

EXTRACTION SPECTROPHOTOMETRIC DETERMINATION OF [(2-CHLOROPHENYL)METHYLENE]PROPANE DINITRILE WITH REACTIVE DYES OF THE STABILIZED DIAZONIUM SALT TYPE

Emil HALÁMEK and Zbyněk KOBLIHA

Department of Chemistry, Military University, 682 03 Vyškov

Received June 21, 1991

Accepted October 6, 1991

A spectrophotometric method has been developed for the determination of [(2-chlorophenyl)methylene]propane dinitrile (CS) stabilized by the diazonium salts Fast Red TR and Fast Blue B after extraction with chloroform. Mass spectrometry, ^1H and ^{13}C NMR and elemental analysis confirmed the presence of the hydrazoform of the azo dye formed by reaction with malononitrile, as a product of the alkaline hydrolysis of substance CS, and the diazonium salt Fast Red TR.

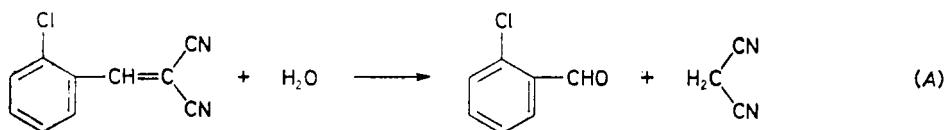
[(2-Chlorophenyl)methylene]propane dinitrile was first synthesized in 1928 by the American Company Corson and Stoughton¹, after whom it has the code name CS; it was widely employed in Indochina during the war from 1964–1969 (see refs^{2,3}). It is also used by police forces to suppress violence in a wide range of technical agents, including the use of solutions of this substance in water cannons. Similarly, it is used as an effective filling for special agents and munitions for antiterrorist units. Although substance CS is designed for temporary immobilization of living organisms, its use is not harmless. Resorption in the organism results in metabolic oxidation leading to hydrolytic splitting off of cyanide^{4–6} and the rapid development of cyanide poisoning. This substance has been shown to have carcinogenic properties^{7,8}, which has led in some countries to laws against its use by police forces.

In addition to difficult physical^{9,10} and physical chemical methods^{11,12}, a number of colorimetric and spectrophotometric can be used to analyse CS. These methods are often based on determination of the product of alkaline hydrolysis¹³. [(2-Chlorophenyl)methylene]propane dinitrile (*o*-chlorobenzylidene malononitrile) and malononitrile react with chloranil to yield green-blue compounds that were determined photometrically at 680–700 nm (ref.¹⁴). Similarly, Stachlewska-Wroblowa suggested that substance CS could be determined by a colour reaction with quinones¹⁵. Substance CS has been determined qualitatively by the thin-layer chromatographic method^{16,17}. It also reacts with *m*-dinitrobenzene to form an orange-yellow colour with maximum absorbance at 408–432 nm (see ref.¹⁸). The reaction is not specific; all compounds containing active hydrogen atoms in a methylene group yield the

colour¹⁹. Similarly, substance CS reacts with 1,3,5-trinitrobenzene in dimethylformamide medium to form an orange product.

The method based on the reaction of substance CS with formaldehyde in concentrated sulfuric acid²⁰ leads to the formation of a yellow reaction product. The oxidation reaction of substance CS with potassium permanganate has become the basis for the spectrophotometric determination. The epoxide formed is hydrolyzed to form cyanide which is converted by chlorination to cyanogen chloride, which undergoes the König-Zinck reaction²¹ with pyridine and barbituric acid²² to yield the polymethine dye, absorbing at 584 nm. Substance CS forms a yellow colour with ninhydrin. Ninhydrin first acts as a weak oxidant and the cyanide ion formed by hydrolysis reacts with the reagent to form a coloured compounds¹⁹.

Because of a number of drawbacks of these methods, especially low sensitivity, low reagent stability and insufficient reproducibility, this work was carried out in order to develop a determination method based on the formation of azo dyes through the reaction of stabilized diazonium salts – Fast Red TR and Fast Blue B – with the malononitrile component formed from substance CS by hydrolysis²³:



An attempt was also made to identify the reaction product of substance CS and Fast Red TR.

EXPERIMENTAL

Chemicals and Instruments

The work was carried out using [(2-chlorophenyl)methylene]propane dinitrile (substance CS) manufactured by VOZ-072 Zemianské Kostofany, standardized by titration of excess potassium cyanide ($c = 0.1 \text{ mol l}^{-1}$) with a standard 0.1 mol l^{-1} solution of silver nitrate with potentiometric indication of the equivalence point (98.3%). Malononitrile (MN), diazonium compounds – Fast Red TR [bis(4-chloro-2-methylbenzene diazonium) tetrachlorozincate (C.I. 37085) (R)], Fast Blue B [3,3'-dimethoxybiphenyl-4,4'-bis diazonium tetrafluoroborate (C.I. 37235) (B)] – and the other chemicals were at least of p.a. purity, Lachema Brno.

The infrared spectra were measured on a Specord IR 74 instrument (Zeiss, Jena) in chloroform. ^1H and ^{13}C NMR spectra were measured in hexadeuteriodimethyl sulfoxide, using tetramethylsilane as a standard, on an NMR-FT, KNM-FX 100 pulse spectrometer (JEOL, Japan). The mass spectra (GC-MS) were measured on a Hewlett-Packard type 5995 A instrument with electronic ionization.

Elemental analysis (C, H, N, Cl) was carried out in the laboratory for organic analysis of the Institute of Chemical Technology in Prague. Potentiometric measurements were carried out and the buffer solutions²⁴ were checked using an MV 870 pH meter (Präcitronic, Dresden) and silver

ISE CRYTUR type 4717, glass type 01-21 and calomel type RCE 101 electrodes (Monokrystaly, Turnov). Photometric measurements were carried out on a Spekol 11 single-beam spectrophotometer (Zeiss, Jena).

Procedure

The functional dependence $A = f(\lambda)$ (Fig. 1) was determined by preparing a methanol solution of substance CS and malononitrile (MN) with a concentration of 5 mmol l^{-1} , and methanol solutions of Fast Red TR ($c_R = 3 \text{ mmol l}^{-1}$) and Fast Blue B ($c_B = 2 \text{ mmol l}^{-1}$).

An amount of 1.9 ml of borate buffer solution with pH 8.0 (R) or 9.4 (B), 0.1 ml of reagent and 0.1 ml of a solution of substance CS or MN were pipetted into a test tube. After standing for 5 min at $t = 20^\circ\text{C}$, the sample was extracted into 2 ml of chloroform by shaking for 2 min. The absorption of the extract was read against pure solvent. The effect of the pH was studied by determination of the $A = f(\text{pH})$ dependence (Fig. 2). Amounts of 1.9 ml of buffer solution with pH from 3.0 to 11.0, in steps of 0.5 pH units and 0.1 ml of reagent ($c_R = 3 \text{ mmol l}^{-1}$, $c_B = 2 \text{ mmol l}^{-1}$) were pipetted into test tubes; 50 μl of a solution of substance CS or MN ($c_{\text{CS},\text{MN}} = 5 \text{ mmol l}^{-1}$) were added using a micropipette. After standing for 10 min at $t = 20^\circ\text{C}$ and subsequent extraction with 2 ml of chloroform, the absorbance of the organic phase was measured at $\lambda = 370 \text{ nm}$ (R) or 420 nm (B).

The stability of the diazonium salts in the buffer solutions was determined. An amount of 0.1 ml of solution of the diazonium salt ($c_R = 3 \text{ mmol l}^{-1}$, $c_B = 2 \text{ mmol l}^{-1}$) was pipetted into 1.9 ml of buffer solution at pH 8.0 (R) or 9.4 (B), the mixture was tempered on a water bath

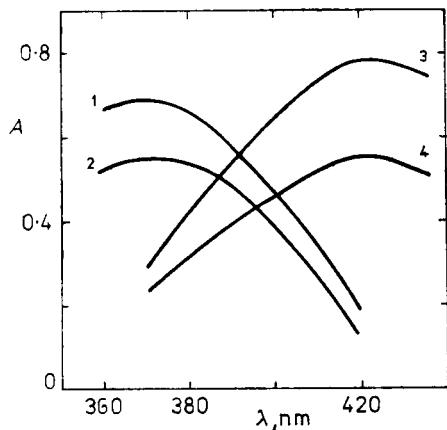


FIG. 1

Absorption curve for malononitrile (1, 3) and substance CS (2, 4), $c_{\text{MN,CS}} = 0.24 \text{ mmol l}^{-1}$ with Fast Red TR (1, 2), $c = 0.14 \text{ mmol l}^{-1}$ and Fast Blue B (3, 4), $c = 0.1 \text{ mmol l}^{-1}$

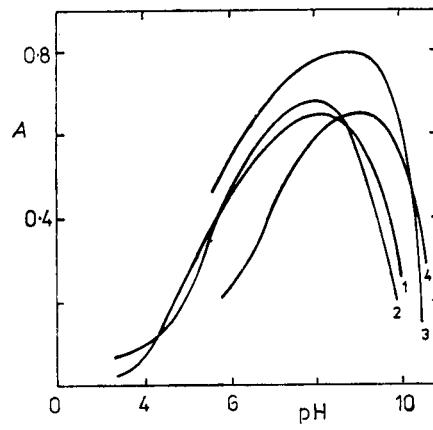


FIG. 2

Dependence of the absorbance of the reaction products of malononitrile (1, 3) and substance CS (2, 4) on the pH, $c_{\text{CS,MN}} = 0.12 \text{ mmol l}^{-1}$ with Fast Red TR (1, 2), $c = 0.14 \text{ mmol l}^{-1}$ and Fast Blue B (3, 4), $c = 0.1 \text{ mmol l}^{-1}$, for $\lambda = 370$ and 420 nm

for a period of to 40 min at $t = 20^\circ\text{C}$ and the absorbance was read at 5-minute intervals at $\lambda = 370 \text{ nm}$ (R) or 420 nm (B).

The kinetics of formation of the reaction products was followed in dependence on the temperature (Figs 3, 4, 5). An amount of 1.9 ml of buffer with pH 8.0 (R) or 9.4 (B), 0.1 ml of reagent ($c_{\text{R,B}} = 1 \text{ mmol l}^{-1}$) and 100 μl of a solution of substance CS or MN ($c_{\text{CS,MN}} = 5 \text{ mmol l}^{-1}$) were pipetted into a test tube and tempered at a temperature from 5 to 55°C for a period from 2 to 45 min. At two- or five-minute intervals, extraction was carried out into 2 ml of chloroform

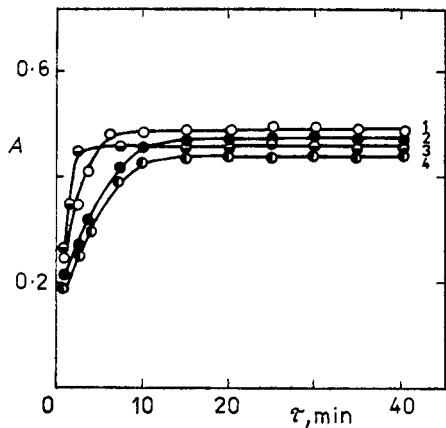


FIG. 3

Kinetics of the reaction of $25.7 \mu\text{mol l}^{-1}$ malononitrile (1, 3) and $25.0 \mu\text{mol l}^{-1}$ of substance CS (2, 4) with 0.1 mmol l^{-1} Fast Red TR (1, 2) and Fast Blue B (3, 4), $t = 25^\circ\text{C}$

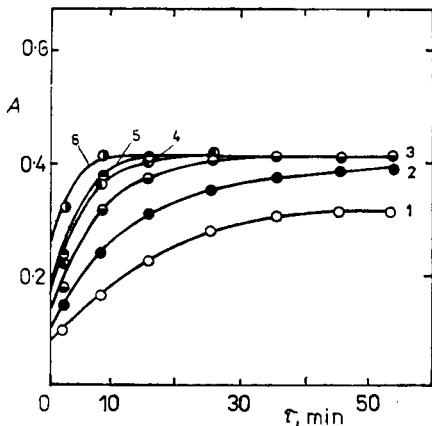


FIG. 4

Kinetics of the reaction of $25.0 \mu\text{mol l}^{-1}$ of substance CS with $46 \mu\text{mol l}^{-1}$ Fast Red TR for t ($^\circ\text{C}$): 1 5, 2 10, 3 15, 4 20, 5 25, and 6 30; pH 8.0, $\lambda = 370 \text{ nm}$

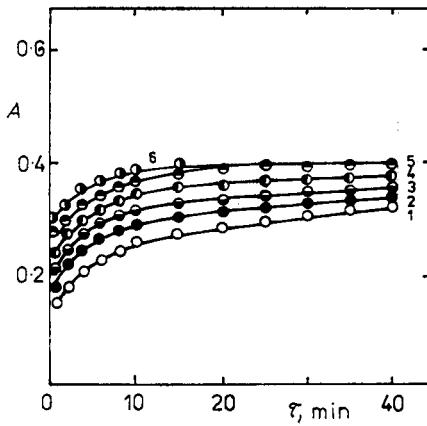


FIG. 5

Kinetics of the reaction of $25.0 \mu\text{mol l}^{-1}$ of substance CS with $50 \mu\text{mol l}^{-1}$ Fast Blue B for t ($^\circ\text{C}$): 1 5, 2 10, 3 15, 4 20, 5 25, and 6 30; pH = 9.4; $\lambda = 420 \text{ nm}$

by shaking for 2 min, the aqueous phase was drawn off and the absorbance of the chloroform extract was measured for $\lambda = 370$ nm (R) or 420 nm (B).

The stoichiometric ratio of the test substance with the reagent was determined by preparing 1 mmol l⁻¹ methanol solutions of substances CS, MN and the diazonium salts R and B. A micropipette was used to transfer 20 to 180 μ l of a solution of substance CS or MN in steps of 20 μ l into a test tube, and the solution was diluted with a solution of the diazonium salt to 200 μ l; 1.8 ml of buffer of pH 8.0 (R) or 9.4 (B) was added. After reaction for 20 min at $t = 25^\circ\text{C}$, the absorbance of the chloroform extract was measured.

To prepare the reaction product of CS and MN with Fast Red TR, 15 ml of buffer with pH 8.0, 3 ml of methanol solution of substance CS or MN ($c_{\text{CS},\text{MN}} = 10 \text{ mmol l}^{-1}$) and 12 ml of a solution of the diazonium salt ($c_{\text{R}} = 20 \text{ mmol l}^{-1}$) were shaken together and, after a reaction time of 20 min at $t = 25^\circ\text{C}$, the mixture was extracted with 30 ml of chloroform by shaking for 2 min. The solvent was evaporated off, product dried in a vacuum and repeatedly recrystallized from diethyl ether. The purity of the substance was checked by TLC on Silufol plates, Kavalier Votice (activated for 2 h at $t = 120^\circ\text{C}$). An amount of 5 μ l of the substance in methanol ($c = 1 \text{ mg ml}^{-1}$) was applied to the start and was separated using a pyridine-butyl acetate-water (2 : 2 : 1 vol. %) mobile phase. The reaction products of substances CS and MN with the studied diazonium salts both exhibited two spots with $R_{F1} = 0.50$ and $R_{F2} = 0.88$, one of which apparently corresponds to the hydrazone (V) and the other to the azo dye (IV) (Eq. (D)).

IR Spectrum: 3 300 (NH), 2 240 and 2 220 (C≡N); 1 530 (C≡N).

¹H NMR Spectrum: 2.9, 3 H (CH₃); 7.38, 3 H (Ar-H).

¹³C NMR Spectrum: 17.4 (CH₃); 86.0 (C≡N); 109.7 and 114.2 (C≡N); 126.9 and 121.9 (CH).

Mass Spectrum (*m/z*, %): 218 (M⁺ 75), 165 (100), 125 (70), 77 (89), 51 (93). For C₁₀H₇N₄Cl (219.6) calculated: 54.94% C, 3.23% H, 16.21% Cl, 25.62% N; found: 55.00% C, 3.30% H, 16.21% Cl, 25.49% N.

To measure the $A = f(c_{\text{CS},\text{MN}})$ dependence, an amount of 1.9 ml of buffer with pH 8.0 (R) or 9.4 (B), 0.1 ml of reagent ($c_{\text{R}} = 2 \text{ mmol l}^{-1}$, $c_{\text{B}} = 1 \text{ mmol l}^{-1}$) and, using a micropipette, 20 to 200 μ l of test substance CS or MN ($c_{\text{CS},\text{MN}} = 0.5 \text{ mmol l}^{-1}$) in steps of 20 μ l were mixed together. After tempering for 20 min on a water bath at $t = 5$ to 30°C, cooling and extraction with 2 ml of chloroform by shaking for 2 min, the absorbance of the organic layer was measured at $\lambda = 370$ nm (R) or 420 nm (B) against the pure solvent.

RESULTS AND DISCUSSION

Preliminary experiments with substance CS and its hydrolysis products¹³ – malononitrile (MN) and *o*-chlorobenzaldehyde – demonstrated that, in alkaline medium, substances CS and MN react with Fast Red TR to yield a yellow product that can be extracted into chloroform, with an absorbance maximum at $\lambda = 370$ nm and, for Fast Blue B, $\lambda = 420$ nm (Fig. 1).

To determine substances CS and MN, the absorbance maximum was read from the $A = f(\text{pH})$ dependence in Fig. 2 for the product formed in the aqueous phase at pH 8.0 using a phosphate buffer and, for Fast Blue B, using a borate buffer at pH 9.4. The blank experiments yielded a constant absorbance value over the whole studied pH range.

The stability methanol solutions of the stabilized diazonium salts was determined

by measuring the $A = f(\tau)$ dependence. The reagent solutions can be used for the determination for at least 40 min after preparation, yielding constant chloroform extract absorbance values against the blank.

In the studied concentration range 5 to 500 $\mu\text{mol l}^{-1}$ of diazonium salt, optimum concentrations of Fast Blue B of 50 $\mu\text{mol l}^{-1}$ and of Fast Red TR of 100 $\mu\text{mol l}^{-1}$ in the reaction mixture were found for the determination of MN and substance CS.

A study was made of the kinetics of the reaction of substances CS and MN with the diazonium salts. The $A = f(\tau)$ dependence for $t = 25^\circ\text{C}$ in Fig. 3 depicts the quantitative progress of the reaction for the reaction of MN with Fast Blue B after 4 min and with Fast Red TR after 7 min of tempering of the reaction mixture. The maximum absorbance of the extract of the product of substance CS with diazonium salts was identical after 15 min. Thus, the rate-controlling process is not the formation of the coloured product but hydrolysis of substance CS with malononitrile.

Figures 4 and 5 depict the rate of the reactions of substance CS with the studied diazonium salts at reaction mixture temperatures of 5 to 30°C. After 20 min, constant absorbance values were read for the chloroform phase for $t = 20, 25$ and 30°C after reaction with Fast Red TR and for $t = 25$ and 30°C for Fast Blue B. A 20-minute reaction time was selected for further studies of the determination conditions.

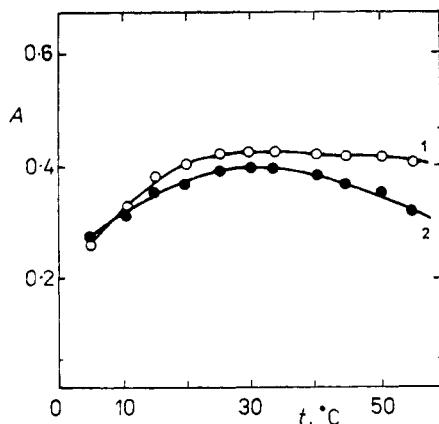


FIG. 6

Dependence of the absorbance of the product of the reaction of 25.0 $\mu\text{mol l}^{-1}$ of substance CS with 46.6 $\mu\text{mol l}^{-1}$ of Fast Red TR (1) and with 50.0 $\mu\text{mol l}^{-1}$ Fast Blue B (2) on the temperature, tempered for 20 min

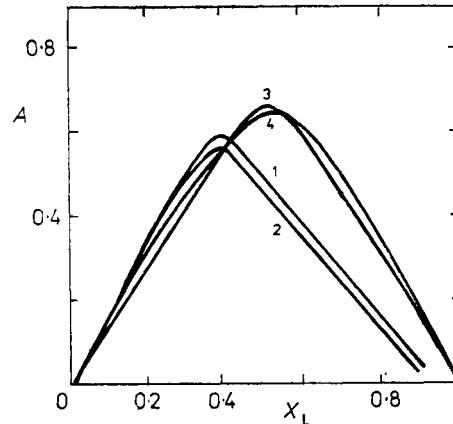


FIG. 7

Dependence of the product of the reaction of malononitrile (1, 4) and of substance CS (2, 3) with Fast Blue B (1, 2) and Fast Red TR (3, 4) at a mole fraction of the reagent of X_L , $t = 25^\circ\text{C}$, tempered for 20 min

In the determination of substance CS with Fast Blue B it is suitable to temper the reaction mixture at $t = 25^\circ\text{C}$. Reaction with Fast Red TR is not greatly affected by the temperature above 20°C , Fig. 6. The blank absorbance values are constant for both reagents.

It can be seen from the dependence of the absorbance of the chloroform extract on the mole fraction of the reagent x_L (Fig. 7) that substances CS and MN react with Fast Red TR in a stoichiometric ratio of 1 : 1 and with Fast Blue B in a ratio of 2 : 1.

Calibration curves were constructed by measuring the organic phase after previous 20-minute tempering of the reaction mixture of substance CS with the studied diazonium salts at temperatures of 5 to 30°C . The parameters of the calibration straight lines are given in Table I. The determinations conform to the Lambert-Beer law and, even in the least favourable case, the correlation coefficient had a value of 0.994. The best results were obtained in the determination of substance CS with Fast Red TR, for which a determination limit of 190 to 230 ng of substance CS in 1 ml was calculated even at a reaction mixture temperature of 5 to 15°C . The dependence $A = f(c_{\text{MN}})$ was measured for $t = 20^\circ\text{C}$ and tempering time of 20 min.

TABLE I

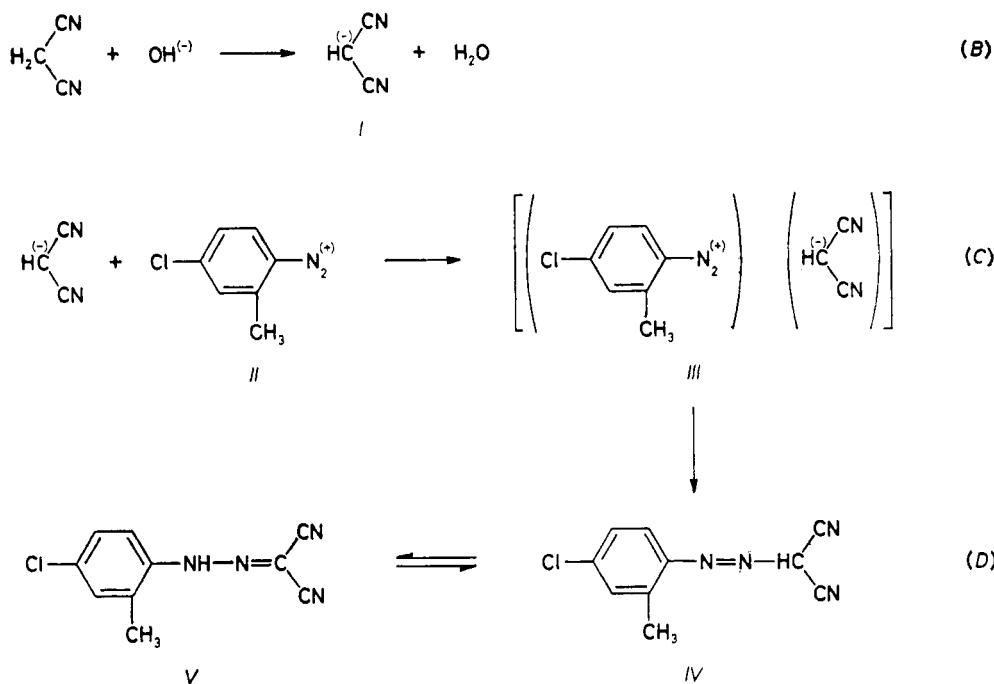
Parameters of the extraction spectrophotometric determination of substance CS with the diazonium salt Fast Red TR (R) and Fast Blue B (B) for $t = 5$ to 30°C

| t $^\circ\text{C}$ | Reagent | Parameter ^a | | | | |
|-------------------------|---------|-----------------------------|-------|--|--------------------------------|--------------------------------|
| | | k 1 mol^{-1} | q | ϵ $1 \text{ mol}^{-1} \text{ cm}^{-1}$ | L_D $\mu\text{g ml}^{-1}$ | L_Q $\mu\text{g ml}^{-1}$ |
| 5 | R | 12 000 | 0 | 12 000 | 0.08 | 0.22 |
| | B | 11 500 | 0.016 | 11 400 | 0.45 | 0.84 |
| 10 | R | 16 400 | 0.016 | 16 400 | 0.07 | 0.19 |
| | B | 13 100 | 0.013 | 13 000 | 0.40 | 0.48 |
| 15 | R | 18 500 | 0 | 18 700 | 0.07 | 0.23 |
| | B | 14 700 | 0.015 | 14 700 | 0.35 | 0.49 |
| 20 | R | 18 500 | 0.021 | 18 500 | 0.06 | 0.18 |
| | B | 15 300 | 0.013 | 15 300 | 0.42 | 0.57 |
| 25 | R | 19 000 | 0 | 19 100 | 0.07 | 0.21 |
| | B | 15 900 | 0.021 | 15 800 | 0.44 | 0.45 |
| 30 | R | 19 000 | 0 | 19 100 | 0.06 | 0.19 |
| | B | 15 700 | 0.012 | 15 700 | 0.27 | 0.45 |

^a k and q are the parameters of the linear dependence of absorbance A on concentration c_{CS} ($A = kc + q$), ϵ molar absorption coefficient at $\lambda = 370 \text{ nm}$ (R) or 420 nm (B), L_D detection limit and L_Q determination limit²⁵.

The extraction spectrophotometric method with Fast Red TR for $\lambda = 370$ nm ($\epsilon = 20\,000\,1\text{ mol}^{-1}\text{ cm}^{-1}$) permits the determination of 80 ng, while the method employing Fast Blue B for $\lambda = 420$ nm ($\epsilon = 18\,500\,1\text{ mol}^{-1}\text{ cm}^{-1}$) permits the determination of 200 ng MN in 1 ml.

The hydrolysis of substance CS in slightly alkaline medium leads to the formation of a reactive aldehyde and malononitrile²⁶. On the basis of this fact, an ionic mechanism was proposed for the reaction of substance CS with Fast red TR (Eqs (B), (C), (D)). The anion of malononitrile (*I*) and the diazonium salt (*II*) react to produce the ion associate (*III*) as the reaction intermediate, which is converted by consequence coupling into the azo dye (*IV*). The product formed exists predominantly in the hydrazo-form (*V*) (ref.²⁷).



REFERENCES

1. Corson B. B., Stoughton R. W.: *J. Am. Chem. Soc.* **50**, 2825 (1928).
2. Mrázek J.: *Právo mezinárodní bezpečnosti a odzbrojení*. Academia, Praha 1990.
3. Robinson J. P., Leitenberg M.: *The Problem of Chemical and Biological Warfare*, Vol. I., p. 186. Stockholm International Peace Research Institute, Stockholm 1971.

4. Stern J., Malherbe H., Green R. H.: *Biochem. J.* **52**, 114 (1952).
5. Jones G. R. N., Israel M. S.: *Nature* **228**, 1315 (1970).
6. Jones G. R. N.: *Nature* **235**, 257 (1970).
7. Dressler G. Z.: *Militärmedizin* **6**, 272 (1980).
8. Franke S.: *Lehrbuch der Militärchemie*, Vol. 1., p. 159. Militärverlag, Berlin 1977.
9. Hasan Saad S. M., Abdalla J. M., Nashed N. E.: *Mikrochim. Acta* **2**, 27 (1984).
10. Enqvist J., Manninen A., Ravio P., Kokko M., Kuronen P., Hesso A., Rautio M., Häkkinen A. M., Taivainen U. M., Kantolahti E., Sipponen K., Koskinen L., Laitinen R., Björk H., Kettunen R.: *Systematic Identification of Chemical Warfare Agents*, Vol. B.4. The Ministry for Foreign Affairs of Finland, Helsinki 1983.
11. Rautio M., Koskinen L., Kauppinen J., Stenman F., Savolainen A. L., Raunemaa T., Kokko M., Hassinen E., Pyysalo H., Laitinen R., Inkeroinen P., Kantolahti E.: *Trace Analysis of Chemical Warfare Agents*, Vol. C.2. The Ministry for Foreign Affairs of Finland, Helsinki 1985.
12. Enqvist J., Manninen A., Piispanen H., Juutilainen T., Kokko M., Kironen P., Sivonen A., Kenttämaa H., Kahkamaa E., Rautio M., Taivainen U. M., Kalervo L., Sipponen K., Kinnunen P., Gahmberg C., Virtanen J., Koskinen L., Sunila P., Laitinen R., Jarva M., Helle H., Ravio P., Björk H.: *Systematic Identification of Chemical Warfare Agents*, Vol. B.3. The Ministry for Foreign Affairs of Finland, Helsinki 1982.
13. Franke S.: *Lehrbuch der Militärchemie*, Vol. 2, p. 369. Militärverlag, Berlin 1977.
14. Stachlewska-Wroblowa A.: *Biul. WAT. J. Dobrowskiego* **21**, 109 (1972).
15. Stachlewska-Wroblowa A.: *Biul. WAT. J. Dobrowskiego* **22**, 105 (1973).
16. Lundemann W. D., Stutz M. H., Sass S.: *Anal. Chem.* **41**, 679 (1969).
17. Haddadin M., Khalidi U.: *Anal. Chem.* **46**, 2072 (1974).
18. Issa F. M., Yssa Y. M., Yossef H. Z., Issa R. M.: *Fresenius Z. Anal. Chem.* **277**, 125 (1975).
19. Makles Z.: *Thesis*. VACHZ, Moscow 1978.
20. Spasov G., Stoev S., Ivanov I., Georgiev V., Nikolova G.: *Chim. Ind. (Sofia)* **49**, 109 (1977).
21. König W.: *J. Prakt. Chem.* **69**, 105 (1904).
22. Asmus E., Garschhhagen Z.: *Z. Anal. Chem.* **138**, 144 (1953).
23. Kobliha Z., Halámek E. (Military University Vyškov): Czech. 2700, 1988.
24. Küster F. W., Thiel A.: *Chemicko-analytické výpočetní tabulky*. Academia, Praha 1988.
25. Eckschlager K.: *Chem. Listy* **83**, 1009 (1989).
26. Pearson R. G., Dillon R. L.: *J. Am. Chem. Soc.* **75**, 24 (1953).
27. Lyčka A.: *Collect. Czech. Chem. Commun.* **49**, 2801 (1984).

Translated by M. Štulíková.