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## Study of 1,4-naphthoquinones Properties by Spectroscopic Methods

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**Abstract.** The quinone fragment is not only quite common for many synthetic and natural compounds, but also has a connection with the occurrence of various types of biological activity. This fact is related to their ability to accept one or two electrons with the formation of the corresponding radical anion or radical dianion. Since the electron-acceptor ability of quinone can be changed by the direct addition of the substituent to its structure, the analysis of the spectral characteristics of alkyl- or arylamino derivatives of 1,4-naphthoquinone is of great interest. In this work, the spectral characteristics of 2,3-dichloro-1,4-naphthoquinone (NQ) and compounds synthesized on its basis, namely, 2-chloro-3-((4-hydroxyphenyl)amino)-1,4-naphthoquinone (NQ1) and 4-((3-chloro-1,4-naphthoquinon-2-yl)amino)phenyl sulfurofluoridate (NQS) were studied by spectrophotometry. The influence of pH on the intensity and absorption maximum wavelength of the analyzed compounds was studied. Under selected working conditions linear dependences of absorbance on concentration in the ranges  $3 \cdot 10^{-6} - 2 \cdot 10^{-5}$ ;  $3 \cdot 10^{-7} - 2 \cdot 10^{-6}$  and  $2 \cdot 10^{-7} - 2 \cdot 10^{-6}$  M were obtained with the regression equations  $y = 0,0417x + 0,0477$  ( $R^2 = 0,9993$ );  $y = 0,389x + 0,0095$  ( $R^2 = 0,9997$ ); and  $y = 0,3215x + 0,0452$  ( $R^2 = 0,9997$ ) for NQ, NQ1 and NQS respectively. A method of quantitative determination of compounds in the substance by spectrophotometry was developed with the detection limits (LOD) of NQ, NQ1 and NQS equal to  $8,8 \cdot 10^{-7}$ ,  $9,4 \cdot 10^{-8}$  and  $7,3 \cdot 10^{-8}$  M, respectively. The accuracy of the proposed technique was verified by the standard addition method method.

**Keywords:** spectrophotometry, 2,3-dichloro-1,4-naphthoquinone, 2-chloro-3-((4-hydroxyphenylamino)-1,4-naphthoquinone, 4-((3-chloro-1,4-naphthoquinon-2-yl)amino)phenyl sulfurofluoridate.

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## Изучение свойств 1,4-нафтохинонов спектроскопическими методами

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**Аннотация.** Органические соединения, имеющие в своей структуре хиноновый фрагмент, достаточно часто встречаются во многих синтетических и природных объектах. Их биологическая активность связана со способностью принимать один или два электрона с образованием соответствующего анион-радикала или дианион-радикала. Так как электроакцепторная способность хинона может быть изменена добавлением заместителя в его структуру, анализ спектральных характеристик алкил- или ариламинопроизводных 1,4-нафтохинона представляет большой интерес. В данной работе методом ИК-спектроскопии были получены спектральные характеристики 2,3-дихлор-1,4-нафтохинона (NQ) и синтезированных на его основе соединений, а именно 2-хлор-3-((4-гидроксифенил)амино)-1,4-нафтохинона (NQ1) и 4-((3-хлор-1,4-нафтохинон-2-ил)амино)-фенилсульфотридата (NQS), а также методом спектрофотометрии изучены спектры поглощения и описаны оптические свойства этих соединений. Проведено исследование влияния pH раствора на интенсивность и длину волны максимума поглощения исследуемых соединений. При подобранных рабочих условиях получены линейные зависимости интенсивности оптической плотности от концентрации в диапазонах  $3 \cdot 10^{-6} - 2 \cdot 10^{-5}$ ;  $3 \cdot 10^{-7} - 2 \cdot 10^{-6}$  и  $2 \cdot 10^{-7} - 2 \cdot 10^{-6}$  М с уравнениями регрессии  $y = 0,0417x + 0,0477$  ( $R^2 = 0,9993$ );  $y = 0,389x + 0,0095$  ( $R^2 = 0,9997$ ); и  $y = 0,3215x + 0,0452$  ( $R^2 = 0,9997$ ) для NQ, NQ1 и NQS соответственно. Разработана методика количественного определения соединений в субстанции методом спектрофотометрии с пределами обнаружения (LOD) NQ, NQ1 и NQS, равными  $8,8 \cdot 10^{-7}$ ,  $9,4 \cdot 10^{-8}$  и  $7,3 \cdot 10^{-8}$  М соответственно. Правильность методики количественного определения анализируемых соединений в субстанции проверялась методом «введено-найдено».

**Ключевые слова:** спектрофотометрия, 2,3-дихлор-1,4-нафтохинон, 2-хлор-3-((4-гидроксифенил)амино)-1,4-нафтохинон, 4-((3-хлор-1,4-нафтохинон-2-ил)амино)-фенилсульфотридат.

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## Introduction

A large number of synthetic and natural organic compounds containing quinone fragment in their structure have various types of biological activity, namely, they exhibit anti-inflammatory, antituberculosis, antimalarials, antitumor properties, etc. [1–4].

1,4-Naphthoquinones are the most important representatives of the quinone series and are used in modern manufacturing in various industries ranging from pharmaceuticals to dyes [5, 6].

Such methods as absorption spectrophotometry and/or electrochemistry (polarography and voltammetry) are used to evaluate the influence of structural changes on the reactivity of 1,4-naphthoquinones. This is due to the presence of electronic effects induced by substituents of the quinone nucleus, which can cause noticeable shifts in the half-wave potential values or affect the mechanism, reversibility and/or rate of electrode processes [7].

The properties and functions of 1,4-naphthoquinones are closely related to their redox potential, which allows them to participate in electron transport within the cell membrane. Therefore, the study of the influence of donor and acceptor properties of substituents on the electronic properties of compounds may provide a key to understanding the mechanisms of their biological activity. Such information will be useful for the design of new molecules with greater and more specific biological activity.

2,3-dichloro-1,4-naphthoquinone (NQ) (Fig. 1) was chosen as the basic compound on the basis that it is sufficiently stable, readily available and also known as a key raw material in organic, medicinal and industrial chemistry. NQ (trade names are “Dichlone” and “Phygon”) is a broad-spectrum fungicide containing a quinone group and belongs to the class of aromatic diketones in which the carbonyl groups form a part of a ring system. This pesticide can be used for fruits, vegetables, agricultural crops, garden plants treatment, and for residential and commercial outdoor application [8, 9].

New derivatives of 1,4-naphthoquinone, namely 2-chloro-3-((4-hydroxyphenyl)amino)-1,4-naphthoquinone (NQ1) and 4-((3-chloro-1,4-naphthoquinon-2-yl)amino)phenyl sulfurofluoridate (NQS), were synthesized based on NQ (Fig. 1) [10, 11]. New synthesized compounds are suspected to have cytotoxic activity.

This work is devoted to investigation of the optical properties of new 1,4-naphthoquinone derivatives by spectroscopic methods and development a spectrophotometric technique for the determination of NQ, NQ1 and NQS in the substance. Spectrophotometry is considered one of the most convenient analytical methods due to its inherent simplicity, low cost and wide availability in most quality control systems and clinical laboratories [12–14].

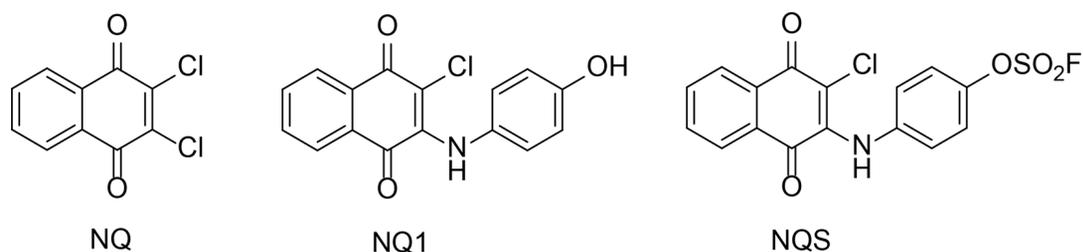


Fig. 1. Chemical structures of NQ, NQ1 and NQS

## Instruments, materials and research methods

### *Instruments*

The infrared absorption spectra of the synthesized compounds were recorded on Agilent 660 FTIR spectrometer in a KBr disk.

For pH measurement the pH-meter/ionomer “ITAN” (“Tomanalyt”, Tomsk) was used.

The absorption spectra of the studied compounds were recorded in quartz cuvettes with an optical path length of 1 cm using Cary60 spectrophotometer.

### *Reagents*

2,3-Dichloro-1,4-naphthoquinone (NQ) is a commercial compound manufactured by Sigma-Aldrich, USA (CAS 117–80–6).

2-Chloro-3-((4-hydroxyphenyl)amino)-1,4-naphthoquinone (NQ1) and 4-((3-chloro-1,4-naphthoquinon-2-yl)amino)phenyl sulfurofluoridate (NQS), were synthesized according to the method described in the work [15].

Potassium bromide (KBr) was used to obtain infrared absorption spectra.

Analyzed compounds were dissolved in 1 cm<sup>3</sup> of dimethylformamide (DMF), transferred into 50 cm<sup>3</sup> volumetric flasks and brought to the mark with 96 % ethanol. Despite the good solubility of the analyzed substances in DMF, the absorption spectra in this solvent were characterized by low intensity and a wide range of absorption. Therefore, it was decided to use DMF as a solvent and 96 % ethanol as a background solution.

The required pH value was reached using 1 M hydrochloric acid (HCl) and 1 M sodium hydroxide (NaOH)

### *Experimental procedure*

#### *IR spectroscopy*

A small amount of KBr was ground in an agate mortar until creamy, then the analyzed compound was added and the grinding was continued by stirring thoroughly. The sample was then quantitatively transferred into tablet press. The resulting tablet was transferred into an Agilent 660 FTIR spectrometer and the IR spectrum was recorded.

#### *Spectrophotometry*

Solutions of analyzed compounds with concentration range from  $5 \cdot 10^{-6}$  to  $2 \cdot 10^{-5}$  mol l<sup>-1</sup> were prepared in 50 cm<sup>3</sup> volumetric flasks. 3 cm<sup>3</sup> of 96 % ethanol was placed in 1 cm quartz cuvette as a blank solution. After proving the purity of the blank solution, the absorption spectra of the analyzed compounds were taken in the wavelength range 200–800 nm with an error of  $\pm 0.1$  nm and the absorption maximum wavelength was recorded.

## Results and discussion

The use of IR spectroscopy for nondestructive analysis of both biological samples and pharmaceuticals is actively used in many studies. Fig. 2 shows the IR spectra of the analyzed compounds in KBr.

Since all the studied compounds have a benzene fragment in their structure, IR spectroscopy showed characteristic absorption bands for the phenyl group: valence ( $3100\text{--}2950$  cm<sup>-1</sup>), in-plane

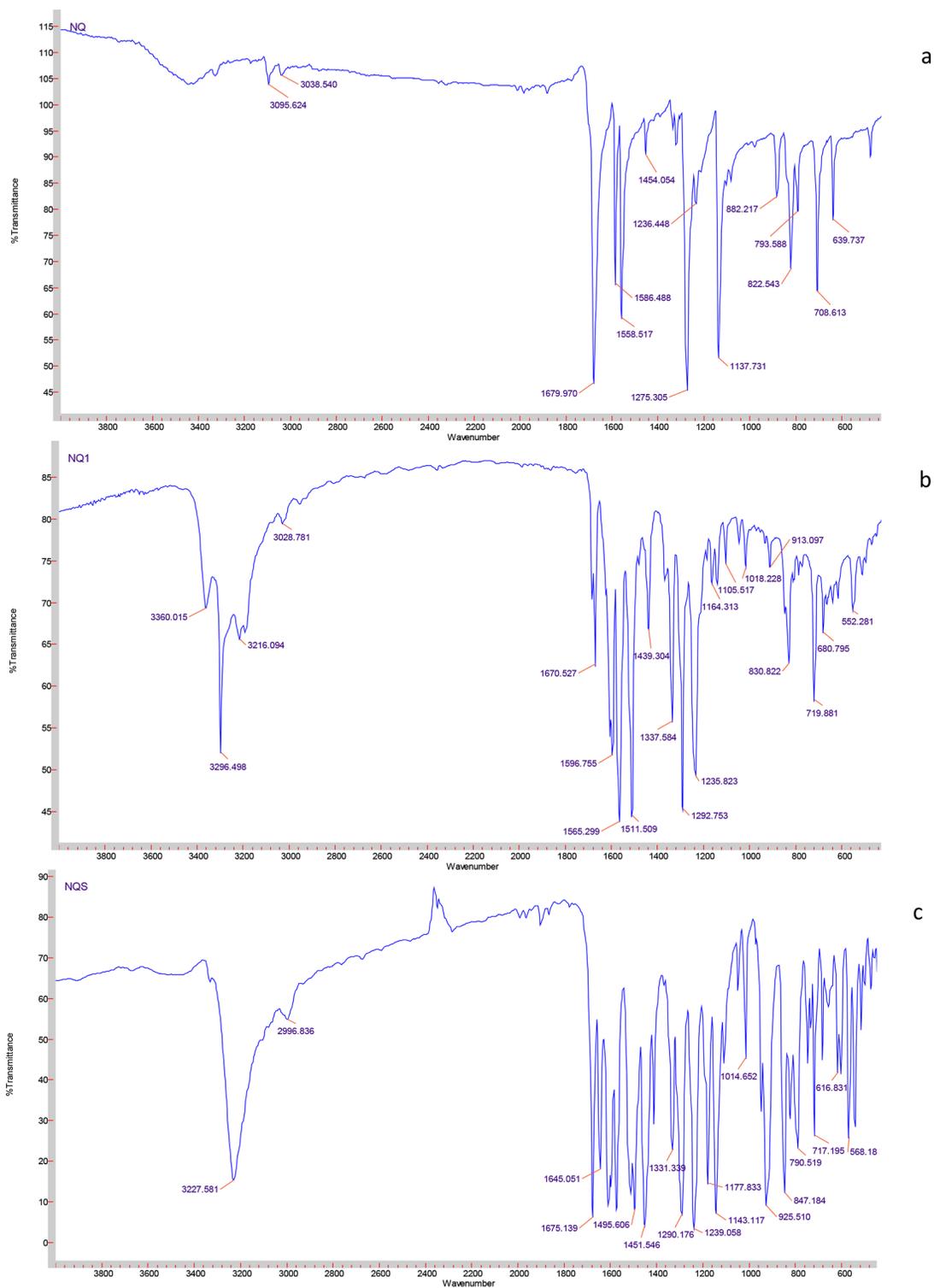


Fig. 2. IR spectra a) NQ b) NQ1 c) NQS in KBr

(1300–1000  $\text{cm}^{-1}$ ) and out-of-plane deformation (900–675  $\text{cm}^{-1}$ ) C-H vibrations, as well as overtones 2000–1650  $\text{cm}^{-1}$  and skeletal vibrations of the C–C cycle (1600–1585 and 1500–1400  $\text{cm}^{-1}$ ). In addition, the absorption bands caused by valence and deformation vibrations of C–CO-C (1300–1200  $\text{cm}^{-1}$ ), valence vibrations of C=O with intense absorption in the region of 1870–1540  $\text{cm}^{-1}$ , and valence vibrations of the C–Cl bond in the region of 1100–1040  $\text{cm}^{-1}$  are common for all analyzed substances.

For compounds NQ1 and NQS, absorption bands caused by valence (3350–3310  $\text{cm}^{-1}$ ), deformation (1550–1450  $\text{cm}^{-1}$ ) and wagging (909–666  $\text{cm}^{-1}$ ) vibrations of the N-H bond as well as an absorption band caused by valence vibrations of the C-O bond in the region of 1260–1000  $\text{cm}^{-1}$  are observed.

For NQ1 it is also worth noting the appearance of absorption bands due to valence (3350–3330  $\text{cm}^{-1}$ ) and plane deformation (1420–1330  $\text{cm}^{-1}$ ) vibrations of the O-H bond.

NQS is characterized by two intense absorption bands in the region of 1350–1300 and 1160–1120  $\text{cm}^{-1}$  due to antisymmetric and symmetric valence vibrations of the  $\text{SO}_2$  group, respectively, and vibrations in the region starting at 1000  $\text{cm}^{-1}$  from the S-F bond.

*Investigation of optical properties of the analyzed compounds  
by spectrophotometry*

Absorption spectra of the analyzed compounds in the wavelength range 200–800 nm were recorded (Fig. 3).

When considering the UV absorption spectra of the investigated compounds, their similarity can be observed. For example, all the investigated compounds exhibit an absorption band in the

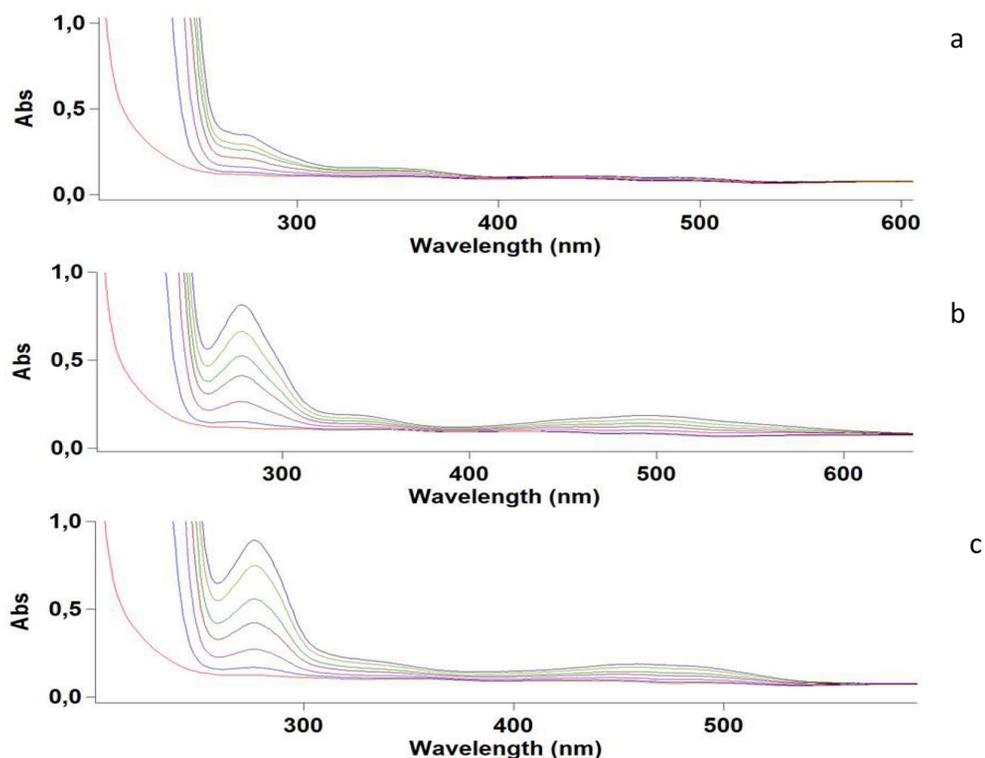


Fig. 3. Absorption spectra a) NQ b) NQ1 c) NQS in 96 % ethanol with concentrations from  $5 \cdot 10^{-6}$  to  $2 \cdot 10^{-5}$   $\text{mol l}^{-1}$

range of 250–280 nm, which corresponds to the intense  $\pi \rightarrow \pi^*$  electronic transition in benzene and naphthoquinone. The presence of a benzene fragment in the structure of the compounds is confirmed by the presence of characteristic absorption bands of the phenyl group as described earlier. Additionally, a weak  $n \rightarrow \pi^*$  transition band is observed in the longer wavelength region of 330–350 nm.

The introduction of substituents into the benzene ring that are conjugated with the  $\pi$ -system of the aromatic ring ( $-\text{NH}_2$ , absorption bands at 3350–3310  $\text{cm}^{-1}$  are typically associated with the region where vibrational bonds N-H of the primary amino group manifest), noticeably influences the intensity and position of these absorption bands. Specifically, it leads to a bathochromic shift of these absorption bands and increases their intensity. This phenomenon is observed for compounds NQ1 and NQS.

For NQ1 and NQS, a wide but not very intense absorption band in the visible region, between 470 and 500 nm, is characteristic. This absorption band is typical for amino-substituted quinones and can be attributed to charge transfer electronic transitions and weak  $n \rightarrow \pi^*$  electronic transitions of the carbonyl group in the quinone.

In the derivatives under consideration, this absorption is significantly shifted to the visible region compared to the spectrum of 1,4-naphthoquinone. Indeed, upon adding substituted aniline to 1,4-naphthoquinone, a bathochromic shift of this absorption band is observed with changes in intensity and width [16].

Also, it is worth noting that the direct influence on this absorption band is exerted by the modification of substituents. For example, upon introducing a fluorine and sulfur atom into the NQS compound (two intense absorption bands in the range of 1350–1300 and 1160–1120  $\text{cm}^{-1}$  for the  $\text{SO}_2$  group, and an absorption band at 1000  $\text{cm}^{-1}$  for the S-F bond), a shift towards shorter wavelengths was observed due to the electron-withdrawing effect of the substituents.

#### *Investigation of pH influence of the blank solution on the optical properties of analyzed compounds*

The pH value of the blank solution has a significant influence on the absorption peak position and its maximum intensity. The intensity of the absorption peak with a maximum wavelength 280 nm decreases with increasing pH (alkaline medium). Maximum intensity value is observed at pH = 4 for all three compounds. Acidification of the solution does not cause significant changes of the signal intensity. It should also be noted that there is no influence of pH on the absorption maximum wavelength, its shift occurs at  $\pm 2$  nm, which cannot indicate a bathochromic shift when the pH of the solution is changed [17].

Due to the fact that the absorption peak at a wavelength 280 nm has a more pronounced shape and intensity, it was chosen as the analytical signal. Analytical procedures for quantitative determination of the studied compounds were developed individually for NQ, NQ1 and NQS due to significant overlap of their absorption spectra.

After selecting the optimal pH value for the determination of NQ, NQ1 and NQS, the calibration curves were plotted (Fig. 4).

Quantitative characteristics of NQ, NQ1 and NQS determination in model solutions are presented in Table 1.

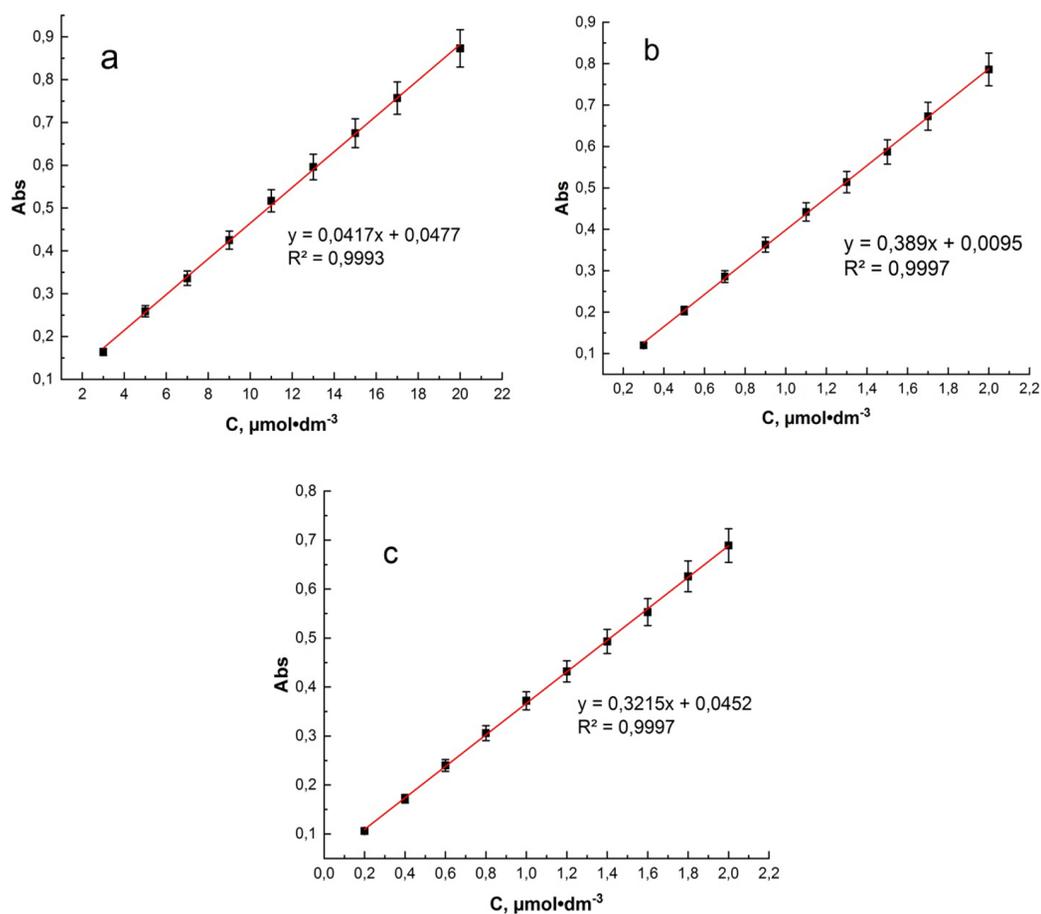


Fig. 4. Dependence of absorbance on the concentration a) NQ b) NQ1 c) NQS in 96 % ethanol (pH = 4)

Table 1. Quantitative characteristics of NQ, NQ1 and NQS determination in model solutions (n=5; P=0,95)

Compound	Wavelength, nm	Regression equation	Linear concentration range, $\text{mol}\cdot\text{l}^{-1}$	Limit of detection LOD, $\text{mol}\cdot\text{l}^{-1}$
NQ	277	$y = 0,0417x + 0,0477$ $R^2 = 0,9993$	$3\cdot 10^{-6} - 2\cdot 10^{-5}$	$8,8\cdot 10^{-7}$
NQ1	280	$y = 0,389x + 0,0095$ $R^2 = 0,9997$	$3\cdot 10^{-7} - 2\cdot 10^{-6}$	$9,4\cdot 10^{-8}$
NQS	278	$y = 0,3215x + 0,0452$ $R^2 = 0,9997$	$2\cdot 10^{-7} - 2\cdot 10^{-6}$	$7,3\cdot 10^{-8}$

The graphs are linear in the reported concentration ranges. The accuracy of the proposed method for quantitative determination of NQ, NQ1 and NQS in the substance was verified by the standard addition method (Table 2).

Table 2 shows that the data obtained by the spectrophotometry are satisfactory. Therefore, it can be concluded that the method can be used for the determination of analyzed compounds in substances.

Table 2. Quantitative determination of NQ, NQ1 and NQS in substance (n=5; P=0,95)

Compound	Added, mol l <sup>-1</sup>	Found (mol l <sup>-1</sup> )	Sr (RMS)	t <sub>calc</sub>	t <sub>table</sub>
NQ	5·10 <sup>-6</sup>	5,9·10 <sup>-6</sup>	0,25	0,78	2,78
	1·10 <sup>-5</sup>	1,06·10 <sup>-5</sup>	0,19	0,52	
	1,5·10 <sup>-5</sup>	1,54·10 <sup>-5</sup>	0,17	0,38	
NQ1	5·10 <sup>-7</sup>	6,1·10 <sup>-7</sup>	0,15	0,1	
	1·10 <sup>-6</sup>	1,08·10 <sup>-6</sup>	0,13	0,07	
	1,5·10 <sup>-6</sup>	1,55·10 <sup>-6</sup>	0,12	0,04	
NQS	5·10 <sup>-7</sup>	6,13·10 <sup>-7</sup>	0,13	0,1	
	1·10 <sup>-6</sup>	1,08·10 <sup>-6</sup>	0,12	0,07	
	1,5·10 <sup>-6</sup>	1,56·10 <sup>-6</sup>	0,1	0,05	

### Conclusions

In this work the spectral characteristics of the analyzed compounds such as IR and absorption spectra were obtained and described. The optimum pH value 4.0 for the quantitative determination of the compounds was selected. A method for spectrophotometric determination of NQ, NQ1 and NQS in substances with detection limit of NQ, NQ1 and NQS  $8,8 \cdot 10^{-7}$ ,  $9,4 \cdot 10^{-8}$  and  $7,3 \cdot 10^{-8}$  M, respectively, was developed.

### Competing of interests

The authors declare the absence a conflict of interest warranting disclosure in this article.

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