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A Simple Spectrophotometric Determination of Cyanides by *P*-nitrobenzaldehyde and Tetrazolium Blue

V. Pitschmann[1], Z. Kobliha[2*] and I. Tušarová[1]

[1] *Oritest spol. s r.o, Prague, Czech Republic*
[2] *NBC Defence Institute (Vyškov), University of Defence, Brno, Czech Republic*

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Abstract:

The article describes a simple and a very sensitive spectrophotometric method for determining free cyanides, based on their catalytic reaction with p-nitrobenzaldehyde. Cyanide ions allow for a transformation of p-nitrobenzaldehyde into its transitory reactive cyanohydrin, which reduces a redox indicator, the tetrazolium blue, into a purple-coloured diformazan derivative. The product's absorption maximum is 520 nm, the determination limit found by calculation is 0.005 $mg \cdot l^{-1}$ *and molar absorptivity is* $4.86 \times 10^5\ l \cdot mol^{-1} \cdot cm^{-1}$*. Determination is obstructed by the presence of strong reductive substances.*

Keywords:

Spectrophotometry, cyanides, p-nitrobenzaldehyde, tetrazolium blue.

1. Introduction

Cyanides are systemic or blood poisons. Their effect on human organism is based on blocking the cell breathing, the substance of which is the transport of oxygen from blood into tissues. Cyanides are also efficient inhibitors of metabolic processes (glucose metabolism, function of thyroid hormones, enzymatic activity, and influence on the Calvin cycle). Lethal dose for humans (LD_{50}) is approximately $1\ mg \cdot kg^{-1}$ perorally. Each year, approximately 2 million tons of cyanides are produced in the world, a significant part of which is used by the chemical and steel industries, precious metals mining and galvanotechnics.

Due to large amounts of cyanides in circulation and their high toxicity, it is necessary to regard the cyanides as very significant industrial toxic substances.

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* *Corresponding author: NBC Defence Institute (Vyškov), University of Defence, Kounicova 65, 662 10 Brno, Czech Republic, phone: +420 973 452 311, E-mail: Zbynek.Kobliha@unob.cz*

Hydrogen cyanide is one of the secondary chemical warfare agents and a potential terrorist weapon. Cyanides are also the most frequently abused chemicals for criminal reasons. Therefore a great attention is paid to their analysis [1, 2].

Cyanides can be determined by different electrochemical, chromatographic, fluorimetric, and other instrumental methods. To determine cyanides in water, especially the colorimetric and spectrophotometric methods are advantageous, as they are noted for simplicity and availability of instrumentation [3-6]. Among these methods, there are also procedures which make use of the cyanides reaction with benzaldehyde into a transitory active cyanohydrin (mandelonitrile) or benzoin, which reduces the *o*-dinitrobenzene, creating a purple *o*-nitrophenylhydroxylamine dianion [7, 8]. As the cyanides regenerate in the process – it is a catalytic reaction – a low detection limit is attained thereby. Even better results can be attained by using the *p*-nitrobenzaldehyde instead of benzaldehyde [9-14]. Other agents have been suggested instead of the *o*-dinitrobenzene, namely the 2,3,5-triphenyltetrazoliumchloride providing red triphenylformazan [9] or luminol, which is still suitable for the chemiluminescent determination of cyanides [15].

This article describes a modified, simple, and highly sensitive method of spectrophotometric determination of cyanides based on their interaction with *p*-nitrobenzaldehyde, when the formed cyanohydrin reduces the tetrazolium blue to a coloured diformazan derivate. In the past, using tetrazolium blue was suggested for example for a spectrophotometric determination of corticosteroids and other substances with the ketone functional group, reducing sugars, tea tannins, or eventually some pharmaceutical preparations designed for treatment of the Parkinson disease. Different compounds of the tetrazolium structure, such as the 2,3,5-triphenyltetrazoliumchloride, are also used for a spectrophotometric determination of sulphides and their precursors or some chemotherapeutic substances used in oncology.

2. Experimental Part

2.1. Agents and Chemicals

Potassium cyanide was used as a standard, the purity of which was checked potentiometrically [16]. The stock solution was prepared by dissolving 0.1 g of potassium cyanide in redistilled water. Working solutions were prepared by diluting the basic solution by redistilled water. The following agents were prepared to determine the cyanides: $0.1 \div 1$ % solution of *p*-nitrobenzaldehyde in methanol, $0.01 \div 0.1$ % solution of tetrazolium blue chloride [3,3′-(3,3-dimethoxy-4,4-biphenylene)-bis(2,5-diphenyl-2*H*-tetrazoliumchloride)] in methanol and aqueous solution of sodium hydroxide in 0.01 to 0.5 $\text{mol} \cdot \text{l}^{-1}$ concentration. All the chemicals used were at least analytically grade (Aldrich-Sigma-Fluka).

2.2. Apparatus

Spectrophotometric measurements were carried out with Aquamate apparatus (Thermo Spectronic, UK). Potentiometrical measurements were carried out with pH-meter HI 4222 (Fisher Scientific, CZ) with a cyanide ISE (Monokrystaly Turnov, CZ).

2.3. Procedures

A tube was filled with measured 2 ml of a standard solution with added 0.2 ml of sodium hydroxide solution, 2 ml of *p*-nitrobenzaldehyde solution, 2 ml of tetrazolium blue solution, and completed by 10 ml of methanol. After 30 minutes, the formed red-purple colour was measured at a wavelength of 520 nm as opposed to methanol.

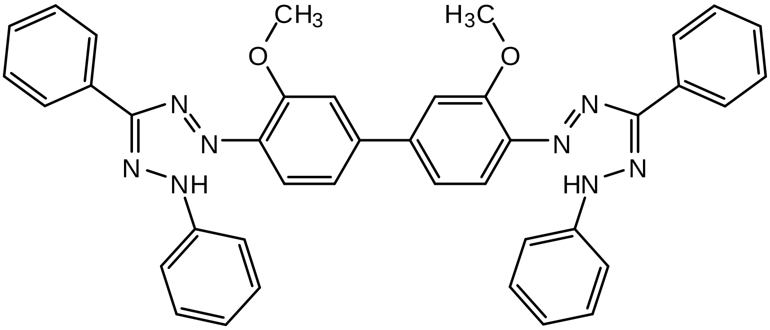
The values for creating a calibration graph were acquired by measuring a series of standard working solutions of cyanides in different concentrations. Values measured in blind determinations with distilled water were subtracted from values measured in the individual standards. Statistical evaluation of the method was carried out in the usual way; the concentration of cyanides in the formulas was determined directly from the calibration graph, with regard to the dilution or by the Lambert-Beer law.

3. Results and Discussion

3.1. Chemical Reaction, Colouring and its Stability

Cyanide determination is based on the reaction with *p*-nitrobenzaldehyde into a reactive cyanohydrin and the subsequent reduction of the tetrazolium blue [9]. The final product of the reaction is a purple coloured compound derived from diformazan with a maximum absorption at 520 nm (Scheme 1). Fig. 1 shows a typical dependence of absorbance on the wavelength.

The colour fully develops within 25 minutes and the formed colour is stable for 60 to 80 minutes. Dynamic dependence of absorbance on time is depicted by Fig. 2.



Scheme 1 Final reaction product

3.2. Optimizing Reaction Conditions

Analytic reaction only takes place in alkaline medium. Additions of Na_2CO_3, $NaHCO_3$, KOH and NaOH were tested, whereas NaOH seems to be most suitable. The best results were attained when adding 0.2 ml of 0.1 $mol \cdot l^{-1}$ NaOH per 10 ml of the solution. Lower NaOH concentration has an adverse effect on the detection limit, with greater concentrations of NaOH, there are competitive reactions and the agent tends to get darker. The sequence of the agents added has no influence on the process of the reaction and the result of the determination. Fig. 3 shows a characteristic process of the dependence of absorbance on added NaOH.

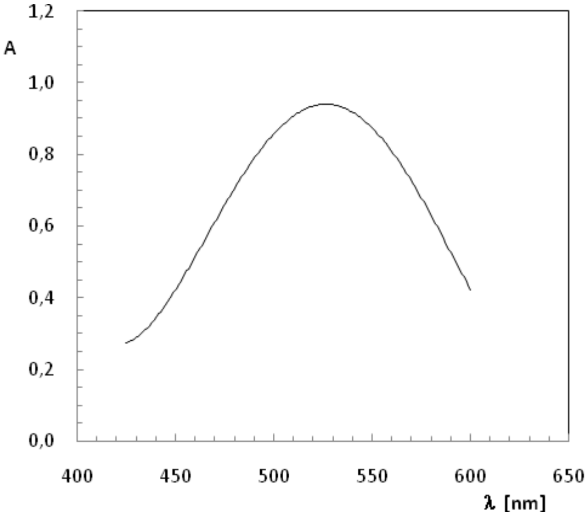


Fig. 1 Typical dependence of absorbance on the wavelength (0.06 mg·l^{-1} CN^{-}, 20 °C)

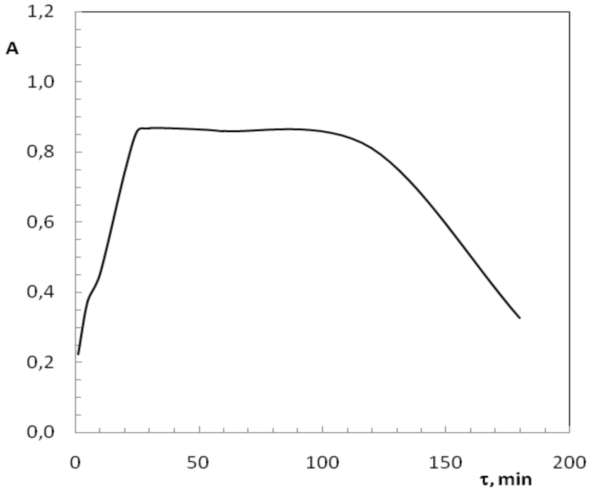


Fig. 2 Dependence of absorbance on time (0.04 mg·l^{-1} CN^{-}, 20 °C)

An influence of the agents' concentration on the process and the result of the cyanides determination were studied. To determine trace concentrations of cyanides, a 0.6 % solution of *p*-nitrobenzaldehyde and 0.06 % solution of tetrazolium blue is optimal. Higher concentrations cause lower stability of solutions of reagents and colour of the product. In contrast, lower concentrations result in a narrower range of the determination of cyanides.

Reaction temperature significantly influences the determination of cyanides. Heating the reaction system causes acceleration in the occurrence of the colouring, and attaining higher values of absorbance. By increasing the temperature by 5 °C, the absorbance value increases by 20 to 40 %, which corresponds to other procedures with *p*-nitrobenzaldehyde [11]. However, increased temperature causes more expressive disturbing influence of extraneous chemical substances.

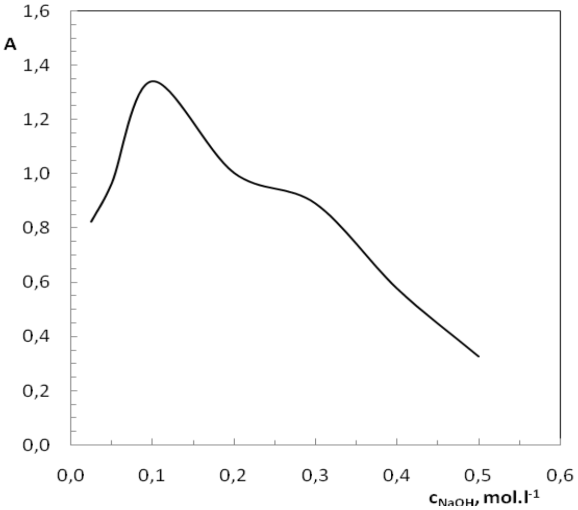


Fig. 3 Dependence of absorbance on adding 0.2 ml of sodium hydroxide (0.06 mg·l^{-1} CN^{-}, 20 °C)

3.3. Calibration Graph and Determination Limit

Fig. 4 shows the dependence of absorbance on the cyanide concentration. The determination limit found by calculation is 0,005 mg·l^{-1}. Samples with cyanide concentration greater than 0.06 mg·l^{-1} it is necessary to dilute by water. The sensitivity of the method expressed by the calibration dependence guideline (molar absorptivity) at 520 nm is 4.86×10^5 l·mol^{-1}·cm^{-1}, a coefficient of determination $R^2 = 0.9991$. Sandell's sensitivity is 0.0005 mg·l^{-1} for $\Delta A = 0.001$. Relative standard deviation (RSD) for eight repetitive measurements of the assay containing cyanides of 0.01 mg·l^{-1} concentration is 5.24 %.

3.4. Disturbing Effects

The influence of extraneous substance on the result of the determination was studied in detail. Examples of these results are shown in Tab. 1. Disturbing effects of extraneous substances are, in general, combinations of their interaction with *p*-nitrobenzaldehyde and tetrazolium blue, or eventually with the cyanides in samples. The same colouring as in the case of cyanides is evoked by sulphides, sulphites, and other strong reduction substances, which directly reduce the tetrazolium blue into the diformazan derivative. Substances with active chlorine and bromine react with cyanides to cyanogen chloride or cyanogen bromide, which do not have a catalytic effect. In case of fixed cyanides (namely the stable complexes with Fe, Co), it is necessary to release hydrogen cyanide via distillation and to capture it in an alkaline absorption solution, using standard procedures [17].

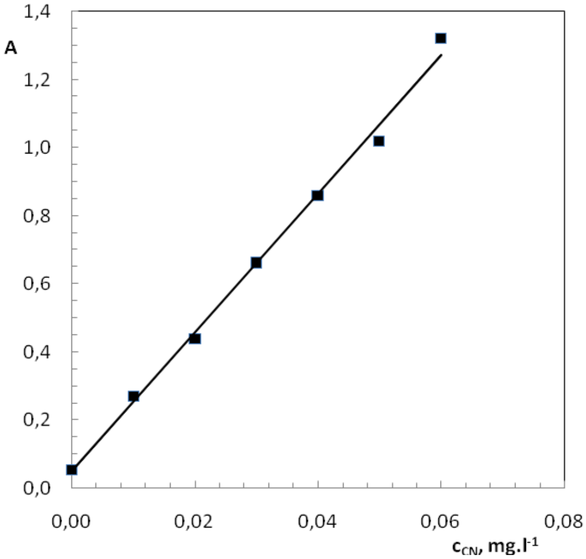


Fig. 4 Dependence of absorbance on the cyanide concentration (520 nm, time of measurement 30 minutes, 20 °C)

Tab. 1 Study of interferences (0.5 µg of CN$^-$ inserted into 10 ml of solution, results rounded off)

Extraneous substance	**Extraneous substance, inserted, µg**	**CN$^-$ found, µg**
ClO^-	0.1	0.4
NO_2^-	100	0.5
S^{2-}	10	0.6
SO_3^{2-}	10	0.6
SCN^-	100	0.5
NH_4^+	100	0.5
Formaldehyde	100	0.5
Acetone	1000	0.6
Phenol	100	0.5
Thioacetamide	100	0.6

4. Conclusion

The suggested method can be used for fast and very sensitive laboratory determination of free cyanides in waters. It is apparent from Tab. 2, that the method can determine cyanides in comparable or lower concentrations than other spectrophotometric methods. However, it is necessary to take into account the disturbing effect of strong reduction substances, and to strictly comply with the reaction conditions, namely the alkalinity of the medium.

Results of the cyanides determinations were compared in real samples (samples of previously unknown concentration of cyanides) with a reference method based on the transfer of cyanides into cyanogen chloride (impact of chloramine), and its reaction with pyridine and the barbituric acid [17]. A good conformity was found between the proposed and the reference method, with the results differing by no more than 11.1 % (Tab. 3).

Tab. 2 Comparison with selected spectrophotometric methods for cyanide determination

Method – agents	**Determination limit (mg·l^{-1})**	**Ref.**
Phenolphtalin, Cu(II)	0.005	5
Pyridine, barbituric acid, chloramine	0.005	17
Na isonicotinate, Na barbiturate, chloramine	0.05	3
Pyridine, benzidine, bromine	0.05	17
Ninhydrin	0.02	4
Chromogenic disulphides	0.007	6
p-Nitrobenzaldehyde, *o*-dinitrobenzene	0.001	9
p-Nitrobenzaldehyde, *o*-dinitrobenzene	0.05	13
p-Nitrobenzaldehyde, 2,3,5-triphenyltetrazoliumchloride	0.03	9
p-Nitrobenzaldehyde, tetrazolium blue	0.005	Proposal

Tab. 3 Results of cyanide determination in real samples

Assay	**CN$^-$ found, mg.l^{-1}**	
	Proposed method	**Reference method**
1	0.036	0.032
2	0.081	0.073
3	0.095	0.086
4	Not found	Not found
5	0.127	0.116

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