Complex formation and liquid-liquid extraction of the ion-pair of molybdenum(VI) with 3,5-dinitrocatechol and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide

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The complex formation and the ion-association of the ion-pair formed between the anionic chelate of molybdenum(VI)–3,5-dinitrocatechol (3,5-DNC) with the cation of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) in the liquid-liquid extraction system Mo(VI)–3,5-DNC–MTT–H_2O–$CHCl_3$ was studied by spectrophotometry. The optimum conditions for the chelate formation and extraction of the ion-associated complex Mo(VI)–3,5-DNC–MTT were established. The molar ratio of the components in the ion-associated complex Mo(VI)–3,5-DNC–MTT was determined by independent methods. The association process in aqueous phase and the extraction equilibria were investigated and quantitatively characterized. The following key constants of the processes were calculated: association constant, distribution constant, extraction constant and recovery factor. Based on this, a reaction scheme, a general formula and a structural formula of the complex were suggested.

Keywords: molybdenum(VI), ion-associated chelate, extraction equilibria, UV-Vis spectroscopy

INTRODUCTION

Molybdenum(VI) forms complexes with various organic ligands, such as polyphenols and their functional derivatives, polyhydroxycarboxylic acids, aminopolycarboxylic acids, hydroxamic acids, amines (primary, secondary and tertiary), 8-hydroxyquinoline and its derivatives, aldehyde hydrazones, oximes, β-diketones, fluorones, hydroxyazodyes, biomolecules (chitosan, chitin, D-glucosamine, L-alanine, L-phenylalanine) [1–9]. Molybdenum (VI) gives colored chelates with aromatic compounds, containing two or more hydroxyl groups in *o*-position relative to each other. The colored anionic chelates of molybdenum (VI) form ion-associated complexes with bulky organic cations, like methyltrioctylammonium, cetyl-pyridinium, cetyltrimethylammonium, tetraphenyl-phosphonium [1, 10–12].

The structure and properties of tetrazolium salts determine their ability to form ion-associated complexes. The bulky hydrophobic organic substituents in the molecules of the tetrazolium salts increase the extractability of the ion associated complexes. The presence of a quaternary nitrogen atom in the molecules of the tetrazolium salts determines the ability to form ionic associates in the aqueous phase without protonation, as opposed to the amines [13–15]. Tetrazolium salts are used as reagents for the preparation of various ion-associated complexes of metals, e.g. W(VI), Ge(IV), Tl(III), Nb(V), V(V), Ga(III), Co(II) [15–19].

The liquid-liquid extraction is a part of the chemistry of the solutions and the coordination compounds. It is applied to study the processes of complex formation and the extraction equilibria. The extraction spectrophotometry is a relatively simple, convenient, rapid to perform and inexpensive method for preparation and characterization of new complex compounds, as well as for their application in the chemical analysis [20–24].

The aim of this research was to study spectrophotometrically the extraction equilibria of the complex formation of the ion-pair formed between the anionic chelate of Mo(VI)–3,5-dinitrocatechol (3,5-DNC) and the cation of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) in the liquid-liquid system Mo(VI)–3,5-DNC–MTT–H_2O–$CHCl_3$.

EXPERIMENTAL

Reagents and apparatus

$Na_2MoO_4 \cdot 2H_2O$ (Fluka AG, p.a.): an aqueous 2.08×10^{-2} mol dm^{-3} solution was prepared. 3,5-Dinitrocatechol (3,5-DNC) (Sigma–Aldrich, p.a.): 3,5-DNC was dissolved in $CHCl_3$ to give a

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1.0×10⁻³ mol dm⁻³ solution. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (Merck, Germany, p.a.): an aqueous 2.0×10⁻³ mol dm⁻³ solution was prepared. H_2SO_4 (95-97% for analysis, Merck): a 2 mol dm⁻³ solution was prepared. The concentration of H_2SO_4 was determined titrimetrically. A Camspec M508 spectrophotometer (UK), equipped with 10 mm path length cells, was employed for measurement of the absorbance. The organic solvent $CHCl_3$ was additionally distilled.

Procedure for establishment of the optimum condition for complex formation

The required aliquots of the solutions of Mo(VI), MTT and H_2SO_4 were introduced into 250 cm³ separatory funnels. The resulting solutions were diluted with distilled water to a total volume of 10 cm³. A required aliquot of a chloroform solution of 3,5-DNC was added and then the organic phase was adjusted to a volume of 10 cm³ with chloroform. The funnels were shaken for a fixed time (up to 240 s). A portion of the organic extract was filtered through a filter paper into a 1 cm cell and the absorbance was measured against a blank. The blank extraction was performed in the same manner in the absence of molybdenum.

Procedure for determination of the distribution constant

The distribution constant (K_D) was determined from the ratio $K_D = A_1/(A_3 – A_1)$, where A_1 and A_3 are the absorbance (measured against blanks) obtained after a single and triple extraction, respectively. The single extraction and the first stage of the triple extraction were performed under the optimum conditions for complex formation (Table 1, column 1). The organic layers were transferred into 25 cm³ calibrated flasks and the flask from the single extraction was brought to volume with chloroform. The second stage of the triple extraction was performed by adding 7 cm³ of chloroform to the aqueous phase that remained after the first stage. After extraction, the obtained extract was added to this first stage of the triple extraction. The third stage of the triple extraction was performed in the same manner as for the second stage and the extract was added to those of the first two stages. The volume of the flask was brought to the mark with chloroform. The calibrated flasks were shaken before the spectrophotometric measurements [25].

RESULTS AND DISCUSSION

Optimum extraction-spectrophotometric conditions

The absorption spectrum of the extract of the studied ion-pair formed between the anionic chelate of Mo(VI) with 3,5-DNC and the tetrazolium cation

in $CHCl_3$ was characterized by an absorption maximum in the visible range (λ_{max} = 405 nm) (Fig. 1).
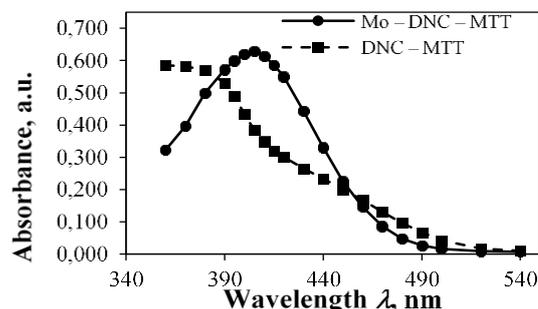

Fig. 1. Absorption spectra of the complex Mo(VI)–3,5-DNC–MTT and of the blank sample 3,5-DNC–MTT in $CHCl_3$ $C_{Mo(VI)}$ = 2.08×10⁻⁵ mol dm⁻³; $C_{3,5\text{-DNC}}$ = 2.0×10⁻⁴ mol dm⁻³, C_{MTT} = 2.0 × 10⁻⁴ mol dm⁻³; C_{H2SO4} = 4.0 × 10⁻¹ mol dm⁻³;λ = 405 nm; τ = 2 min.

The influence of the acidity of the aqueous phase on the extraction of the anionic chelate Mo(VI)–3,5-DNC into the organic phase in the form of an ion-pair with the tetrazolium cation was investigated. The maximum and constant extraction of the ion-associated complex is achieved in strongly acidic solution of (0.2–0.5) mol dm⁻³ H_2SO_4. The results showed that the extraction equilibrium is achieved for shaking time of not less than 90 s. A longer shaking time did not affect the absorbance. The experiments were performed for 2 min. The concentrations of the reagents are the most important factor influencing the extraction equilibria. The chelate formation of Mo(VI)–3,5-DNC requires 6.7–fold excess of 3,5-DNC ($C_{3,5\text{-DNC}} \geq$ 1.4×10⁻⁴ mol dm⁻³) and 7.7–fold excess of MTT ($C_{MMT} \geq$ 1.6×10⁻⁴ mol dm⁻³) for maximum association and extraction. The optimum experimental conditions for the extraction of the ion-associated complex are summarized in Table 1, column 1.

Beer's law, apparent molar absorptivity and other analytical characteristics

The range of obedience to Beer's law, i.e. the linear relationship between the molybdenum concentration in the aqueous phase ($C_{Mo(VI)}$, µg cm⁻³) and the absorbance of the ion-association complex in the organic phase after extraction was studied using regression analysis under the optimum conditions for complex formation. The equation of a straight line was found to be Y = 0.3254 X − 0.0016 with a correlation coefficient squared 0.9996. Further analytical characteristics, such as apparent molar absorptivity ε', adherence to Beer's law, Sandell's sensitivity, limit of detection and limit of quantification, are shown in Table 1, column 2.

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Table 1. Optimum extraction-spectrophotometric conditions and analytical characteristics of the system Mo(VI)–3,5-DNC–MTT–H$_2$O–CHCl$_3$

Optimum conditions	Analytical characteristic
Absorption maximum (λ_{max}) 405 nm	Apparent molar absorptivity (ε') $(3.13 \pm 0.07) \times 10^4$ dm^3 mol^{-1}cm^{-1}
Volume of the aqueous phase 10 cm^3	True molar absorptivity (ε) $(3.12 \pm 0.04) \times 10^4$ dm^3 mol^{-1}cm^{-1}
Volume of the organic phase 10 cm^3	Sandell's sensitivity (SS) 3.07 ng cm^{-2}
Concentration of H$_2$SO$_4$ in the aqueous phase 0.4 mol dm^{-3} (0.2 ÷ 0.5)	Adherence to Beer's law up to 3.99 µg cm^{-3}
Shaking time (τ) 2 min	Relative standard deviation (RSD) 1.84%
Concentration of 3,5-DNC $\geq 1.4 \times 10^{-4}$ mol dm^{-3}	Limit of detection (LOD) 0.0977 µg cm^{-3}
Concentration of MTT $\geq 1.6 \times 10^{-4}$ mol dm^{-3}	Limit of quantification (LOQ) 0.3257 µg cm^{-3}

Molar Ratios of the Complex, Reaction Scheme and Suggested General Formula

The mobile equilibrium method and the straight-line method of Asmus were applied to prove the molar ratios Mo(VI):3,5-DNC and Mo(VI):MTT [26]. The results from the application of these methods are shown in Figs. 2÷4, respectively. On the basis of the results it can be concluded that Mo(VI), 3,5-DNC and MTT interact in molar ratio 1:2:2. The molar ratio was confirmed by an independent method – the method of Likussar-Boltz [27].
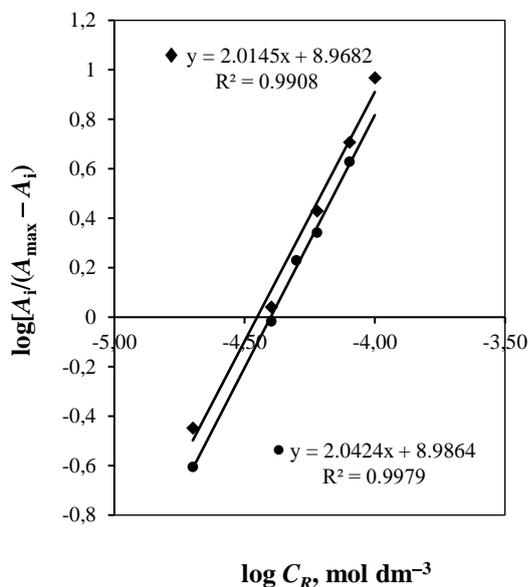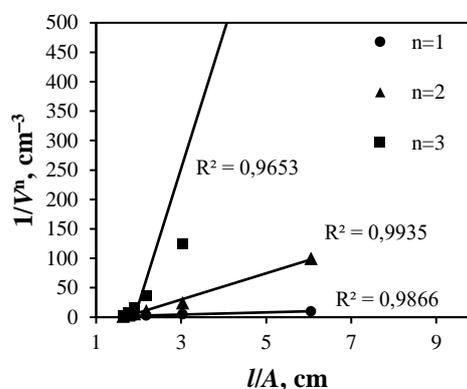
Fig. 3. Determination of the molar ratio (n) Mo(VI):MTT by the method of Asmus. $C_{Mo(VI)} = 2.08 \times 10^{-5}$ mol dm^{-3}; $C_{3,5-DNC} = 2.0 \times 10^{-4}$ mol dm^{-3}; $C_{H_2SO_4} = 4.0 \times 10^{-1}$ mol dm^{-3}; V – volume of MTT, cm^3; $\lambda = 405$ nm; $\tau = 2$ min.
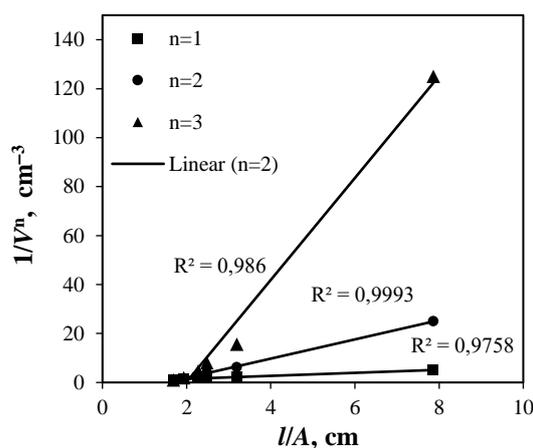
Fig. 2. Straight lines by the mobile equilibrium method for determination of the molar ratios Mo(VI) : 3,5-DNC and Mo(VI) : MTT. $C_{Mo(VI)} = 2.08 \times 10^{-5}$ mol dm^{-3}; $C_{H2SO4} = 4.0 \times 10^{-1}$ mol dm^{-3}; $\lambda = 405$ nm; $\tau = 2$ min ● Mo(VI) : 3,5-DNC, $C_{MTT} = 2.0 \times 10^{-4}$ mol dm^{-3}; ■ Mo(VI) : MTT, $C_{3,5-DNC} = 2.0 \times 10^{-4}$ mol dm^{-3}.

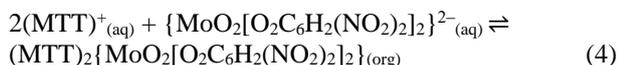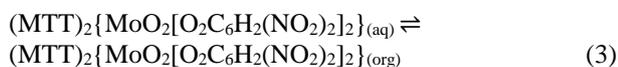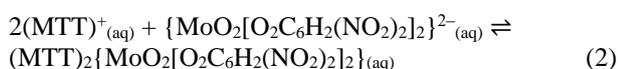**Fig. 4.** Determination of molar ratio (n) Mo(VI) : 3,5-DNC by the method of Asmus. $C_{Mo(VI)} = 2.08 \times 10^{-5}$ mol dm^{-3}; $C_{MTT} = 2.0 \times 10^{-4}$ mol dm^{-3}; $C_{H_2SO_4} = 4.0 \times 10^{-1}$ mol dm^{-3}; V – volume of 3,5-DNC, cm^3; $\lambda = 405$ nm; $\tau = 2$ min.

The carried out experiments showed that the complex formation and the extraction of the ion-associated complex occurred in strongly acidic solution. Under these conditions, the complex formation of anionic chelate Mo(VI)–3,5-DNC is given by Eq. (1):

$$MoO_4^{2-} + 2(HO)_2C_6H_2(NO_2)_2 \rightleftharpoons \{MoO_2[O_2C_6H_2(NO_2)_2]_2\}^{2-} + 2\ H_2O \qquad (1)$$
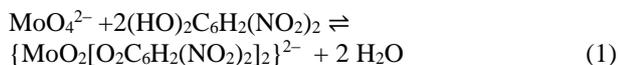
Having in mind the reaction of chelate formation of Mo(VI)–3,5-DNC and molar ratio indicated above, it can be suggested that the formation of the ion-associate in the aqueous phase, its distribution between the aqueous and the organic phases and its extraction in chloroform are given by the following Eqs. (2÷4).

$$2(MTT)^+{}_{(aq)} + \{MoO_2[O_2C_6H_2(NO_2)_2]_2\}^{2-}{}_{(aq)} \rightleftharpoons (MTT)_2\{MoO_2[O_2C_6H_2(NO_2)_2]_2\}_{(aq)} \qquad (2)$$

$$(MTT)_2\{MoO_2[O_2C_6H_2(NO_2)_2]_2\}_{(aq)} \rightleftharpoons (MTT)_2\{MoO_2[O_2C_6H_2(NO_2)_2]_2\}_{(org)} \qquad (3)$$

$$2(MTT)^+{}_{(aq)} + \{MoO_2[O_2C_6H_2(NO_2)_2]_2\}^{2-}{}_{(aq)} \rightleftharpoons (MTT)_2\{MoO_2[O_2C_6H_2(NO_2)_2]_2\}_{(org)} \qquad (4)$$

Hence, the ion-pair formed between the anionic chelate of Mo(VI)–3,5-DNC with the tetrazolium cation can be represented by the general formula $(MTT)_2\{MoO_2[O_2C_6H_2(NO_2)_2]_2\}$.

Equilibrium constants, True molar absorptivity and recovery factor

The values of the equilibrium constants and the recovery factor, describing quantitatively the equilibrium in the aqueous phase and the extraction of the ion-associated complex in the organic phase are presented in Table 2.

The association constant β and the true molar absorptivity ε were determined by the method of Komar-Tolmachev from Eq. (5) [26]:

$$\beta = (1/n)^n / [\varepsilon\ (tg\ \alpha)^{n+1}] \qquad (5)$$

where l is the cuvette thickness (l = 1 cm); n – the molar ratio between the components

independently determined (e.g. by the mobile equilibrium method or the straight-line method of Asmus) (n = 2); ε – the true molar absorptivity; tg α – angular coefficient of the straight line.

The true molar absorptivity ε was determined by the method of Komar-Tolmachev (Fig. 5) from the equation of a straight line Y = 1.4805 X + 3.2075 ($\varepsilon = 1/ (3.2075 \times 10^{-5})$) and its value is given in Table 1, column 2.
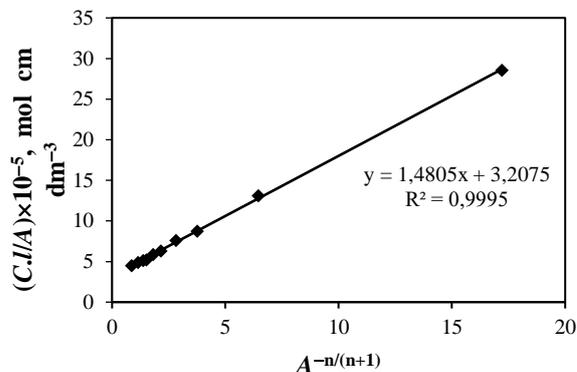

Fig. 5. Dependency of $(C.l/A)$ on $A^{-n/(n+1)}$ (method of Komar–Tolmachev) ; $C = C_{Mo(VI)}$ mol dm^{-3}; $C_{MTT} = 2\ C_{Mo(VI)}$ mol dm^{-3}; $C_{3,5\text{-DNC}} = 2.0 \times 10^{-4}$ mol dm^{-3}; A – absorbance; l – cell thickness, $l = 1$ cm; n = 2.

The distribution constant (K_D) was determined by Eq. (6), where A_1 and A_3 are the absorbance (measured against blanks) obtained after a single and triple extraction, respectively.

$$K_D = \{(MTT)_2\{MoO_2[O_2C_6H_2(NO_2)_2]_2\}\}_{(org)}/ \{(MTT)_2\{MoO_2[O_2C_6H_2(NO_2)_2]_2\}\}_{(aq)} = A_1/(A_3-A_1) \quad (6)$$

The recovery factor $R\%$ and the extraction constant K_{ex} were determined from Eq. (7) and Eq. (8), respectively:

$$R\% = 100\ K_D / (K_D + 1) \qquad (7)$$

$$\log K_{ex} = \log K_D + \log \beta \qquad (8)$$

where β was determined by the method of Komar-Tolmachev.

Table 2. Values of the equilibrium constants and the recovery factor

Equilibrium constant and recovery factor	Value
Equilibrium (Eq. 2) - Association constant β $\beta = (MTT)_2\{MoO_2[O_2C_6H_2(NO_2)_2]_2\}_{(aq)}/ \{[(MTT)^+]^2{}_{(aq)} \times \{\{MoO_2[O_2C_6H_2(NO_2)_2]_2\}^{2-}\}_{(aq)}\}$	$\log \beta = (9.39 \pm 1.07)^a$ $\log \beta = (9.54 \pm 0.21)^b$
Equilibrium (Eq. 3) - Distribution constant K_D $K_D = \{(MTT)_2\{MoO_2[O_2C_6H_2(NO_2)_2]_2\}\}_{(org)} / \{(MTT)_2\{MoO_2[O_2C_6H_2(NO_2)_2]_2\}\}_{(aq)}$	$\log K_D = (1.25 \pm 0.01)^c$
Equilibrium (Eq. 4) - Extraction constant K_{ex} $K_{ex} = \{(MTT)_2\{MoO_2[O_2C_6H_2(NO_2)_2]_2\}\}_{(org)} / \{\{[MTT]^+\}^2{}_{(aq)} \times \{\{MoO_2[O_2C_6H_2(NO_2)_2]_2\}^{2-}\}_{(aq)}\}$	$\log K_{ex} = (10.64 \pm 1.08)^d$ $\log K_{ex} = (9.89 \pm 0.04)^e$
Recovery factor $R\%$	$R = (94.65 \pm 0.14)\%^f$

[a] Calculated by Komar-Tolmachev method (Eq.(5)); [b] Calculated by Holme-Lagmyhr method [28]; [c] Calculated by Eq. (6); [d] Calculated by Eq. (8), where β is determined by the Komar-Tolmachev method [26]; [e] Calculated by Likussar-Boltz method [27]; [f] Calculated by Eq. (7).
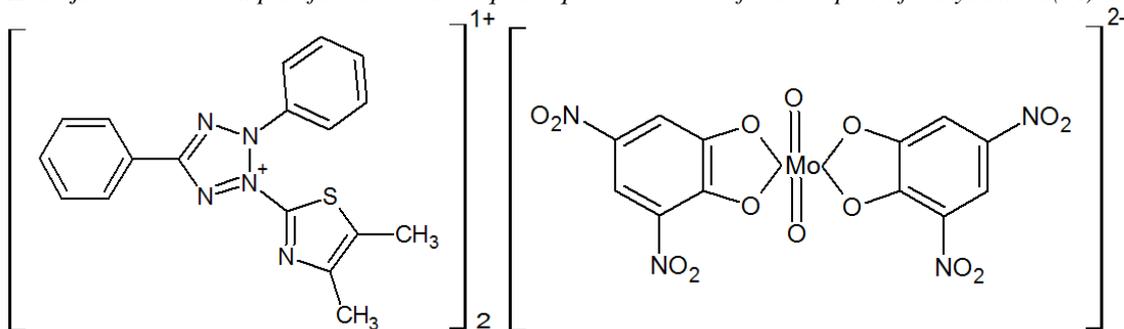
Fig. 6. Structural formula of the ion-associated complex Mo(VI)–3,5-DNC–MTT

The results obtained by independent methods are statistically similar and confirm the proposed scheme of the process of complex formation of the ion-pair in the aqueous phase, its distribution between the aqueous and the organic phases and its extraction in chloroform. Based on this, the proposed structure of the ion-associated complex is represented in Fig. 6.

The values of the equilibrium constants, the recovery factor and the analytical characteristics indicate that the ion-pair formed between the anionic chelate of Mo(VI) with 3,5-DNC and the tetrazolium cation is characterized by sufficiently high stability and good extraction. The presence of hydrophilic nitro groups in the molecule of the ion-associated complex stabilizes the ion-pair in the aqueous phase. The availability of the phenyl radicals in the tetrazolium salt increases the extractability of the ion-associated complex.

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