
**THE COULOMETRIC TITRATION OF THE DERIVATIVES OF
N,N-DIMETHYL-4-AMINOAZOBENZENE BY GENERATED
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Conditions for the coulometric generation of titanium(III) ions in buffer medium in the presence of sodium citrate were found on the basis of a study of the electrochemical behaviour of the Ti(III)/Ti(IV) system. A current efficiency of 100% was attained in acetic acid solution ($c = 1 \text{ mol l}^{-1}$) containing citric acid ($c = 0.2 \text{ mol l}^{-1}$) and titanium(IV) chloride ($c = 0.1 \text{ mol l}^{-1}$), with pH adjusted to 6 using sodium hydroxide, in the current density range 0.5 to 10 mA/cm² using a mercury electrode and 1 to 2 mA/cm² using a paraffin-impregnated spectrographic carbon electrode. It was found that methanol does not affect the current efficiency at contents of up to 33% (v/v) and conditions were found for the coulometric titration of small amounts (0.2–2 mg) of some genotoxic derivatives of N,N-dimethyl-4-aminoazobenzene.

Derivatives of N,N-dimethyl-4-aminoazobenzene are among the best known genotoxic azocompounds^{1–4} and are often used as model substances in various toxicological studies. N,N-dimethyl-4-aminoazobenzene itself has been demonstrated to have carcinogenic action in some laboratory animals¹. It follows from QSAR correlation between the biological activity and the structure of its derivatives that the carcinogenicity of these substances generally increases with increasing lipophilicity⁵. However, newer sources⁶ have pointed out that the more polar substance, methyl red (N,N-dimethyl-4-amino-2'-carboxyazobenzene) has genotoxic activity.

A number of analytical methods have been developed in connection with the biological activity of N,N-dimethyl-4-aminoazobenzene and its derivatives, permitting the sensitive and selective determination of these substances in the working or natural environment and in various biological materials^{7–9}. The most sensitive methods include modern polarographic and voltammetric procedures^{3,7,8} and HPLC with electrochemical detection⁹ is most selective. These methods require

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a suitable absolute method for control of the concentration of the stock solutions for construction of a calibration curve. Thus, the volumetric determination of the derivatives of azobenzene with divalent chromium¹⁰ or trivalent titanium¹¹ and determination by constant potential coulometry¹² were originally developed.

This work deals with the coulometric titration of selected genotoxic derivatives of N,N-dimethyl-4-aminoazobenzene by generated titanium(III) ions. Unsubstituted N,N-dimethyl-4-aminoazobenzene was selected as a model substance as a representative nonpolar, strongly lipophilic and thus the most toxic derivative, along with its 4'-hydroxy derivative representing the products of its metabolic transformation, which usually includes C-hydroxylation, and its 2'-carboxy derivative as a readily available representative of more polar derivatives with weaker genotoxic effects.

Most of the coulometric methods for the determination of azocompounds described so far are based on their titration by sufficiently strong reducing agents, such as trivalent titanium¹³⁻¹⁹, divalent chromium²⁰ or dithionate²¹. A coulometric titration with hexavalent chromium²² has also been described, based on oxidation, titration with generated bromine²³, involving substitution of the aromatic nuclei, and constant potential coulometry based on direct reduction of the azogroup on a large-area mercury electrode^{12,24}. The different conditions necessary for the generation of the titanium(III) ions and for their reaction with the test substance led to the use of external coulometric generation¹⁵⁻¹⁹. Coulometric titration with strong reducing agents²⁵ and application to the determination of azocompounds²⁶ is discussed in detail in the cited reviews. Volumetric methods for the determination of azocompounds are described in monographs²⁷⁻²⁹.

It followed from an earlier study¹¹ that the rate of the reduction of the derivatives of N,N-dimethyl-4-aminoazobenzene by trivalent titanium in the presence of various complexing agents increases in the order: EDTA < fluoride < tartrate < citrate. The rate of the reduction in sodium citrate medium ($c = 0.1 \text{ mol l}^{-1}$) at pH 5.5 is sufficient for direct potentiometric titration. Thus, this work deals with the coulometric titration in sodium citrate medium at pH 6. Lingane and Iwamoto³⁰ studied the coulometric generation of titanium(III) ions in complexing medium (sodium citrate with pH adjusted to 1.0), and Bourg et al.^{31,32} studied this generation in EDTA at pH 3.9

The electrochemical behaviour of the Ti(IV)/Ti(III) system in the presence of citrate was studied to determine the optimal conditions for the coulometric generation of titanium(III) ions for reaction with the genotoxic derivatives of N,N-dimethyl-4-aminoazobenzene.

EXPERIMENTAL

Reagents

Stock solution of titanium(IV) chloride^{3,3}: 20 ml of titanium(IV) chloride (Riedel-de Haën, F.R.G.) was measured into a beaker and distilled water was added with constant stirring. After completion of the violent hydrolysis, the solution was transferred quantitatively to a 50 ml volumetric flask and filled to the mark with distilled water.

Base electrolyte: 1.92 g of citric acid was dissolved in 25 ml of acetic acid ($c = 2 \text{ mol l}^{-1}$) and the pH was adjusted with a sodium hydroxide solution ($c = 10 \text{ mol l}^{-1}$) to a value of 6.2. The required amount (1.4 ml) of the stock solution of titanium(IV) chloride was then added so that the final pH value decreased to 6.0; the solution was then diluted to 50 ml. The final solution contained 0.2 mol l^{-1} citric acid, 0.1 mol l^{-1} Ti(IV) and 1 mol l^{-1} acetic acid.

Apparatus

The coulometric measurements were carried out on an OH 404 coulometric analyzer (Radelkis, Budapest) with the following generation electrodes: large-area mercury electrode (surface area 9.08 cm^2), platinum foil electrode (2 cm^2), gold foil electrode (1.4 cm^2) spectrographic carbon disk electrode (0.785 cm^2), paraffin-impregnated spectrographic carbon disk electrode (0.785 cm^2) and glassy carbon cylindrical electrode (0.98 cm^2). The carbon electrodes were prepared by fixing the spectrographic (Elektrokarbon, Topolčany) or glassy (GC 20, Tokai, Japan) carbon with picein in a glass tube. Electrical contact consisted of mercury and a platinum wire. The carbon was impregnated with melted paraffin at decreased pressure for five hours. The electrode surface was polished with velvet and an emulsion (Alumina Emulsion 3, Gb. Klees, F.R.G.). The anode was always a platinum foil electrode (2.5 cm^2) separated from the cathode space by a frit. Constant potential coulometric measurements were carried out using a saturated calomel reference OH-9327 electrode (Radelkis, Budapest). Biamperometric indication of the equivalence point was carried out using two platinum foil electrodes (applied voltage of 100 mV); potentiometric indication employed a platinum foil electrode (0.56 cm^2) and a saturated calomel reference electrode. Volumetric determination was carried out using an ABU 1b automatic burette with a TTT 1c titrator (both from Radiometer, Copenhagen).

The voltammograms and polarograms were recorded on a PA 4 polarographic analyzer with an XY-4105 recorder (Laboratorní přístroje, Prague) in a three-electrode arrangement with a saturated calomel reference and platinum wire auxiliary electrode. An SMDE 1 hanging mercury drop electrode (Laboratorní přístroje, Prague), stationary platinum and gold disk electrode with a diameter of 2.5 mm, stationary spectrographic carbon disk electrode and paraffin-impregnated spectrographic disk electrode, both with a diameter of 1 cm, and a stationary glassy carbon disk electrode with a diameter of 2 mm were used as working electrodes.

Spectrophotometric measurements were carried out on a Specord UV-VIS spectrophotometer (Carl Zeiss, Jena) in 0.5 cm quartz cuvettes and the pH was controlled using a PHM 62 instrument (Radiometer, Copenhagen) with glass and saturated calomel electrodes.

Procedures

The voltammetric curves were measured by DC voltammetry in unstirred solution at a polarization rate of 20 mV s^{-1} using the above working electrodes. The polarization curves in stirred solutions were measured point-by-point with an interval of 50 to 100 mV using the appropriate coulometric generation electrodes.

The efficiency of the generation of titanium(III) ions was studied in the base electrolyte as described above by generating a given amount of titanium(III) ions at a current density of 0.5 to 10 mA/cm², which was immediately titrated with a standard solution of potassium dichromate with biamperometric indication.

The coulometric titration of the derivatives of N,N-dimethyl-4-aminoazobenzene was carried out by adding 15 ml methanol and 5 ml of a solution of the studied substance ($c = 1 \text{ mmol l}^{-1}$) to 50 ml of the base electrolyte and titrating visually to decolouration of the solution using a mercury or paraffin-impregnated spectrographic carbon generation electrode at a current density of 2 mA/cm².

Oxygen was removed from all the solutions by bubbling for ten minutes with nitrogen purified by passing through a solution of chromium(II) ions in hydrochloric acid over zinc amalgam. Nitrogen was passed over the surface of the studied solution during the coulometric titrations and during the polarographic and voltammetric measurements. All measurements were carried out at laboratory temperature.

RESULTS

Electrochemical Behaviour of the Ti(IV)/Ti(III) System in the Presence of Citrate

The voltammetric curves of the base electrolyte alone and of the same solution containing tetravalent titanium ($c = 0.1 \text{ mol l}^{-1}$) at various working electrodes in unstirred solution are given in Fig. 1. It can be seen that platinum, gold and spectrographic carbon are not suitable materials for the generation electrode. On the other hand, glassy carbon could be a suitable material (Fig. 1d); tetravalent titanium is reduced at this electrode at a potential of about 400 mV more positive than that for the reduction of the base electrolyte. Spectrographic carbon (Fig. 1e) is also suitable, with a potential difference of more than 200 mV. Mercury is clearly the best material for the generation of titanium(III) ions (Fig. 1f), with a potential difference between the reduction of tetravalent titanium and the base electrolyte of about 600 mV.

Figure 2 depicts the polarization curves for the studied system on the generation electrodes in stirred solutions (i.e. under conditions identical with those used in the coulometric generation of titanium(III) ions), measured point-by-point. The above base electrolyte was used with the solid electrodes. For the sake of comparison, the polarization curves were measured at a large-area mercury electrode in this base electrolyte at pH 5 and 6.

It can be seen from Fig. 2a that 100% current efficiency cannot be attained on platinum even at very low currents. On the other hand, it can be expected from the measured polarization curves for gold (Fig. 2b), glassy carbon (Fig. 2c) and paraffin-impregnated spectral carbon (Fig. 2d) that the current efficiency can approach 100% at low generation currents and current densities of up to 2 mA/cm². It follows from Figs 2e and 2f that 100% current efficiency can be expected at mercury at much higher currents (cca up to 35 mA at pH 5 and 55 mA at pH 6, corresponding to cur-

rent densities of 4 and 6 mA/cm², respectively). This is apparently connected with the larger hydrogen overpotential at the mercury electrode and the larger electrode area compared with the solid electrodes used. It follows from comparison of Figs 2e and 2f that a medium with higher pH is preferential from the point of view of the current efficiency. The potential of the reduction of tetravalent titanium is practically the same in media with pH 5 and 6, while that for the reduction of the base electrolyte is shifted by about 100 mV to more negative values.

*Titration of Titanium(III) Ions in the Presence of Citrate
by Various Oxidizing Agents*

The current efficiency for the generation of titanium(III) ions under the given conditions was found by volumetric determination of the generated trivalent titanium by a suitable oxidizing agent. The greatest problem in the search for a suitable oxidizing agent is indication of the equivalence point²⁶. (It should be recalled that, when

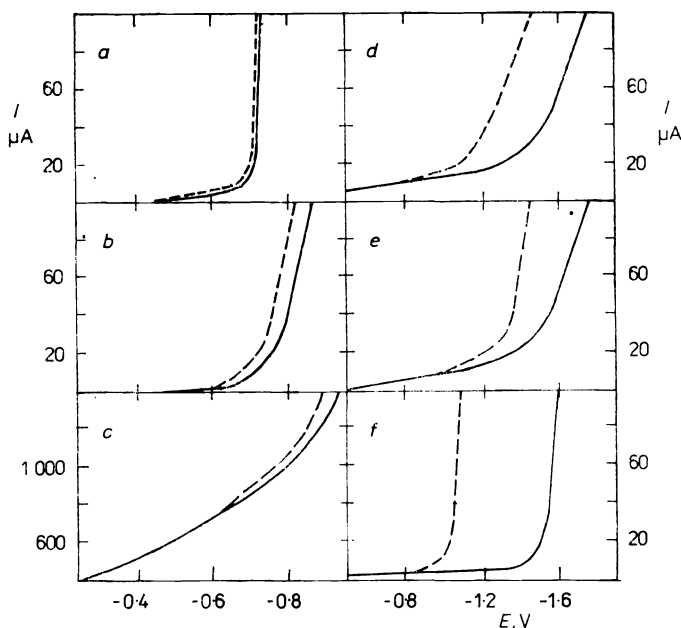


FIG. 1

Voltammetric curves of the base electrolyte in the absence (full line) and presence (dashed line) of Ti(IV) at a stationary platinum (d), gold (b), spectrographic carbon (c), glassy carbon (d), paraffin-impregnated spectrographic carbon (e) disk and hanging mercury drop (f) electrodes, at pH 6

a mercury generation electrode is employed, the metallic mercury present can greatly affect the titration of titanium ions by the given oxidation agent.) Of the reagents tested (ferric ions, hexacyanoferrite, dichromate) and equivalence point indication methods (equilibrium potentiometry with a platinum or mercury cup indication electrode, biamperometry or bipotentiometry with two platinum electrodes, polarometry, etc.), the best results were obtained using a standard solution of potassium dichromate ($c = 1.6 \text{ mmol l}^{-1}$) with biamperometric indication with two platinum foil electrodes and an applied voltage of 100 mV. When this method is employed for the titration of titanium(III) ions generated at a large-area mercury electrode with a current of 5 mA for 1 000 s, the consumption of $5.30 \pm 0.06 \text{ ml}$ (average of 7 determinations from which the standard deviation was also calculated) corresponded exactly to the theoretically expected value assuming 100% current efficiency in the generation of the titanium(III) ions (5.30 ml). The somewhat anomalous shape of the titration curve (Fig. 3a) is apparently connected with the oxidation of metallic

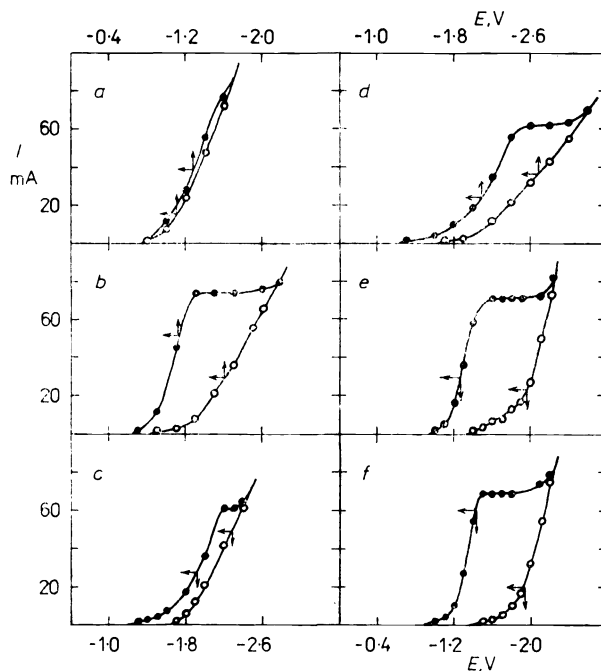


FIG. 2

Polarization curves in stirred solution in the absence (○) and presence (●) of tetravalent titanium at the coulometric platinum (a), gold (b), glassy carbon (c) and paraffin-impregnated spectrographic carbon (d) electrodes at pH 6 and at the large-area mercury electrode at pH 5 (e) and pH 6 (f)

mercury by dichromate with formation of insoluble mercury compounds after the equivalence point. When this method is employed to titrate titanium(III) ions generated by a current of 1 mA for 2 500 s at a paraffin-impregnated spectrographic carbon electrode, a consumption of 2.65 ± 0.05 ml (average of 7 determinations) was found, in exact agreement with the theoretical value (2.65 ml). The biamperometric curves were again rather anomalous (Fig. 3b), apparently connected with passivation of the indication electrodes, gradual formation of complexes of the reagents with citrate and the overall complexity of the reaction medium.

The Current Efficiency in the Generation of Titanium(III) Ions in the Presence of Citrate

Using the above base electrolyte at various electrodes and various current densities, a given amount of titanium(III) ions was generated and was immediately titrated with a standard solution of potassium dichromate with biamperometric indication with an applied potential of 100 mV. The current efficiency and generation current with the charge passed is given for each system in Table I. It can be seen that mercury is the best material for the generation electrode and that a current efficiency of 100% can be attained at this electrode in the generation of titanium(III) ions in a wide range of current densities (0.5 to 10 mA/cm²). This range is much narrower on a paraffin-impregnated spectrographic carbon electrode (1 to 2 mA/cm²) and 100% current efficiency was not attained at all at platinum and gold electrodes. It is interesting that 100% current efficiency was also not attained at a cylindrical glassy carbon

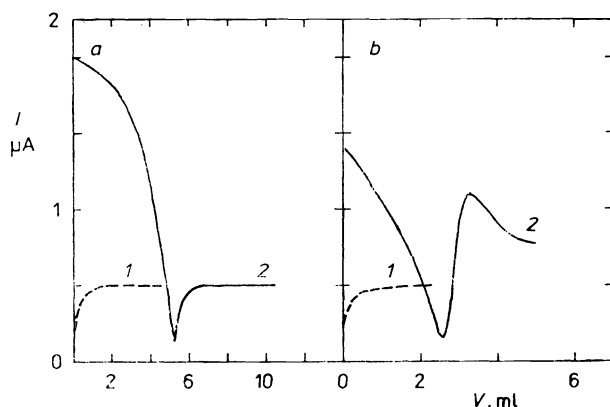


FIG. 3

Biamperometric titration of titanium(III) ions generated at the mercury (a) and paraffin-impregnated carbon (b) electrodes by a standard potassium dichromate solution ($c = 0.001666$ mol \cdot l^{-1}). 1 titration of the catholyte alone; 2 titration of generated titanium(III) ions

TABLE I
Current efficiency in the generation of titanium(III) ions at various electrodes

J^a mA cm ⁻²	I^b mA	Q^c mC	η^d %
Mercury electrode, $A = 9.08 \text{ cm}^2$			
0.5	4.5	2 580	100
1	9.1	2 500	100
2	18.2	5 000	100
5	45.4	5 030	100
10	90.0	5 029	100
Paraffin-impregnated spectrographic carbon electrode, $A = 0.785 \text{ cm}^2$			
0.5	0.4	2 007	78
1	0.8	2 501	100
2	1.6	2 510	100
5	3.9	2 500	98
10	7.9	4 999	66
Glassy carbon electrode, $A = 0.98 \text{ cm}^2$			
0.5	0.49	2 510	91.7
1	0.98	2 512	88.5
2	1.96	2 499	78.6
5	4.9	2 603	63.7
10	9.8	2 515	26.7
Gold electrode, $A = 1.4 \text{ cm}^2$			
0.5	0.7	2 508	53
1	1.4	2 505	54
2	2.8	5 056	37
5	7.0	5 021	35
10	14	5 394	31
Platinum electrode, $A = 2 \text{ cm}^2$			
0.5	1.0	2 700	3
1	2.0	2 510	7
2	4.0	3 000	6
5	10	2 498	5
10	20	5 000	4

^a Current density; ^b generation current; ^c charge passed; ^d current efficiency.

electrode, visible in the evolution of gas bubbles at the surface of this electrode and especially at the edges.

A water – methanol (2 : 1) mixture was also studied because of the increased solubility of the studied substances. It was found that 100% current efficiency can be attained in this medium at current densities of 1 and 2 mA/cm² when the mercury or paraffin-impregnated spectrographic carbon electrodes were used.

*Direct Coulometric Titration of the Derivatives of
N,N-dimethyl-4-aminoazobenzene by Titanium(III) Ions in Citrate Medium*

Conditions for 100% current efficiency for the generation of titanium(III) ions at the mercury and paraffin-impregnated spectrographic carbon electrodes were employed in the direct titration of methyl red, N,N-dimethyl-4-aminoazobenzene and N,N-dimethyl-4-amino-4'-hydroxyazobenzene, which are then reduced according to

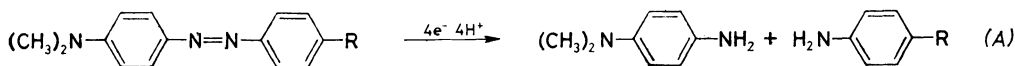
TABLE II

Accuracy and reproducibility of the determination of the derivatives of N,N-dimethyl-4-aminoazobenzene with trivalent titanium

Added mg	Found ^a %	<i>s_r</i> %
N,N-Dimethyl-4-amino-2'-carboxyazobenzene		
2.0198	100.15	0.60
1.3465	100.16	0.81
0.6733	101.38	1.12
0.2693 ^b	101.34	1.15
N,N-Dimethyl-4-aminoazobenzene		
1.6898	94.72	0.33
1.1265	99.80	1.42
0.5633	100.53	1.80
0.2253 ^b	101.06	0.94
N,N-Dimethyl-4-amino-4'-hydroxyazobenzene		
1.8098	99.91	1.00
1.2065	100.63	0.75
0.6033	102.21	0.50
0.2413 ^b	103.26	0.98

^a Average of 7 determinations from which the standard deviation *s_r* was calculated; Ti(III) generated at the mercury electrode at a current density of 2 mA/cm²; ^b current density 1 mA/cm².

the equation:



It followed from preliminary experiments that the instant of solution decolouration can be used for visual indication of the equivalence point. A mixed water-methanol (2 : 1) medium was employed for solubility reasons. The results given in Tables II and III reflect the accuracy and reproducibility of the results. The large negative error connected with the determination of the largest amount of N,N-dimethyl-4-aminoazobenzene is a result of the limited solubility of the substance in this medium. It was found spectrophotometrically that, when larger amounts of this substance are present, they are gradually precipitated from the generation solution, resulting in the decreased values found. This error is more marked when solid electrodes are used because their smaller areas lead to longer analysis times than that for the mercury electrode. The values found for methyl orange (N,N-dimethyl-4-amino-4'-

TABLE III

Accuracy and reproducibility of the determination of the derivatives of N,N-dimethyl-4-aminoazobenzene with trivalent titanium

Added mg	Found ^a %	s_r %
N,N-Dimethyl-4-amino-2'-carboxyazobenzene		
2.0198	99.21	0.78
1.3465	100.74	1.15
0.6733	101.63	1.13
N,N-Dimethyl-4-aminoazobenzene		
1.6898	80.76	1.09
1.1265	99.41	1.25
0.5633	102.73	1.81
N,N-Dimethyl-4-amino-4'-hydroxyazobenzene		
1.8098	100.44	1.25
1.2065	102.42	0.68
0.6033	102.63	1.65

^a Average of 7 determinations from which the standard deviation s_r was calculated; Ti(III) generated at a paraffin-impregnated spectrographic carbon electrode at a current density of 2 mA/cm².

-sulfoazobenzene) are 10–15% lower than the theoretical values, possibly connected with the strong $-I$ effect of the sulfo group. Consequently, this method cannot be applied automatically to further derivatives of N,N-dimethyl-4-aminoazobenzene without testing its precision and accuracy.

Although visual detection is possible, a suitable method for objective determination of the equivalence point was sought. Potentiometric indication with a platinum indicator electrode involved slow potential equilibration, the titration curves obtained were very drawn-out and were not suitable for determination of the equivalence point. Biamperometric indication with a pair of platinum foil electrodes yielded a titration curve of the $_ /$ type. However, the current increase after attainment of the equivalence point is not very marked so that determination of the equivalence point was not very accurate.

Spectrophotometric and polarographic determination of the equivalence point were also studied²⁶. Both differential pulse polarography and spectrophotometry in the visible region can be used for objective determination of the equivalence point. However, the precision of the determination of the equivalence point by these techniques ($\pm 2\%$) is not much greater than visual determination.

REFERENCES

1. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man: Some Aromatic Azocompounds, Vol. 8, p. 55. IARC, Lyon 1975.
2. Miller J. A., Miller E. C.: Adv. Cancer Res. 1, 339, (1953).
3. Miller J. A., Miller E. C., Firnger E. C.: Adv. Cancer Res. 17, 387 (1954).
4. Badger G. M.: Adv. Cancer Res. 2, 73 (1954).
5. Hansch C., Fujita T.: J. Am. Chem. Soc. 86, 1616 (1964).
6. Lewis J. R. (Ed.): Registry of Toxic Effects of Chemical Substances — 1978 Edition. DHEW NIOSH Publ. 79–100, Cincinnati 1979.
7. Barek J., Kelnar L.: Collect. Czech. Chem. Commun. 50, 712 (1985).
8. Barek J., Hrnčič R.: Collect. Czech. Chem. Commun. 51, 2083 (1986).
9. Burcinová A., Štulík K., Pacáková V.: J. Chromatogr. 389, 387 (1987).
10. Barek J., Berka A., Borek V.: Microchem. J. 27, 49 (1982).
11. Barek J., Berka A., Borek V.: Collect. Czech. Chem. Commun. 47, 495 (1982).
12. Barek J., Berka A., Zima J.: Collect. Czech. Chem. Commun. 50, 1819 (1985).
13. Slovák Z., Borák J.: Chem. Prum. 18, 82 (1968).
14. Mitev S., Demyanov N., Agasyan P. K.: God. Vissn. Khim.-Tekhnol. Inst. Burgas 11, 335 (1976); Chem. Abstr. 88, 182079 (1978).
15. Parsons J. S., Seaman W.: Anal. Chem. 27, 210 (1955).
16. Mitev S., Agasyan P. K.: Zavod. Lab. 41, 396 (1975).
17. Mitev S., Agasyan P. K.: Zh. Anal. Khim. 29, 974 (1974).
18. Early J. V., Ma T. S.: Mikrochim. Acta 685 (1960).
19. Papier J.: Rev. Met. 51, 723 (1954).
20. Kostromin A. I., Makarova L. L., Shulkina V. M.: Zh. Anal. Khim. 32, 933 (1977).
21. Munemori M.: Talanta 1, 110 (1958).
22. Kostromin A. I., Makarova L. L.: Zh. Anal. Khim. 30, 1225 (1975).

23. Kadrnal J., Růžička E.: *Acta Univ. Palacki. Olomuc., Fac. Rerum Nat.* **49** (Chem. 15), 191 (1976).
24. Chikrizova E. G., Podolenko A. A.: *Zavod. Lab.* **30**, 791 (1964).
25. Stučka V.: *Chem. Listy* **75**, 949 (1981).
26. Kvapilová H.: *Thesis*. Charles University, Prague 1988.
27. Kolthoff I. M., Elving P. J. in: *Treatise on Analytical Chemistry*, Part II, Vol. 15, p. 454. Wiley, New York 1976.
28. Ashworth M. R. F.: *Titrimetric Organic Analysis*, Part I: Direct Methods. Interscience, New York 1964.
29. Ashworth M. R. F.: *Titrimetric Organic Analysis*, Part II: Indirect Methods. Interscience, New York 1965.
30. Lingane J. J., Iwamoto R. T.: *Anal. Chim. Acta* **13**, 465 (1955).
31. Bourg P., Astruc M., Bonastre J.: *Anal. Chem.* **47**, 538 (1975).
32. Bourg P., Astruc M., Bonastre J.: *Analisis* **3**, 252 (1975).
33. Arthur P., Donahue J. F.: *Anal. Chem.* **24**, 1612 (1952).

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