSSION
Dissociation characteristics

Figure 1 demonstrates the effect of pH on the absorbance of the solutions of AH, CoA_3, and FeA_2. The dissociation constants calculated for AH using Eqs. 23 and 24 and for CoA_3 using Eqs. 25 and 26 agree. To prevent decomposition of the moderately weak complex FeA_2 the series was carried out in 0.001 M solutions of iron(II) sulphate. Hydrolysis of iron cations made fixing the value for E_A impossible. So, only Eq. 26 was apropos for FeA_2. The values of the characteristics found are presented in Table 1.
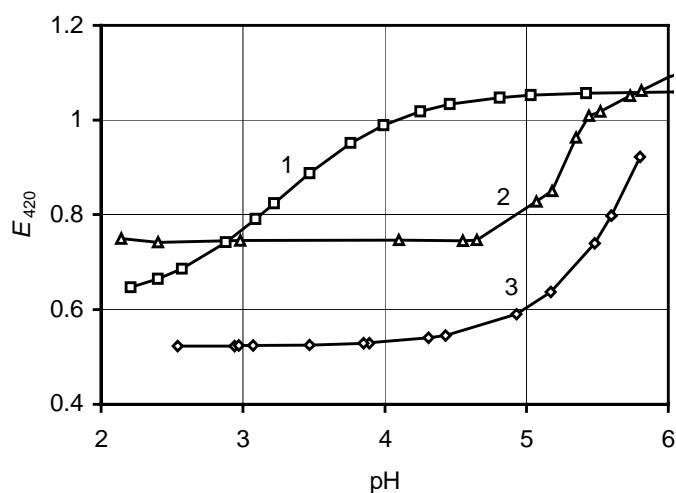

Fig. 1. Effect of pH on the absorbance at acidic dissociation of weak acids. 1 – AH (5E–04 M), 2 – concentrate of CoA_3, 3 – concentrate of FeA_2.

Table 1. Acidic dissociation constants and absorptivities

Characteristic	AH	CoA$_3$	FeA$_2$
K_1	5.48E–04	–	–
K_2	–	5.90E–06	4.73E–07
ε_1 (420 nm)	1220	–	–
ε_2 (420 nm)	3120	–	–
$q = \varepsilon_4 / \varepsilon_3$	–	1.54	5.09

Effect of C_{Me} on E_{H}

Estimation of the conditional constants ε_H and β_H

Figure 2 depicts the effect of the initial concentrations of cobalt and iron(II) sulphates on the absorbance when nitrite concentration is 5×10^{-5} M, 2,5-DMR concentration is 0.01 M, $pH_{Co} = 4.0$, and $pH_{Fe} = 3.3$.

The conditional stability constant and molar absorptivity were computed from Eq. 3 by the least squares method in Microsoft Excel. For this aim, various values of ε_{H} were fed until the least mean squares deviation of β_{H} from the mean β_{H} was obtained at various values of C_{Me}. The calculation process is illustrated by Table 2 for the cobalt complex. The characteristics obtained for both complexes are gathered in Table 3.
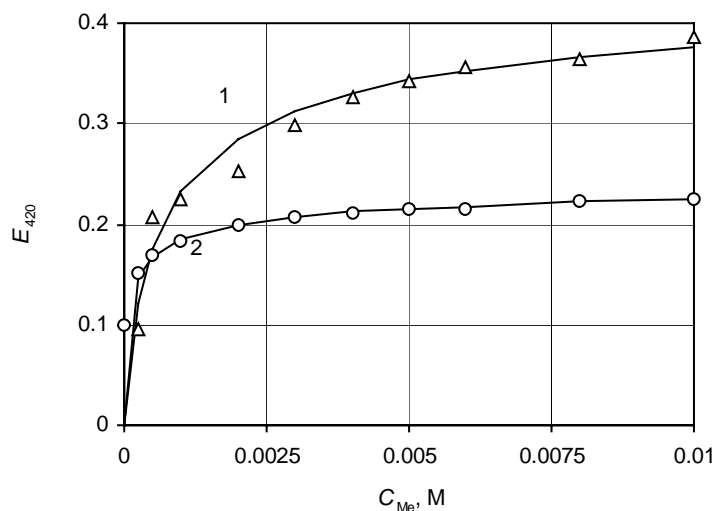

Fig. 2. Effect of the initial concentration of complexing cations on the absorbance. Points – experimental, curves – calculated using conditional constants; 1 – iron(II), 2 – cobalt compounds. $pH_{Co} = 4.0$, $pH_{Fe} = 3.3$, $C_{\text{N}} = 5E$–05 M.

Table 2. Estimation of the conditional constants β_H and ε_H for the cobalt complex. $C_N = 5E\text{--}05$ M, pH = 4.0, $n = 3$. When $\varepsilon_H = 15\,800$, $s_\beta = 10.1\%$

C_{Co}, M	E_H	β_H	s^2	E_{calc}
2.50E–04	0.152	4.23E+12	4.21E+23	0.147
5.00E–04	0.168	3.66E+12	5.75E+21	0.167
0.001	0.184	3.44E+12	1.97E+22	0.185
0.002	0.198	3.30E+12	8.1E+22	0.2
0.003	0.206	3.38E+12	4.17E+22	0.207
0.004	0.211	3.41E+12	3.01E+22	0.212
0.005	0.215	3.53E+12	3.24E+21	0.215
0.006	0.215	2.94E+12	4.18E+23	0.217
0.008	0.223	3.93E+12	1.21E+23	0.222
0.01	0.226	4.02E+12	1.88E+23	0.225
Mean		3.59E+12	1.33E+23	

Table 3. Characteristics of the complexes and insoluble salts

Characteristic	CoA_3	FeA_2	$Co(CoA_3)_2$	$Fe(FeA_2)_2$
ε_H	15 800	19 000	–	–
β_H	3.59E+12	1.89E+07	–	–
ε_3	15 500	10 880	–	–
ε_4	23 870	55 380	–	–
β	823E+11	4.13E+08	–	–
ε_5	–	–	26 900	75 800
SP	–	–	1.15E–15	6.20E–19

Effect of C_{Me}

The theoretical curves in Fig. 2 computed introducing the conditional constants in Table 3 into Eq. 2 are in good agreement with the experimental plots of E_H versus C_{Me} for cobalt complexes (curve 3) and in more or less satisfactory agreement with those for iron complexes.

Effect of pH on E in clear solutions

Figure 3 depicts the effect of pH on the absorbance for 5×10^{-5} M nitrite when C_{Me} is 0.002 M.

Estimation of ε_3, ε_4, and β

The values of stability constants for the formation of CoA_3 and FeA_2 and of molar absorptivity for their molecular and anionic species were computed in Excel using Eq. 5 and the constants in Table 1. For this aim, various values of ε_3 were fed into Eq. 10 to calculate the sum of x_3 and x_4 for Eq. 5 until the least mean squares deviation of β from its mean was obtained at various pH values. After that, the values of x_4, x_3, x_2, and x_1 were found by Eqs. 6–9. As an example, the calculation process for the iron complex is presented in Table 4. For iron complexes some illogical negative values of x_1 and x_2 were obtained
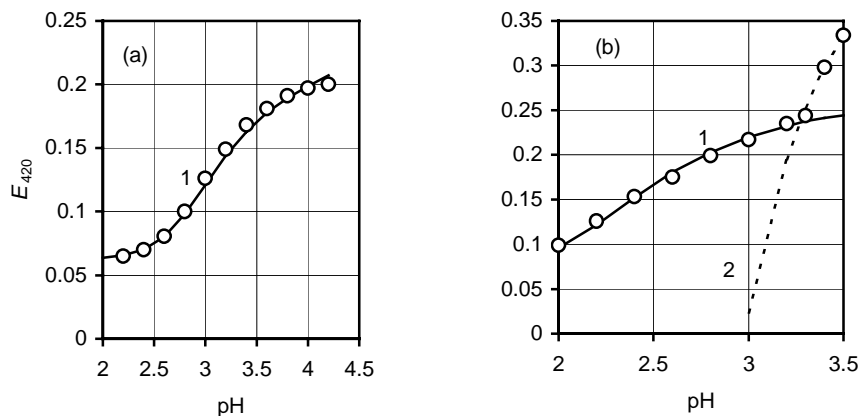
Fig. 3. Effect of pH on the absorbance for cobalt (a) and iron(II) (b) compounds. Points – experimental, curves – calculated; 1 – clear solutions, 2 – turbid solutions. C_N = 5E–05, C_{Me} = 0.002 M.

since turbidity occurred at higher pH values (shadowed rows in Table 4). All the equilibrium concentrations calculated for the cobalt complex were positive.

The characteristic constants of both complexes found for the clear solutions are given in Table 3.

Effect of pH

Figure 3 compares experimental results and theoretical curves computed by Eq. 4 using the constants from Table 3 and calculating the equilibrium concentrations of the species according to Eqs. 4–9. The experimental results for the cobalt complex agree with the predicted curves under the whole pH region studied (Fig. 3a). The experimental results for the iron complex (Fig. 3b) agree with the predicted values in the clear solutions and are significantly higher in turbid solutions.

Effect of pH on E in turbid solutions

Estimation of ε_5 and SP

The characteristic constants for turbid solutions of the iron complex were computed by the least squares method using Eqs. 12 and 19 feeding the values for the solubility product SP until the least mean squares deviation of ε_5 from the mean value of ε_5 was obtained. The values of ε_5 and SP found are presented in Table 3.

Effect of pH

The dotted curve in Fig. 3b calculated using Eqs. 14–18 and the constants from Table 3 is in good accordance with the experimental results in turbid solutions but is underestimated by the algorithm for clear solutions.

210

Table 4. Estimation of β and ε_3 for the iron(II) complex.
C_N = 5E–05 M, C_{Fe} = 0.002 M. When ε_3 = 10 880, s_β = 33.4%. Grey background marks turbid solutions

| pH	E	H^+	$x_3 + x_4$	x_2	β	$(\beta - \beta_{mean})^2$	x_1	x_3	x_4
2	0.099	0.01	4.27E–06	2.15E–06	4.60E+08	2.20579E+15	3.93E–05	4.27E–06	2.02E–10
2.2	0.126	0.00631	7.40E–06	2.81E–06	4.67E+08	2.91581E+15	3.24E–05	7.40E–06	5.55E–10
2.4	0.153	0.003981	1.05E–05	3.50E–06	4.28E+08	2.24015E+14	2.55E–05	1.05E–05	1.25E–09
2.6	0.175	0.002512	1.30E–05	4.28E–06	3.55E+08	3.41147E+15	1.96E–05	1.30E–05	2.45E–09
2.8	0.199	0.001585	1.58E–05	4.72E–06	3.56E+08	3.32725E+15	1.36E–05	1.58E–05	4.72E–09
3	0.217	0.001	1.79E–05	5.02E–06	3.55E+08	3.471E+15	9.17E–06	1.79E–05	8.46E–09
3.2	0.235	0.000631	2.00E–05	4.61E–06	4.73E+08	3.48974E+15	5.30E–06	2.00E–05	1.50E–08
3.3	0.244	0.000501	2.11E–05	4.02E–06	6.53E+08	5.74597E+16	3.68E–06	2.11E–05	1.99E–08
3.4	0.298	0.000398	2.83E–05	–3.8E–06	9.61E+08	3.0015E+17	–2.79E–06	2.83E–05	3.36E–08
3.5	0.334	0.000316	3.32E–05	–1.0E–05	1.54E+08	6.7446E+16	–6.00E–06	3.31E–05	4.96E–08
Mean					4.13E+08	2.72072E+15			
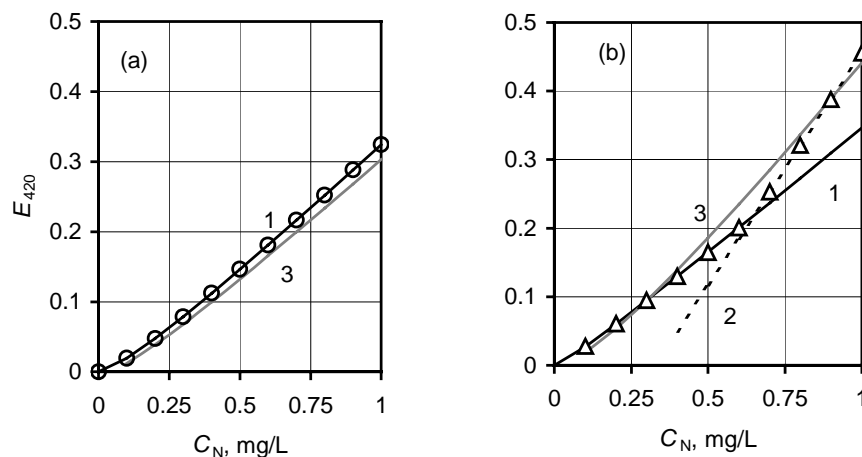
Fig. 4. Effect of nitrite concentration on the absorbance for cobalt (a) and iron(II) (b) compounds. Points – experimental, curves – calculated; 1 – clear solutions, 2 – turbid solutions, 3 – calculated using the conditional constants. C_{Me} = 0.002 M, pH$_{Co}$ = 4.0, pH$_{Fe}$ = 3.3.

Effect of C_N

Figure 4 illustrates the relationship between the nitrite concentration (expressed as nitrogen, mg/L) and absorbance measured in 1 cm cells at 420 nm when C_{Me} is 0.002 M, $C_{2,5DMR}$ is 0.01 M, pH$_{Fe}$ is 3.3, and pH$_{Co}$ is 4.0. Like the pH dependences (Fig. 3a and 3b), the experimental points of the cobalt complex (Fig. 4a) agree with the theoretical curves (1) of clear solutions calculated by Eq. 4 in the whole concentration region studied. For the iron complexes (Fig. 4b) the agreement is observed only at lower concentrations. At higher concentrations of nitrite the experimental results coincide with the dotted calibration graph (2) predicted by Eq. 14 for turbid solutions. Curve (3) calculated using Eq. 2 and the conditional constants from Table 3 is close to the experimentally found calibration curve for the cobalt complex (Fig. 4a), and unsatisfactory for iron solutions. All the curves obtained have a little concavity since the percentage of the nitrite in the complex increases with an increase in the nitrite initial concentration.

Effect of pH on the partial contributions of the species

The regularities described above prove that the effect of pH on E depends on the partial contributions of the species and on the concentration of the reagents. Figure 5 illustrates the partial absorbances of the species formed at an average concentration of nitrite, $C_N = 5 \times 10^{-5}$ M (0.7 mg/L), under the concentrations prescribed for the nitrite test method, $C_{2,5DMR} = 0.01$ M, and $C_{Me} = 0.002$ M. The results obtained demonstrate that when 2,5-DMR is applied, the main contribution to the absorbance at the optimum conditions (pH$_{Co}$ 4.0, pH$_{Fe}$ 3.3) is
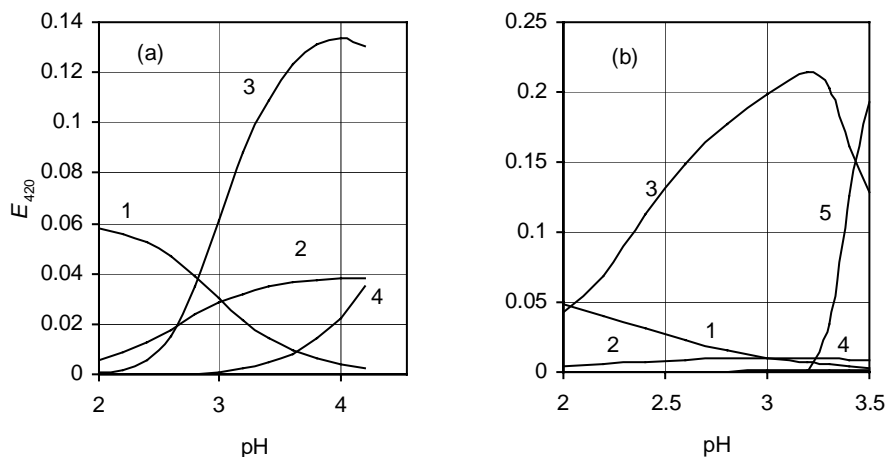
Fig. 5. Effect of pH on the partial absorbances of the species for cobalt (a) and iron(II) (b) compounds. $1 - AH$, $2 - A^-$, $3 - MeA_n$, $4 - MeA_n^-$, $5 - Me(MeA_n)_2$. $C_N = 5E{-}05$, $C_{Me} = 0.002$ M.

given by the molecular species of CoA_3 and FeA_2. Thanks to the comparatively high values of K_2 of the complexes, the contribution of the anionic forms of the complex is small in the pH region before hydrolysis of the cations applied. The anionic species of the iron(II) complex (Fig. 5b) precipitating as an unstable colloid of $Fe(FeA_2)_2$ make a higher region of pH than 3.3 inadvisable. So, considering reproducibility of the test method, the best *Reagent* should consist of $CoSO_4$ and 2,5-DMR, in spite of its about twice lower sensitivity compared with *Reagents* consisting of $FeSO_4$ and 2,5-DMR (Fig. 5a) or 5-MR and $FeSO_4$ or $CoSO_4$ [5]–[7].

CONCLUSIONS

A theoretical basis for photometric estimation of nitrite as cobalt and iron 4-nitroso-2,5-dimethylresorcinolates was developed. The joint effect of pH and the initial concentration of the reagents on the absorbance of the nitrite solution tested is described by two algorithms depending on whether the solution is clear or turbid. The algorithm for cobalt compounds considers contributions of four coloured species resulting from the acidic dissociation of nitrosoalkylresorcinol and the complex AH, A^-, CoA_3, and CoA_3^-. The algorithm for the iron compounds considers formation of analogical four species – AH, A^-, FeA_2, and FeA_2^- – at lower concentrations and pH, and for five species when the insoluble salt $Fe(FeA_2)_2$ can form. The theoretical absorbances computed using the acidic dissociation constants, complex stability constants, salt solubility product, and molar absorptivities found for 4-nitroso-2,5-methylresorcinol and

for its compounds formed with cobalt and iron(II) sulphates agree with the corresponding experimental results.

REFERENCES

1. Standard test method for nitrite–nitrate in water, D 3867-90. In *Annual Book of ASTM Standards*. Vol. 11.01. 1999.
2. Hanson, N. V. (ed.) *Official Standardised and Recommended Methods of Analysis*. The Chemical Society, London, 1973.
3. Horita, K., Wang, G. F. & Satake, M. Column preconcentration analysis–spectrophotometric determination of nitrate and nitrite by a diazotation–coupling reaction. *Analyst*, 1997, **122**, 1569–1574.
4. Schultz, K., Kerber, S. & Kelm, M. Reevaluation of the Griess method for determining NO/NO_2 in aqueous and protein-containing samples. *Nitric Oxide*, 1999, **3**, 225–234.
5. Johannes, I., Mölder, L., Tiikma, L. & Kuusk, K. Meetod nitriti määramiseks. Patent claim 199900117 A. *Eesti Patendileht*, 1999, No. 5, 13.
6. Johannes, I., Tiikma, L. & Kuusk, K. Oil shale resorcinols – effective reagents for determination of nitrite. *Oil Shale*, 2000, **17**, 313–322.
7. Johannes, I. & Kuusk, K. Oil shale resorcinols – effective reagents for nitrite. An algorithm for the influence of pH on absorbance. *Oil Shale*, 2001, **18**, 335–349.
8. Iokhannes, I. K., Molder, L. I., Tiikma, L. V. & Sidoruk, E. A. Nitrosation of 5-methylresorcinol and 2,5-dimethylresorcinol. *R. J. Appl. Chem.*, 1995, **68**, 8, Part 2, 1159–1162.

Nitriti 4-nitroso-2,5-dimetüülresortsinolaadina määramise meetodi teoreetilised alused

Ille Johannes ja Laine Tiikma

Loodi nitriti raud(II)- ja koobalt-4-nitroso-2,5-dimetüülresortsinolaat-kompleksidena (MeA_n) määramise meetodi teoreetilised alused. Tuletati algoritmid, mis kirjeldavad lähteainete kontsentratsiooni ja lahuse pH koosmõju lahuse optilisele tihedusele, võttes arvesse MeA_n püsivust, hägu moodustava soola ($Me(MeA_n)_2$) lahustuvust ning 4-nitroso-2,5-dimetüülresortsinooli ja MeA_n happelist dissotsiatsiooni. Määrati vastavad püsivuskonstandid, lahustuvuskorrutised, happelise dissotsiatsiooni konstandid ja molaarse ekstinktsiooni tegurid.