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Quantitative determination of essential oil terpenoids by the reaction of epoxidation with peroxy decanoic acid

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ABSTRACT

The iodometric titration method was used to study the kinetics of some individual terpenoids epoxidation reactions (α-pinene, limonene, limonene oxide), and the medical-grade turpentine epoxidation with the peroxy decanoic acid in the methylene chloride medium at 297-298 K. The kinetics of the reaction was shown to be governed by the second order kinetic equation. The second order effective constants of the reaction rate were determined: for α-pinene – 3.6 L mol^{-1} min^{-1} (297 K), limonene (to epoxide) – 2,1 L mol^{-1} min^{-1} (295 K), limonene oxide (to dioxide) – 0.052 L mol^{-1} min^{-1} (295 K), for turpentine – 3.9 L mol^{-1} min^{-1} (297 K). The proposed techniques of the quantitative determination of the basic substance content and determination of the iodine number of some terpenoids (α-pinene, limonene, limonene oxide) and the (medical-grade) gum turpentine were based on the data of epoxidation with the peroxy decanoic acid in the methylene chloride medium. The extraction-titrimetric technique was practiced and the possibility of quantitative determination of the (medical-grade) gum turpentine in turpentine ointment 10% (RSD = 3.2%, δ = -1.15%) was demonstrated.

Keywords: *terpenoids (α-pinene, limonene, limonene oxide), turpentine ointment, peroxy decanoic acid, iodometric method, quantitative determination.*

INTRODUCTION

Essential oils (lat. *Olea aetherea*) are the products of the natural, mainly vegetable, origin, – complex multicomponential mixtures of volatile odorants. They belong to different classes of organic compounds, among which mono– and sesquiterpenoids dominate; aromatic and aliphatic compounds are also encountered. The chemical composition of essential oils is rather variable even within one species of plant, and depends upon the area of their growth, climatic conditions, vegetative phase, oil extraction technology, and other factors. Terpenoids and their derivates, being the oil constituents, are presented by compounds of diverse structure: saturated and polyunsaturated, acyclic and cyclic, and oxygen-containing (alcohols, aldehydes, ketones, oxides, esters, lactones, quinones etc.). Moreover, substances of the aliphatic series, aromatic compounds (phenols, phenyipropan derivates), sulfides and nitrogen-containing compounds may dominate in ester oils of some plants [1]. The current widespread use of essential oils both in the natural form and in the form of preparations is based on their application in the therapy (including aromatherapy) of various diseases, specificity of the composition and properties, relation to autoxidation demand strict regulation and evaluation of their quality performance, obligatory finding of the shelf life and control of the storage conditions. To identify oils and eithers a relative value of the certain components retention time, or comparison of chromatographic profiles with the reference chromatogram are used. Also, identity of the oils can be determined by the thin-layer chromatography (TLC), and if needed – using chromatography-mass spectrometry or other instrumental methods (UV-, IR-, NMR– spectroscopy etc.) [2, 3]. The quality control techniques shall include description of the method of quantitative determination of one or several dominant (mainly representative) and specific (attributable to essential oil) components and the prescribed standards of their content. Under the conditions, when the reference samples of individual components are available, the use of gas-liquid chromatography for their

quantitative determination in oil becomes possible simultaneously with their identity verification [3, 4]. At the same time, the important characteristic of the essential oils' quality is the value of the characteristic quantity of the so called numbers. The acidity numbers, ester numbers and saponification number are used to characterize essential oils. Thus, the primary sign of the essential oil adulteration with fatty acids are high values of the ester numbers [2]. At the same time, to our opinion, among the numbers to be determined there can be an important physicochemical parameter of the ester oil quality characteristic – the so called Iodine value (IV), describing the unsaturation degree of the essential oil components, and making it possible not only to confirm the oil quality evaluation, based on findings of the instrumental techniques (additionally verify its identity using an indirect method), prevent it's adulteration or deterioration during storage, but also to perform quantitative determination of the basic (one or several) components of the product or an individual natural odorant, extracted from oil. However, determination of iodine values when characterizing essential oils is not customary. Substances of isoprene structure, found in essential oils, display a very high activity in reactions of substitution by halogens, and are also oxidazible (e.g., aldehyde groups) by halogens. Evidently, it results in overconsumption of halogens, and, subsequently, in obtaining overstated – untrue results of the analysis as to the oil unsaturation degree.

We think that the method based on the epoxidation reaction is the prospective method of determining the degree of unsaturation of the essential oil terpenoids.

The presented paper shows the possibility to use the method of epoxidation with the aid of a relatively stable higher peroxy carboxylic acid – the peroxy decanoic acid, to determine the degree of unsaturation of some essential oils, the most common in medicine. We believe that determination of the iodine value of the essential oils, like that of fatty acids, by the indirect method based on the epoxidation reaction with subsequent conversion into the iodine value, is a rather valuable quantitative characteristic of assessment of the quality of the oils under test, along with the acidity number and the ester number. Besides, taking into account that presently there are no methods for quantitative determination of a series of essential oils, the titrimetric technique proposed by us can be accepted as a basis of the method of quantitative determination of the essential oils like terpenic oil and the medical grade turpentine both individually, and as a component of ointments [2, 3]. The turpentine is used topically in ointments and liniments as an irritant analgesic and aseptic agent for treatment of neuralgias, myositis, and rheumatism. It is used in the form of inhalations for purulent bronchitis, bronchiectasis etc. Pinene is the main component of turpentine (up to 65%). The essential oils and drugs produce a distracting, antiinflammatory, antibacterial [5] and topical irritant effect. They are used for neuralgia, myositis, lumbodynia and rheumatism [6]. We developed a relatively simple technique of the quantitative determination of the turpentine content in 10% turpentine ointment (Unguentum Terebinthinae) composed of:

Turpentine Ointment

Turpentine	10.0
Emulsifier No.1	4.0
Na – carboxymethyl cellulose	0.5
Purified water	100.0

EXPERIMENTAL SECTION

Essential oil compounds, **Chemicals and Reagents**

The items under test were medical-grade turpentine and terpenoids being its basic constituents: refined gum turpentine (or terpene oil, Oleum Terebinthinae rectificatum), a yellowish liquid, boiling T 153-180ºC; d = 860 g L^{-3}, acidity number < 0.7; obtained by stripping a volatile portion of pine pitch (*Pinus silvestris L.)*. It is a mixture of the terpenic carbonhydrates (α-pinene 60–65%, Δ^3-carene 10–12%, limonene 9–10%, α-tenpinene 5–6%, β-pinene 2–3%, camphene 2–3%, γ-terpinene 1–2%). The terpenic immersion oil, manufactured by ″Lubnyhimpharm″, S/N 50791. (–)-α-pinene 99.2 % (according to the data of HPLC), optically pure, d = 874 g L^{-3}, Sigma-Aldrich; (*S*)-(-)-Limonene 96% (according to the data of HPLC), d = 844 g L^{-3}, Sigma-Aldrich; (–)-α-pinene oxide 97.5 % (according to the data of HPLC), Sigma-Aldrich; (+)-Limonene oxide, mixture of *cis* and *trans*-isomers, 98.3% according to the data of gas-liquid chromatography).

Peroxy decanoic acid (peroxy capric acid), H_3C-$(CH_2)_8CO_3H$, 188 g mol^{-1}, 98.6% (based on the data of iodometric titration after recrystallization from hexane), obtained according to D. Swern's procedure [7].

Acetate acid, chemically pure. The acetate acid content is not less than 98%.

Methylene chloride, analytically pure, reagent grade, LAB-SCAN, Ireland. It was additionally purified according to procedure [8], sealed in ampoules and stored in a place protected from light at 8-10ºC.

Preparation of 29.5-30.5% acetic acid solution
The diluted acetate acid: 31.3 pts. wt. of the acetic acid and 68.7 pts. wt. of distilled water are mixed.

Preparation of 5% potassium iodide
5.0 g of potassium iodide are dissolved in the water just boiled and cooled, and the solution is filled up to 100 ml with distilled water. The solution should be colorless.

Preparation of standard sodium thiosulfate solution, $c(Na_2S_2O_3 \cdot 5H_2O)=0.1$ mol L^{-1}, was prepared of the standard-titre fixanal in distilled water in 1 L graduated flask, and the solution volume was filled up to the mark at 20°C.

75 mL cone flasks with stopper plugs were used to carry out the reaction. TC – 80M air bath oven was used for heating and maintaining the necessary temperature of the reaction mixture. IR spectra were taken using Specord M80 spectophotometer in a thin film.

The technique for study of the kinetics of terpenoids and turpentine epoxidation using the peroxy decanoic acid
About 0.1 g of the tested substance (correct hatch) is dissolved in a 75 mL cone flask with a stopper plug in 25.00 (or 20.00) mL of the methylene chloride, about 0.2 g (precise weight) of the peroxy decanoic acid is added, the flask is sealed, thoroughly shaken out, and the time count is started (a stopwatch timer is switched on).

1.00 mL of the obtained solution is taken with a pipette and added to a cone flask after a certain time interval, 4 mL of the diluted acetic acid and 1 mL of 5 % potassium iodide are added with strong shaking, and after that the released iodine is immediately titrated with the reference 0.1 mol L^{-1} sodium thiosulphate solution.

RESULTS AND DISCUSSION

The results of studying the kinetics of individual terpenoids, the medical-grade turpentine epoxidation with the peroxy decanoid acid in the methylene chloride medium at 295-298 K are shown on Fig. 1-7. They demonstrate that, regardless of the nature of the terpenoid and oxidizer, the kinetics of the reactions in the methylene chloride medium is governed by the second order kinetic equation. The quantitative interaction time and reaction stoichiometry were determined according to the kinetics data.

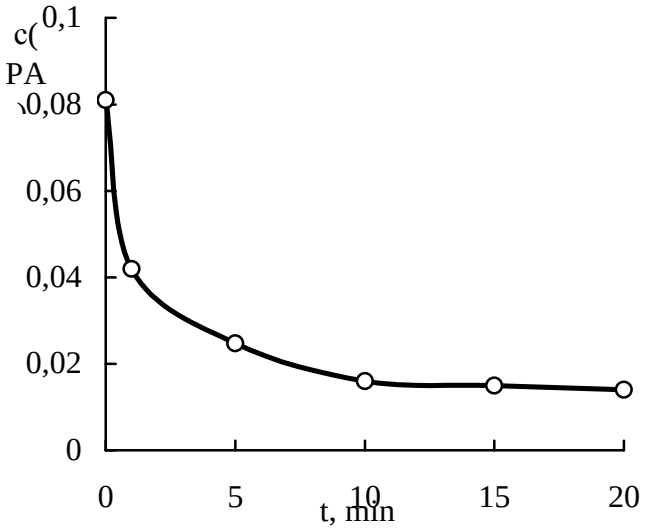

Fig. 1: The kinetic curve of α-pinene epoxidation with the peroxy decanoic acid in the methylene chloride medium at 297 K

It was determined that 1 mol of the peroxy acid is consumed per 1 mol of *α*-pinene. Based on stoichiometry 1 : 1 and the quantitative interaction time of 45 min, the basic substance content, w, in %, was:

$$w = \frac{(V_0 - V_1) \times 0.1 \times K \times M \times V \times 100}{2 \times m \times 1000} = \frac{(1.62 - 0.05) \times 0.1000 \times 136.23 \times 20 \times 100}{2 \times 0.2143 \times 1000} = 99.8\%.$$

$$IV = \frac{(V_0 - V_1)\times 0.1\times K\times 126.93\times V\times 100}{m\times 1000} = \frac{(1.62-0.05)\times 0.1000\times 126.93\times 20\times 100}{0.2143\times 1000} = 186.0$$

(theor.)=186.35

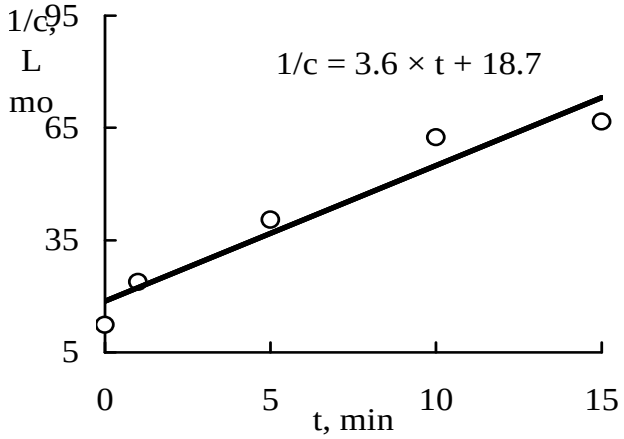

Fig. 2: The reverse concentration anamorphosis of the kinetic curve of *α-pinene* epoxidation with the peroxy decanoic acid. K_{ef} = 3.6 L mol^{-1} min^{-1} (297 K)

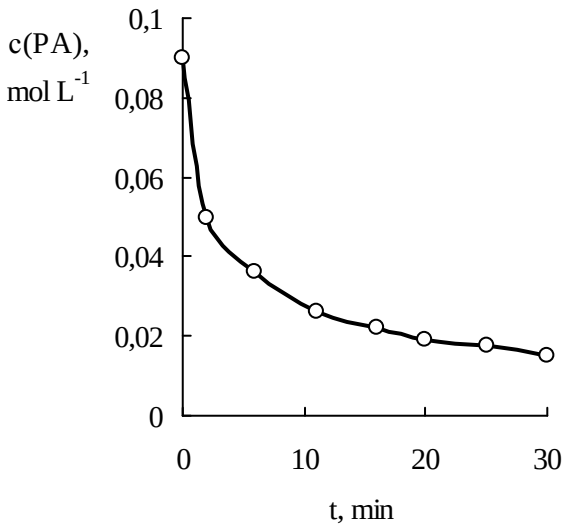

Fig. 3: The kinetic curve of limonene epoxidation with the peroxy decanoic acid in the methylene chloride medium at 295 K

Totally, 2 mol of the peroxy decanoic acid is consumed per 1 mol of limonene. Based on stoichiometry 1 : 1 – formation of limonene epoxide (the volume is found by cutting the extrapolation sections of the kinetic curves) within 5 min, the basic substance content, w, %, equaled:

$$w = \frac{(V_0 - V_1)\times 0.1\times K\times M\times V\times 100}{2\times m\times 1000} = \frac{(1.80-0.62)\times 0.1000\times 136.24\times 20\times 100}{2\times 0.1604\times 1000} = 100.2\%.$$

$$IV = \frac{(V_0 - V_1)\times 0.1\times K\times 126.93\times V\times 100}{m\times 1000} = \frac{(1.80-0.62)\times 0.1000\times 126.93\times 20\times 100}{0.1604\times 1000} = 186.7$$

or 373.5 (based on the data of stoichiometric ratio 1 : 2). IV (theor.)=372.7.

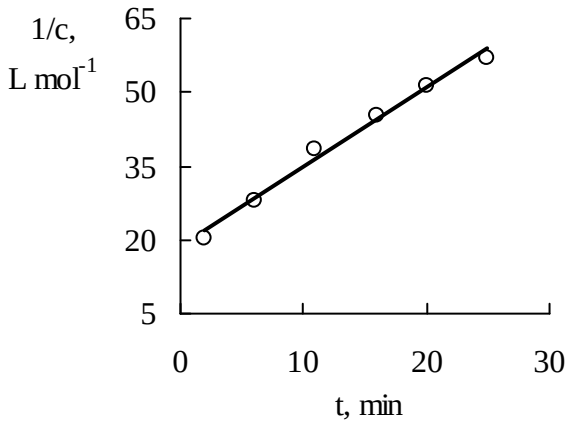

Fig. 4: The reverse concentration anamorphosis of the kinetic curve of limonene epoxidation. k_1=2.1 L mol^{-1} min^{-1} (295 K)

Based on the study of the kinetics of epoxidation by the iodometric titration, it was found that 1 mol of theperoxy decanoic acid was consumed per 1 mol of limonene epoxide (Quantitative interaction time 1.5 h at 295K).

The basic substance content, w, %, equaled:

$$w = \frac{(V_0 - V_1) \times 0.1 \times K \times M \times V \times 100}{2 \times m \times 1000} = \frac{(1{,}87 - 0{,}76) \times 0.1000 \times 152.24 \times 20 \times 100}{2 \times 0.1685 \times 1000} = 100.3\%.$$

$$IV = \frac{(V_0 - V_1) \cdot 0.1 \times K \times 126.93 \times V \times 100}{m \times 1000} = \frac{(1.87 - 0.75) \times 0.1000 \times 126.93 \times 20 \times 100}{0.1685 \times 1000} = 168.7$$

IV (theor.)=166.7

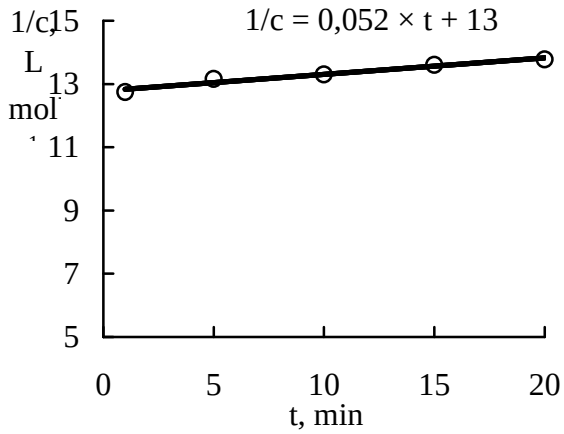

Fig. 5: The reverse concentration anamorphosis of the kinetic curve of limonene epoxide epoxidation up to limonene diepoxide. K_{ef}=0.052 L mol^{-1} min^{-1} (295 K)

The quantitative interaction time – 30 min.

$$IV = \frac{(V_0 - V_1) \times 0.1 \times K \times 126.93 \times V \times 100}{m \times 1000} = \frac{(2.05 - 0.74) \times 0.1000 \times 126.93 \times 20 \times 100}{0.17020 \times 1000} = 197$$

Based on the study of the kinetics at 298 K involving the peroxy decanoic acid in the methylene chloride medium for the terpenic oil, the quantitative interaction time was 25 min.

$$IV = \frac{(V_0 - V_1) \times 0.1 \times K \times 126.93 \times V \times 100}{m \times 1000} = \frac{(1.07 - 0.67) \times 0.1000 \times 126.93 \times 25 \times 100}{0.0929 \times 1000} = 171.$$

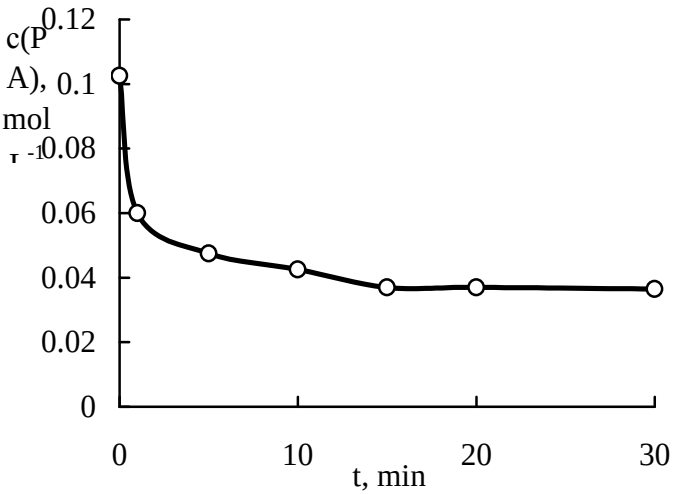

Fig. 6: The kinetic curve of turpentine epoxidation with the peroxy decanoic acid in the methylene chloride medium at 297 K

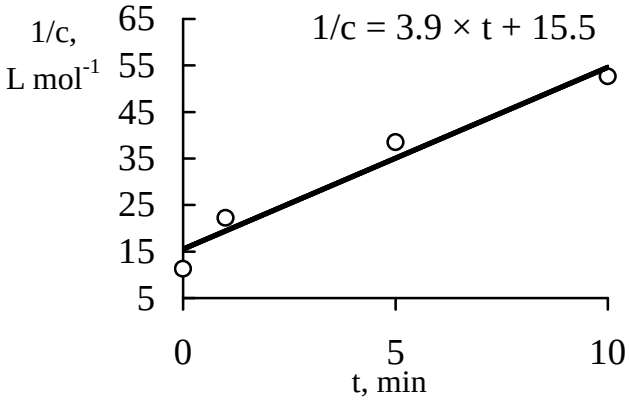

Fig. 7: The reverse concentration anamorphosis of the kinetic curve of turpentine epoxidation. K_{ef}=3.9 L mol^{-1} min^{-1} (297 K)

The procedure of quantitative determination of the medical-grade turpentine content in turpentine ointment 10%

Preparation of 0.05 g mL^{-1} solution of the peroxy decanoic acid in the methylene chloride. About 1.0 g (correct weight) of the peroxy decanoic acid is dissolved in 20.0 mL of the methylene chloride and shaken thoroughly.

Analysis procedure

About 3.0 g (correct hatch) of the ointment is dissolved in 10.0 mL of distilled water, 1 g of sodium sulphate is added and shaken, 10.0 mL of the methylene chloride is added, and the mixture is moved to a separating funnel. The content is shaken during 5 min. After that the bottom layer is separated to a cone flask with a stopper plug. 10 mL of the methylene chloride is added to the water residue, and the terpenic oil is extracted as the first one. The obtained extracts are put together, 1-2 g of the anhydrous sodium sulphate is added, thoroughly mixed up, and the extract is quantitatively transferred to a 20 mL graduated flask using the methylene chloride. The volume is filled up to 20.00 mL with the methylene chloride (in formula V_c). 10,00 mL of 0.05 g mL^{-1} of the methylene chloride solution of the peroxy decanoic acid is added to 10.00 mL of the obtained solution (in the formula V_a, mL), thoroughly mixed and kept for 25 min. Using a pipette, 1.00 mL of the solution is taken and transferred to a cone flask, containing 2 mL of 5% potassium iodide and 5 mL of 30% acetic acid solution. Using a 10 mL microburette, the released 0.1 mol L^{-1} iodine is titrated with the sodium thiosulphate solution (V, mL).

The control experiment is carried out in parallel. 10.00 mL of the methylene chloride is added to 10.00 mL of the peroxy decanoic acid solution and thoroughly mixed. Using a pipette, 1.00 mL of the solution is taken and transferred to a 50 mL cone flask, containing the mixture of 2 mL of 5 % potassium iodide and 5 mL of 30% acetic acid solution, added earlier. The released iodine is immediately titrated with 0.1 mol L^{-1} sodium thiosulphate solution (V_0, mL).

Finding the titre of 0.1 mol L^{-1} of the sodium thiosulphate solution by the substance being determined (gum turpentine). About 0.3 g (precise weight) of the gum turpentine, of which the ointment is made, is dissolved in 20.00 mL of the methylene chloride. 10.00 mL of 0.05 g mL^{-1} of the methylene chloride solution of the peroxy decanoic acid is added to 10.00 mL of the solution, thoroughly mixed and kept for 25 min. Using a pipette, 1.00 mL of the solution is taken and transferred to a cone flask, containing 2 mL of 5% potassium iodide and 5 mL of 30% acetic acid solution. Using a 10 mL microburette, the released 0.1 mol L^{-1} iodine is titrated with the sodium thiosulphate solution (V , mL).

The titre (T) of 0.1 mol L^{-1} of the sodium thiosulphate solution in g per 1.00 mL, by the (medical-grade) gum turpentine is calculated by the formula:

$$T = \frac{m}{(V_0 - V) \times 2 \times 20}$$

where m – the weight of the (medical-grade) gum turpentine, g;

V_0 – the sodium thiosulphate volume, consumed for titration in the control experiment (in the absence of turpentine), mL;

V – the sodium thiosulphate volume, consumed for titration in the working experiment, mL;

Optimum time – 20 - 25 min at 297 – 298 K.

The content of the (medical-grade) gum turpentine in the ointment, %, is found by the formula:

$$w = \frac{(V_0 - V) \times T \times 20 \times 20 \times V_c}{V_a \times m} \cdot 100\% .$$

The metrological characteristics of the results of the quantitative determination of the (medical-grade) gum turpentine content in 10 % turpentine ointment are shown in the table.

Table: The results of the quantitative determination of the (medical-grade) gum turpentine content in 10% turpentine ointment (*n*=7, *P*=0.95)

Turpentine content, g*	$\overline{X} \pm \Delta \overline{X}$	S	$S_{\overline{X}}$	*RSD, %*	*δ,%*	Recovery ± *ε*, %
9.712	9.60 ± 0.28	0.30	0.115	3.2	-1.15	98.85 ± 2.9

**Claimed in the Quality Certificate*

Thus, the practiced technique of terpenoids determination by the reaction of epoxidation using the peroxy decanoic acid in the methylene chloride medium at 295 – 297 K can be used for the quantitative determination of the basic substance content in individual terpenoids, determination of the iodine number (unsaturation degree) of (medical-grade) gum turpentine, and analysis of the turpentine ointment 10%, prepared extempore in conditions of pharmacy.

CONCLUSION

The iodometric titration method was used to study the kinetics of some individual terpenoids epoxidation reactions (α-pinene, limonene, limonene oxide), and the medical-grade turpentine epoxidation with the peroxy decanoic acid in the the methylene chloride medium at 297 – 298 K. The kinetics of the reaction was shown to be governed by the second order kinetic equation. The second order effective constants of the reaction rate were determined: for *α*-pinene – 3.6 L mol^{-1} min^{-1} (297 K), limonene (to monooxide) – 2.1 L mol^{-1} min^{-1} (295 K), limonene oxide (to dioxide) – 0.052 L mol^{-1} min^{-1} (295 K), for turpentine – 3.9 L mol^{-1} min^{-1} (297 K). The proposed techniques of the quantitative determination of the basic substance content and determination of the iodine number of some terpenoids (*α*-pinene, limonene, limonene oxide) and the (medical-grade) gum turpentine were based on the data of epoxidation with the peroxy decanoic acid in the methylene chloride medium. The extraction-titrimetric technique was practiced and the possibility of quantitative determination of the (medical-grade) gum turpentine in turpentine ointment 10 % (RSD = 3.2 % , δ = -1.15 %) was demonstrated.

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