

SPECTROPHOTOMETRIC STUDY OF THE REACTION OF INDOLE WITH NITRITE IONS IN HYDROCHLORIC ACID

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UV/Vis spectrophotometry can be used to study many types of chemical processes, for example acid-base equilibrium, reaction of addition or redox equilibrium. Quantitative determination of indoles are frequently done because of their possible presence in water pollution.

The present work is an investigation of the nitrosation reaction of indole with nitrite ions in water-ethanol solutions acidified with hydrochloric acid.

The effects of the independent variation of concentration of hydrochloric acid solutions from 0.01 to 2.0 mol dm⁻³, or of nitrite ions from 1.0·10⁻⁵ mol dm⁻³ to 3.0·10⁻³ mol dm⁻³ upon indole, were investigated by observing the absorption spectra and stability of the reaction products. Nitrite ions in hydrochloric acid solutions of indole introduce a strong chromophoric group in the aromatic molecule.

Acidic solutions of the nitrosation products exhibit maximum absorption (broad band) at 500 – 530 nm and a second maximum of 400 – 450 nm. By using the Job-method and the molar ratio method it was found that stoichiometric ratio between indole and 3-nitrosylindole is 1:1. Furthermore it was concluded that the position of the introduced group in the former compound is of 3-C atom of indole (in indole red molecule).

Key words: UV/Vis spectra; indole; nitrosation; determination

INTRODUCTION

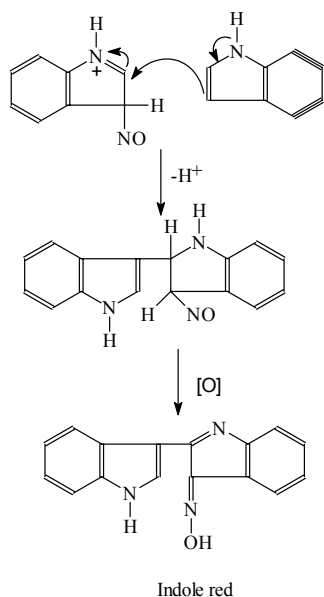
UV/Vis spectroscopy can be used in the purpose of studying many types of chemical reactions, for example, acid-base equilibrium, metal complex association, or redox equilibrium. By spectrophotometric analysis of indole in aqueous solutions, the presence of bacteria *E. coli* was detected. In the grown process of the bacteria *E. coli*, different concentrations of indole or indole derivatives are found [1].

Ehrlich's reagent is widely introduced for qualitative analysis of indole in water [2]. In this paper the Vis spectra of the nitrosation products for indole (I), 3-methylindole (3-MI) and 3-indolylacetic acid (3-IAAH) were obtained. I and 3-MI have a major and shoulder peak (I at 570, 540 nm and 3-MI at 585, 550 nm). The compounds with a

side chain at the 3-C position, like molecules of IAAH and tryptamine have major and minor peaks at two different wavelengths (545 and 575 nm), while 5-hydroxyindoleacetic acid and 5-hydroxytryptamine at 555 and 590 nm. Also, the reaction of nitrite ions with indole for a qualitative test has been widely used. The reaction of nitrosation of indole in aqueous acetic acid has been systematically studied by *Zatti* and *Feratini* in 1890 [3]. As products from this reaction two compounds have been isolated: one is indole red [4] and the other compound is called "dinitrosoindol". *Seidel* [4] found that "dinitrosoindol" decomposes to indole red.

To check the idea that "indole red" is formed by acid-catalyzed condensation of initially formed

3-hydroxy-imino-indolenin with indole we studied the interaction of indole with NaNO_2 in hydrochloric acid solution.



Scheme 1

For the detection of the product in the reaction of indole with nitrite ion (red color) we investigated the UV/Vis spectrophotometry and the reaction of indole with $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$, Salkovski reagent ($\text{FeCl}_3 + \text{HClO}_4$) and *p*-dimethyl-amino-benzaldehyde (PDAB) in hydrochloric acid solutions. *Mitchell* and *Brunstetter* have used the nitrite sulfuric acid solution for the qualitative and quantitative analysis of 2-(3-indolyl) acetic acid (3-IAAH) in aqueous solutions [5]. Also, it was concluded that the product of 3-IAAH decomposition which gives the red color in the *Salkovski* reaction, was not 2-(*N*-hydroxyl, 3-indolyl) acetic acid [6].

The present work was a quantitative study of the nitrosation reaction (nitrite ion with some indoles) in hydrochloric acid solutions. In this work, a possibility for application of this reaction for spectrophotometric determination of indole in natural and waste water (using nitrosation reaction) was investigated. The method was upgraded with suggested procedures.

EXPERIMENTAL

All spectra (UV/Vis) were obtained using HP8452 a recording spectrophotometer equipped with 1.0 cm quartz absorption cells. An advantage of using a Diode-Array Spectrophotometer is that full spectra, rather than the absorbance of a single wavelength can be measured. Indole was subject to

nitrosation reaction at 300 °K for 5, 10, 15, 20, 25 and 30 min (see Fig. 1).

Isobestic point from ten UV/Vis spectra (Fig. 2) of the reaction product of indole with nitrite was obtained by applying the Characteristic Vector Analysis (CVA) [7].

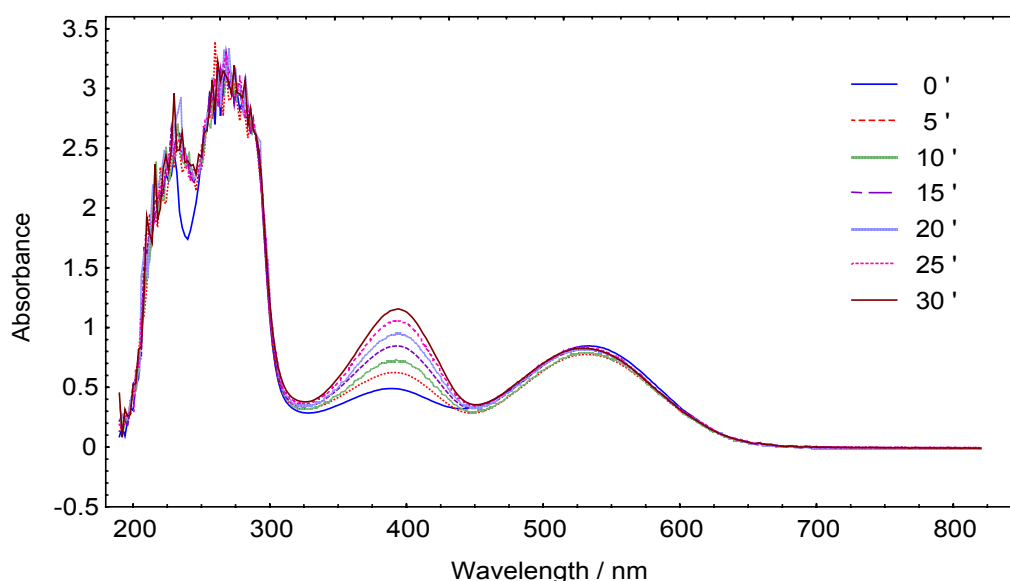


Fig. 1. UV/Vis spectra were obtained for reaction of indole with nitrosyl ions in hydrochloric acid solution ($c = 0.5 \text{ mol dm}^{-3}$). UV/VIS spectra were made at 5 min intervals to 30 min.

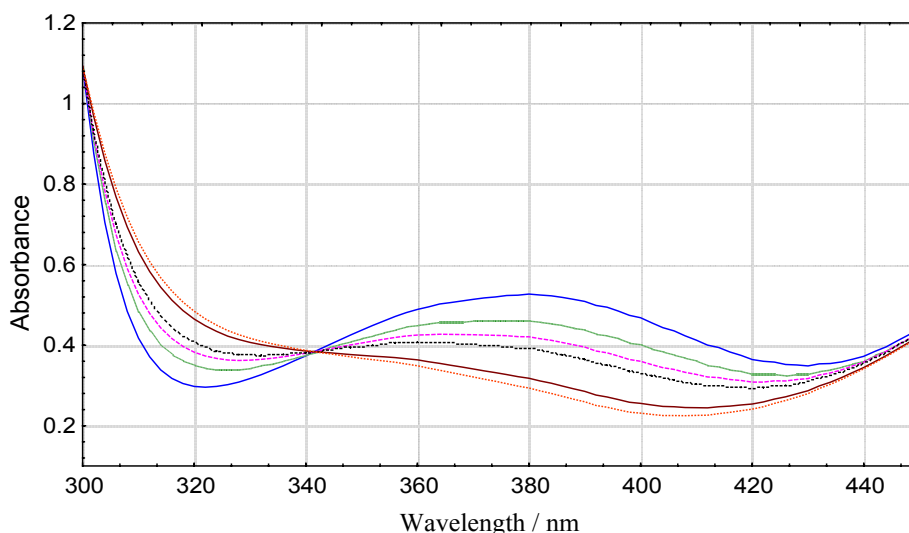


Fig. 2. The reconstituted UV/Vis spectra of reaction product of indole with nitrosyl ion in hydrochloric acid solution (0.5 mol dm^{-3})

Method and procedure

In a series of 25.0 cm^3 flasks different volumes of water/ethanol (1:1) indole solution from 0.5; 1.0; ... to 10.0 cm^3 with concentration of $1.0 \cdot 10^{-3} \text{ mol dm}^{-3}$ were transferred. 5.0 cm^3 of water/ethanol (1:1) nitrite ion solution with concentration $1.0 \cdot 10^{-3} \text{ mol dm}^{-3}$ were added in each flask. Also, 6.5 cm^3 hydrochloric acid solution with concentration of 2.0 mol dm^{-3} were added. Solutions were made up to 25 cm^3 with water/ethanol. The mixtures were transferred to suitable cuvettes and absorbances were read at precisely

timed intervals after the start of addition of hydrochloric acid solutions. This procedure was used for determination of the stoichiometry of the reaction between indole and sodium nitrite (NaNO_2).

On the other hand for determination the stoichiometry of the reaction between indole and sodium nitrites, the *Job's*-method was also used [8, 9]. The series of solutions for *Job's* method were prepared by mixing both components with concentration of $1.0 \cdot 10^{-3} \text{ mol dm}^{-3}$ by varying their ratio from 1:9 to 9:1. Absorbances were measured at 520 nm , $c(\text{HCl})$ was 0.5 mol dm^{-3} and $T/\text{K} = 303$.

RESULTS AND DISCUSSION

UV/Vis spectra

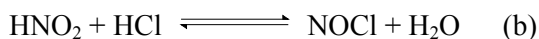
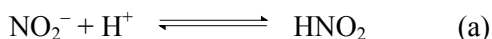
In water/alcohol solutions (1:1) UV/Vis spectrum of indole showed absorption maximum at 196 nm , $\log \varepsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1} = 4.31$, (${}^1\text{B}_a$), and 214 nm , $\log \varepsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1} = 4.49$, (${}^1\text{B}_b$); broad absorption band at $240 - 280 \text{ nm}$, with maximum at 270 nm , $\log \varepsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1} = 3.74$, (${}^1\text{L}_a$) and 286 nm , $\log \varepsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1} = 3.73$, (${}^1\text{L}_b$), all of them resulting from $\pi \rightarrow \pi^*$ transition [10–13]. UV/Vis spectrum of sodium nitrite in water solution showed absorption maxima at 202 nm , $\log \varepsilon / \text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1} = 3.68$, resulting from $\pi \rightarrow \pi^*$; 284 nm $\log \varepsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1} = 1.39$, resulting from $n(\text{O}) \rightarrow \pi^*$ and 354 nm , $\log \varepsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1} = 1.47$, resulting from $n(\text{N}) \rightarrow \pi^*$ [14].

Treatment of sodium nitrite with various concentration solutions of hydrochloric acid was investigated. UV/Vis spectrum of a pure substance (NaNO_2) was obtained in each of those solutions. Slight differences both in location and in the intensity of the $\pi \rightarrow \pi^*$ band (from 202 to 210 nm) was observed with the variation in concentration of hydrochloric acid solution $c(\text{HCl}) = 0.025$ to 1.0 mol dm^{-3} .

Contrary to these small effects a radical change in the UV/Vis spectrum from 300 to 400 nm was observed. The acidic solutions of sodium nitrite (0.5 to 1.0 mol dm^{-3}) shows bands at 210 nm ($\pi \rightarrow \pi^*$) and 358 nm ($n(\text{N}) \rightarrow \pi^*$). The $n(\text{N}) \rightarrow \pi^*$ band of HNO_2 shows an extensive, well-defined vibration fine structure (bands of 338 , 348 , 372 and 386 nm). This separation may be as-

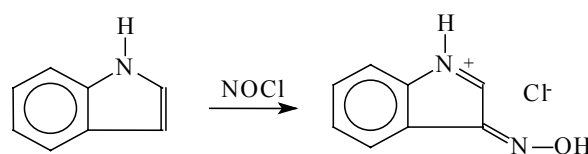
sociated with one of the out of plane N–O bending modes in the HNO_2 .

The relationship between the two forms of NaNO_2 in an aqueous solution is described by the equilibrium (a) for which $K_a^0 = 7.1 \cdot 10^{-4}$ [15] and the treatment of NaNO_2 with HCl at 300°K leading to the formation of reaction products: HNO_2 , and NOCl (see chemical reactions (a) and (b)).



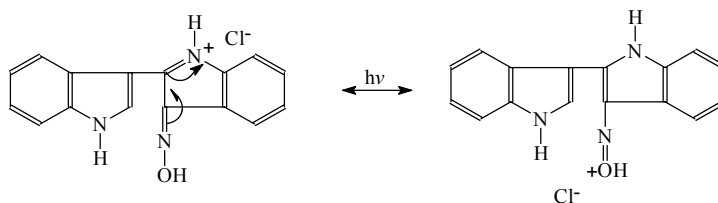
In hydrochloric acid solution with products from reactions (a) and (b), changes are noted in the position and intensity of the ${}^1\text{B}_a$, ${}^1\text{B}_b$, ${}^1\text{L}_a$ and ${}^1\text{L}_b$ bands in the UV spectrum of indole. By increasing the time of the reaction, the intensity of ${}^1\text{B}_a$, ${}^1\text{B}_b$, ${}^1\text{L}_a$ and ${}^1\text{L}_b$ bands decreases [14]. The appearance of new bands at 270(sh) and 280 nm, may be noted in the UV/Vis spectrum. By applying the Characteristic Vector Analysis (CVA) the wavelength (340 nm) of the one isosbestic point (Fig. 2) was obtained. The observed changes in the UV/Vis spectrum are beyond doubt a result of the nitrosation of indole and formation of indole red. Also, the acidic hydrochloric solution of some nitrosa-

tion products exhibit maximums in the UV/Vis spectrum, broad band at 430 and 528 nm (Fig. 1). The band at 528 nm in the UV/Vis spectrum, probably outcoming from indole red, respectively, is due to the intramolecular charge-transfer transition from the Highest Occupied Molecular Orbital (H_B) of one part of the indole red which acts as an electron-donor, to the Lowest Unoccupied Molecular Orbital (V_S) of the other part (indole red) which serves as an electron acceptor. These transitions were denoted as $\text{H}_B \rightarrow \text{V}_S$ by Nagakura and Tanaka [16–20]. The UV/Vis absorption corresponding to the intramolecular charge-transfer may be represented by the following (see Scheme 2).



Scheme 2

Fig. 3 shows the plot of the absorbance at 520 nm against $c(\text{HCl})/\text{mol dm}^{-3}$ obtained during the reaction of indole with 3-nitrosyl indole (Scheme 3) in acidic hydrochloric solutions.



Scheme 3

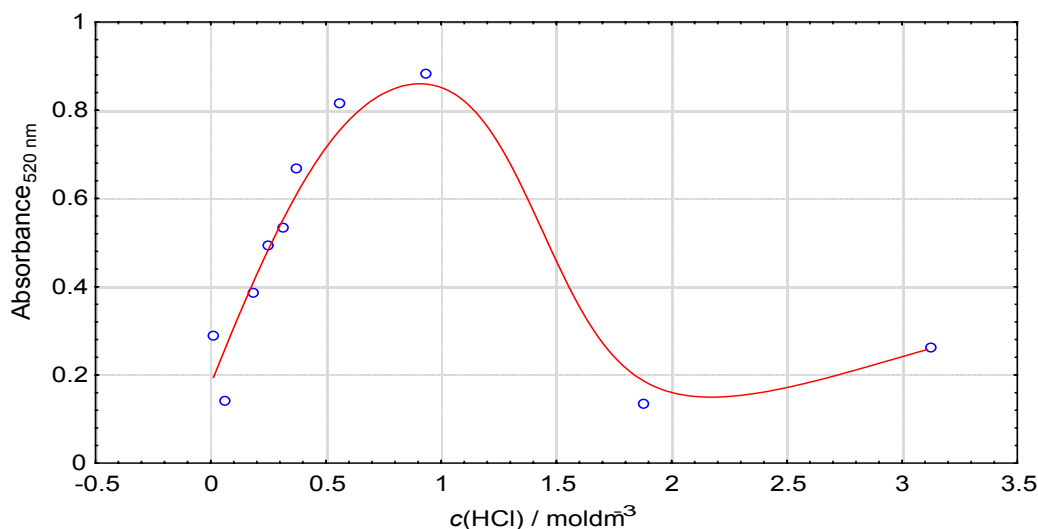


Fig. 3. Plot of absorbance at 520 nm against $c(\text{HCl}) / \text{mol dm}^{-3}$

Constant absorbances are not obtained within the range of 0.05 to 1.0 mol dm⁻³ (see Fig. 3). The formation of the colored species by action of hydrochloric acid is observed.

The UV/Vis analysis of mixtures in the ratio of $V(\text{indole}) : V(\text{NO}_2^-) = 1 : 9$ ($c(\text{HCl}) = 0.5$ mol dm⁻³ and $t = 900$ s) shows the band with λ_{max} at

498 nm. Compared to the spectrum obtained for the ratio of $V(\text{indole}) : V(\text{NO}_2^-) = 9:1$, where the maximum is at 530 nm, the difference is 30 nm. With the appearance of this difference it can not be concluded that in the system more than one product species are formed (see Fig. 4)

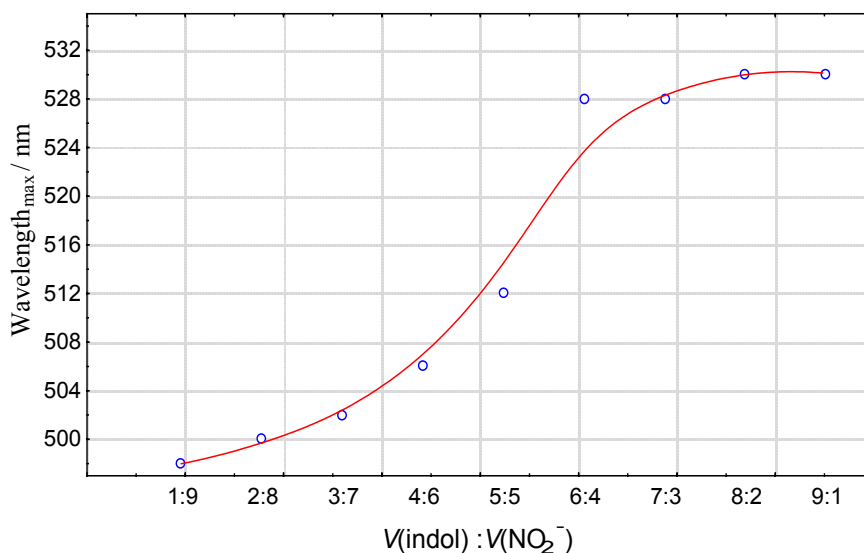


Fig. 4. Plot of λ_{max} against $V(\text{indole}) : V(\text{NO}_2^-)$

Stoichiometric ratio of the reactants

The observation that the absorbance ($A_{530 \text{ nm}}$) is proportional to the formation of reaction product of indole with 3-nitrosylindole ions (see eq. 6 and 7).

The preliminary estimate of the stoichiometry of reaction of indole (A) with 3-nitrosylindole (B),

(from the reaction of indole and decomposition of NaNO_2 or $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$ in the hydrochloric acidic solution) was checked afterwards by the empirical method [15].

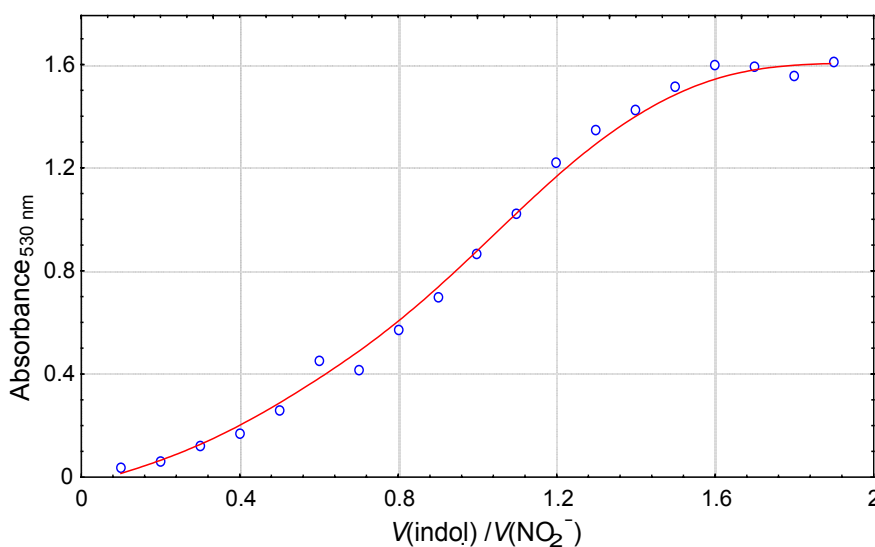
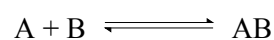


Fig. 5. Effect on absorbance of variations of indole concentration and reaction with 3-nitrosyl indole

With varying c (A – indole) and constant c (B – 3-nitrosyl indole) a line with slope 1.2, intercept 4.45 and $r = 0.989$ was obtained. These data indicate that interaction between indole and 3-nitrosyl indole in constant hydrochloric acid concentration 0.5 mol dm^{-3} , results in the formation of AB product in molar ratio 1:1. The $\log A$ proportional to $\log c(\text{AB})$ of a series of solutions with constant

concentration A and varying concentration of 3-nitrosylindole was plotted against $\log c(\text{B})$, giving a straight line of slope of 1.1 and intercept 4.26, $r = 0.999$. These data also confirmed that the interaction between indole and 3-nitrosylindole in an acidic media, results in the formation of AB product in molar ratio 1:1.

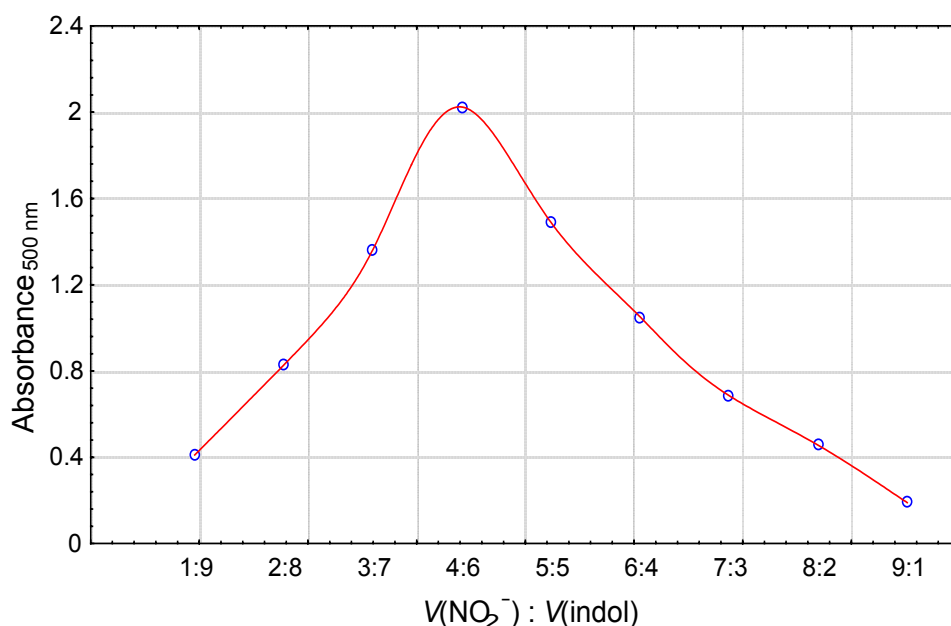


Fig. 6. Absorbances at 520 nm for mixtures solutions in the ratio of $V(\text{NO}_2^-) : V(\text{indole}) = 1 : 9$ to $9 : 1$ at $c(\text{HCl}) = 0.5 \text{ mol dm}^{-3}$ and $t = 900 \text{ s}$

Hence, it is obvious that the stoichiometry can be evaluated by the simple expedient of plotting A vs. $(V(\text{NO}_2^-)/V(\text{indole}))$ (see Fig. 6) and obtaining the maximum of the plot [15]. According to the appearance of the Job's curve it could be concluded that only one product in the system was formed. The obtained curve has an obviously marked maximum at the ratio $V(\text{NO}_2^-) : V(\text{indole}) = 4:6$, $c(\text{B}) : c(\text{A}) = 1:1$.

Determination of molar absorptivity and reaction constant

The equation for a chemical equilibrium



where the capital letters represent the participating chemical species, and the lower-case italic letters are the small integers needed to balance the equation. This equation states that x mol of A (indole) reacts with y mol of B (3-nitrosylindole) and form

z mol of AB. The equilibrium constant expression for this reaction is

$$K_p = \frac{c(\text{AB})}{[c(\text{A}) - c(\text{AB})]^{n+1} \cdot n^n} \quad (1)$$

where $c(\text{AB})$ is the concentration of the product, $c(\text{B})$ is the concentration of the 3-nitrosylindole and n is stoichiometric ratio. The molar absorptivity ε_p of the product is naturally unknown, so that c cannot be evaluated directly. But c is related to ε_p by

$$c = \frac{A}{\varepsilon \cdot l} \quad (2)$$

where l is the length of the cell used in the measurements. The molar absorptivity ε_p value is calculated, using equations

$$\varepsilon_p = \frac{1}{l} \left(\frac{A_i}{c_i} + B \frac{A_i - b \cdot A_j}{c_i(b - B)} \right) \quad (3)$$

$$\frac{c_i}{c_j} = b \quad (4)$$

$$B = \left(\frac{A_i}{A_j} \right)^{\frac{1}{n+1}} \quad (5)$$

The lower-case italic letters x , y and z from the chemical equation are not used for calculation of K_p .

The equation (6) indicates that a plot of $\frac{c(B) \cdot l}{A} \cdot \text{vs} \cdot \frac{1}{\sqrt[n+1]{A^n}}$ should be linear, with slope Q and intercept $\frac{1}{\varepsilon_p}$. The fact that such a plot is linear confirms the conclusion that reaction products has the previously given formula and permits calculation of ε_p and K_p .

$$\frac{c(B) \cdot l}{A} = \frac{1}{\varepsilon_p} + Q \frac{1}{\sqrt[n+1]{A^n}} \quad (6)$$

The Q value is useful for calculation of K_p (Table 1)

$$K_p = \frac{l^n}{n^n \cdot \varepsilon_p \cdot Q} \quad (7)$$

Table 1

K_p values for reaction of indole and 3-nitrosylindole in hydrochloric acid solution

$A + B \rightleftharpoons AB$	
$^a K_p / \text{dm}^{-6} \text{ mol}^{-2}$	$1.0 \cdot 10^5$
$^a \varepsilon_1 / \text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$	$5.4 \cdot 10^3$
$^b K_p / \text{dm}^{-6} \text{ mol}^{-2}$	$4.64 \cdot 10^4$
$^b \varepsilon_1 / \text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$	$3.9 \cdot 10^3$
$^a r$	0.9735
$^a n$	1

^aObtained by the least squares treatment of

$$\frac{c(B) \cdot l}{A} \text{ vs } \frac{1}{\sqrt[n+1]{A^n}}$$

^bNumerical calculation, Ref. [16, 17]

Calibration curves and Beer's law

For spectrophotometric determination of some indoles, examination of the stability of the indoles in various acidic media are reported [11–13]. Various amounts of indole were added to a sample (natural water) and the RSD during the indole determination was checked. Beer's law is obeyed at 530 nm for indole in the concentration range of 14 – 36 $\mu\text{g cm}^{-3}$. The calibration curve from absorbances and concentration of indole was linear ($r = 0.996$).

The slope and intercept were small and identical to those of a calibration graph obtained with deionised water. The detection limit of indole with this method is 8.0 $\mu\text{g cm}^{-3}$. A standard deviation of $\pm 2.5 \mu\text{g cm}^{-3}$ was calculated from a series of 10 replicated measurements for the indole concentration of 21 $\mu\text{g cm}^{-3}$. The small standard deviation of the individual observation and the paired curves are an index of the rather satisfactory precision (Table 2) of the method. Since the sample of natural water did not contain indole at all, we added different volumes of indole solution.

Table 2

The results for indole in the deionised and natural water

Indole	$\gamma_{\text{indole}} / \mu\text{g cm}^{-3}$		
	Added	Found	RSD %
Sample (deionised water)	14.04	14.41	2.6
	16.38	15.57	4.9
	18.72	18.05	3.5
	21.06	21.44	1.8
	23.40	24.36	4.1
	25.74	25.70	0.8
	28.08	29.02	3.3
	32.76	32.58	0.9
Sample (natural water)	35.10	34.42	1.9
	11.00	11.52	4.7
	13.20	12.53	5.2
	15.40	15.18	1.4
	17.60	17.39	1.7
	19.80	19.13	3.3
	22.00	21.90	0.4

Interference's. No interferences are caused by some inorganic ions (Table 3) and indoles: 3-methylindole, 3-acetylindole, 3-formylindole, D-tryptophan, indolyl-2-carboxylic acid, 3-(3-propionic acid), 4-(3-indolyl)butyric acid. The addition of these indoles do not affect the sensitivity of the method. However, milligram amounts (above 20 mg cm⁻³) of 3-IAAH interfered. The identity of the product of reaction of IAAH with HNO₂ had been previously published by Kawana *et al.* [22] and by Acheson *et al.* [23]. Although it is familiar that concentration limits of the Ehrlich's spectrophotometric method are close to determined concentrations of indole in this paper, still Ehrlich's method is less selective due to the mentioned indole derivatives. Additionally from the economic points of view, the expenses for Ehrlich's benzaldehyde reagent (EBR) could affect on the decision for substitution of this method with others. Thus, it

can be concluded that, the proposed method is satisfactory for the rapid practical analysis of different types of water samples. This method is more selective than the other spectrophotometric methods reported for indoles in water.

Table 3

Effect of diverse inorganic ions

Tolerance limit	Diverse inorganic ions
< 0.001 mol dm ⁻³	Na(I), K(I), Ca(II) Mg(II), Sr(II), Fe(II), Fe(III), HCO ₃ ⁻ , CO ₃ ²⁻ , Cl ⁻ , SO ₄ ²⁻ , NO ₃ ⁻ , SiO ₃ ²⁻ , O ₂ , Cl ₂

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Резиме

**ИСПИТУВАЊЕ НА РЕАКЦИЈАТА НА ИНДОЛ СО НИТРИТНИ ЈОНИ ВО РАСТВОРИ
ОД ХЛОРОВОДОРОДНА КИСЕЛИНА СО ПРИМЕНА НА СПЕКТРОФОТОМЕТРИЈАТА**

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и. фак 162, МК-1001 Скопје, Република Македонија***Клучни зборови:** UV/Vis спектри; реакционен продукт; определување; индол

UV/Vis-спектрофотометријата се користи за истражување на повеќе типови хемиски реакции, на пример киселинско базни реакции, реакции на комплексирање или редокс-реакции. Истражувањата на хемиските реакции за квантитативно определување на некои индоли се интензивни затоа што некои од нив можат да бидат вклучени во отпадните води.

Во презентираната работа е истражувана реакцијата на нитрозирање на индол во раствори од хлороводородната киселина и вода-етанол.

Ефектите на независната варијација на концентрацијата на хлороводородните раствори од 0,01 до 2,0 mol dm⁻³ или на нитритни јони од 1,0·10⁻⁵ mol dm⁻³

до 3,0·10⁻³ mol dm⁻³ спрема индол беа следени на основа на изгледот на UV/Vis-спектрите и константата на стабилност на реакциониот продукт.

Киселите раствори од нитрозираниот продукт покажуваат максимална апсорбанца (широка лента) на 500–530 nm и еден втор максимум на бранова должина во интервал од 400–450 nm. Со методот на Job и метод на моларен однос беше најдено дека стехиометрскиот однос помеѓу индол и 3-нитрозилиндол изнесува 1:1. Положбата на групата (NO) во формираното соединение беше на атомот 3-С кај индолот (во молекулата од индол црвено).