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Mass-Spectrometric Characterization of Oligomeric Products from Hydroquinone Oxidation by Hydrogen Peroxide as an Analytical Problem of Particularly Complexity

M. A. Kuznetsov[a] (ORCID: 0000-0002-5458-9004), T. A. Kornilova[a] (ORCID: 0000-0002-3836-143X), E. P. Podol'skaya[b], A. S. Gladchuk[b] (ORCID: 0000-0002-4411-2069), A. K. Buryak[c] (ORCID: 0000-0002-2458-5993), I. V. Minenkova[c] (ORCID: 0000-0003-3323-3294), A. S. Pan'kova[a] (ORCID: 0000-0002-6288-4492), V. G. Makarov[d], A. L. Zagorskii[e] (ORCID: 4000-0001-5318-0971), and I. G. Zenkevich[a, *] (ORCID: 0000-0001-8975-2257)

[a] *Institute for Chemistry, St. Petersburg State University, St. Petersburg, 198504 Russia*
[b] *Institute of Analytical Instrumentation, Russain Academy of Sciences, St. Petersburg, 198095 Russia*
[c] *Frumkin Institute of Physical Chemistry and Electrochemistry, Russain Academy of Sciences, Moscow, 119071 Russia*
[d] *Institute of Pharmacy, Kuzmolovsky, Leningrad oblast, 188663 Russia*
[e] *Graduate School of Management, St. Petersburg State University, St. Petersburg, 199004 Russia*
**e-mail: izenkevich@yandex.ru*

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Abstract—The oxidation of hydroquinone with hydrogen peroxide in the presence of catalytic amounts of $FeSO_4$ results in complex mixtures of oligomers. The average composition of these products is determined by the molar ratio of reagents and varies from $(C_6H_4O_4)_n$ at the hydroquinone to hydrogen peroxide ratio 1 : 3 to $(C_6H_4O_6)_n$ at the ratio 1 : 5. A characteristic feature of MALDI mass spectra for polymers is the periodicity of signals. However, no such periodicity is observed for the products of hydroquinone oxidation within the m/z range <1000 Da, indicating the absence of fixed sequences of structural fragments. This unique feature arises from the variable ratios and irregular positions of polyhydroxyphenylene and polyhydroxybenzoquinone fragments. Additionally, several factors contribute to the lack of mass-spectral periodicity, namely, the potential formation of peroxides, the presence of stable hydrates, various types of linkages, and possible interactions between hydroxylated phenylene and benzoquinone fragments. In contrast, for m/z values >1000 Da, MALDI mass spectra exhibit periodicity with a mass difference of $\Delta(m/z) = 74$. This value likely corresponds to the fragment $C_2H_2O_3$, which is neither a hydroquinone nor a benzoquinone structural unit. Taking into account that an equal $\Delta(m/z)$ value is observed for fragment ions in the EI mass spectrum of tetrahydroxy-*p*-benzoquinone, this gives indirect evidence for the presence of polyhydroxybenzoquinone fragments within the oligomers. A key issue regarding the structure of hydroquinone oxidation products is the type of linkages between the polyhydroxyphenylene and/or polyhydroxybenzoquinone units, which could involve C–C or C–O–C bonds (or both). The available spectral data do not resolve this issue conclusively. However, the presence of a sample of the composition $(C_6H_4O_6)_n$, in which each unit contains six oxygen atoms, suggests that at least one oxygen atom forms a bond between the units, indicating a C–O–C connection. Overall, the unique nature of oligomeric products of hydroquinone oxidation explains their high structural variability and redox properties, which likely contribute to their unique pharmacological characteristics.

Keywords: hydroquinone, hydrogen peroxide, oxidation, formation of oligomers, MALDI mass-spectrometry, structure characterization

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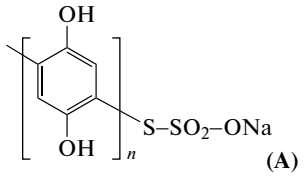
Polyphenolic compounds of plant origin (flavonoids, polyhydroxyaryl carboxylic acids, pigments, and others) and their corresponding quinones exhibit unique chemical and biochemical properties. This primarily relates to the redox characteristics of these compounds, including their ability of both easy oxidation and reduction [1]. These properties determine their biological activity, including antioxidant, antiradical, and antihypoxic effects, as well as their capacity to regulate tissue respiration efficiency and other

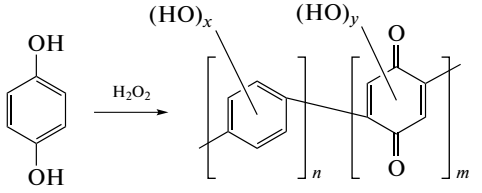
effects. Therefore, it is not surprising that the search for relatively simple and available chemical methods for synthesizing polyphenolic compounds with similar biochemical and pharmacological characteristics has long been considered a relevant task. Among the possible approaches to implementing this concept, methods based on the specific properties of the hydroquinone–*p*-benzoquinone redox system have attracted attention since the early 1970s. The first component of this system, 1,4-dihydroxybenzene (hydroquinone), is easily oxidized by various reagents to form 2,5-cyclohexadien-1,4-dione (*p*-benzoquinone). The standard redox potential of hydroquinone (0.70 V, water, 25°C) is comparable to the potential of the $Fe^{3+} + e^- \rightarrow Fe^{2+}$ system (0.77 V, water). However, the formation of a charge-transfer complex between the hydroquinone and benzoquinone (quinhydrone) leads to an anomalous decrease of this potential to 0.18 V (water, pH 9, 25°C). Additionally, the presence of C=C double bonds in the structure of quinones, conjugated with carbonyl groups, explains their high activity in nucleophilic addition reactions, which can result in the formation of oligomeric products.

A possibility of the polymerization (oligomerization) of hydroquinone requires comment, as it is known to act as an inhibitor of free radical processes [2]. The formation of oligomers is predominantly associated with the oxidation of hydroquinone under the action of various reagents (in the presence of catalysts), such as hydrogen peroxide [3, 4], manganese dioxide [5], and others. In contrast to hydroquinone, *p*-benzoquinone can undergo thermal polymerization, typically conducted in acidic or basic media, or in the presence of transition metal salts or complexes.

Initiators of *p*-benzoquinone oligomerization reactions have included not only alcohols, phenols, amines, and other organic compounds, but also inorganic salts (typically in nonaqueous media), such as hydrosulfites (anion HSO_3^-) or thiosulfates (anion $S_2O_3^{2-}$). The latter method (interaction of *p*-benzoquinone with sodium thiosulfate in acetone) was first proposed for the production of compound Olifen™ (renamed Hypoxen™ in 1999 [6]). However, the history of using such methods has shown that the compounds produced on an industrial scale do not always exhibit consistent properties. This issue prompted the development of alternative methods for synthesizing oligomeric polyphenolic compounds, based on the oxidation of hydroquinone with hydrogen peroxide (compound Epofen™). By now, Hypoxen is used as a biologically active supplement, while Epofen is used in cosmetic products.

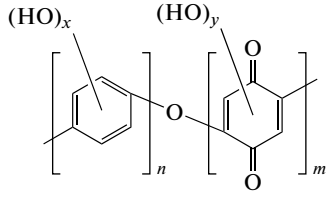


(A)



(B)

$(y = x - 2 \leq 2)$



(C)

$(y = x - 2 \leq 2)$

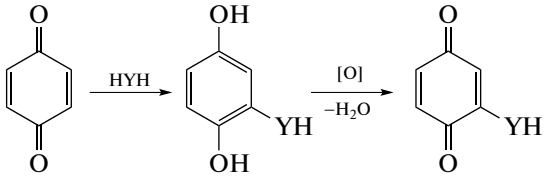
Note than the structure of the products of the reaction of *p*-benzoquinone with sodium thiosulfate is supposed to be determined as sodium poly(hydroxyphenylene)thiosulfonate (A) [6]. In contrast, the structure of oligomeric products of the oxidation of hydroquinone with hydrogen peroxide remains unclear to this day. This uncertainty largely stems from the difficulty in determining the ratio of quinone/hydroquinone fragments in the structure and the nature of a linkage between the elementary units of the oligomers, which may be connected directly (C–C bonds (structure B)) or via an oxygen atom (C–O–C fragments (structure C)). Part of this issue arises from the limited informativeness of current physicochemical methods for characterizing complex oligomeric mixtures, yet this uncertainty still warrants discussion.

The problem of fragment linkage in the oligomeric products of hydroquinone oxidation arises from the fact that, depending on the polymerization conditions, *p*-benzoquinone can form both polyphenylene structures (with C–C bonds) and polyphenyl ethers (with bridging oxygen atoms C–O–C). Most likely, the primary product of hydroquinone oxidation is in all cases a molecular complex of *p*-benzoquinone with the starting hydroquinone in a 1 : 1 ratio—quinhydrone [7, 8]. A dimer of *p*-benzoquinone with C–C bonds has been synthesized, isolated, and used as a component of materials for lithium battery cathodes [9]. Stable trimers of *tert*-butyl-substituted *p*-benzoquinones with C–C bonds have also been isolated [10]. More complex oligomers of *p*-benzoquinone of this type were characterized in [11–17]. However, some publications do not specify the structure of the

oligomerization products of *p*-benzoquinone; by default, it is assumed that they involve the most commonly mentioned C–C ring linkage (e.g., [18–20]). Regardless of the type of the ring linkage, the oligomers exhibit a variable ratio of hydroquinone and benzoquinone fragments, which is determined by the amount of the oxidant used. Due to the presence of these fragments, hydroquinone and benzoquinone oligomers have a deep black color and are prone to both easy oxidation and reduction [11], as hydroquinone and benzoquinone fragments can interconvert.

Alongside the formation of simple C–C linkages, more complex oligomerization mechanisms for *p*-benzoquinones have been proposed, such as the formation of dibenzofuran [21, 22] and even dibenzo-*p*-dioxin fragments [23]. However, such structures could also arise from secondary processes between *p*-benzoquinone and hydroquinone fragments within oligomer chains [20]. Additionally, under excess hydrogen peroxide, 2,5-dihydroxy-*p*-benzoquinone may not form oligomers but instead yield more advanced oxidation products, including dicarboxylic acids [24]. Under milder conditions, the formation of epoxides from C=C bonds in benzoquinones has been observed [25, 26]. Interesting examples of "reverse" processes are known: the photochemical interaction of *p*-benzoquinone with water leads to the formation of hydroquinone and hydrogen peroxide [27, 28].

Finally, the ability of benzoquinones to undergo nucleophilic addition reactions with various reagents HRY or H_2Y results in products with C–Y bonds [29],



Such processes are known to occur with water [30, 31], ethanol, hydrochloric acid [32], amines [33], acetic acid, and other reagents. Initially formed substituted hydroquinones are capable of further oxidation and, subsequently, of undergoing additional nucleophilic addition [29]. This possibility is not always considered, but it was specifically noted, for example, in the review [29]: "Hydroxyl-containing quinones directly add to quinone fragments, resulting in a very interesting class of quinonoid compounds." The sequential occurrence of several such processes can lead to oligomers, where the linkage of rings is facilitated by C–Y–C, most commonly C–O–C fragments, rather than by C–C bonds.

In the early 2000s, the production of a compound Epofen™—an oxidation product of hydroquinone with hydrogen peroxide in the presence of catalytic amounts of iron(II) sulfate—was regulated by TU 9154-001-54773261-02. Initially, this product was attributed to the structure of the monohydrate of 2,4,4'-trihydroxy-diphenyl ether, although this could not be conclusively proven. Since 2006, this product has been protected by a patent [34], in which it was characterized as a mixture of poly(1,4-dihydroxy)phenylene (polyhydroquinone). However, the formation of oligomers with exclusively such a structure is unlikely in an oxidative medium (with excess hydrogen peroxide).

Thus, the issue of not only determining but also at least clarifying the structure of oligomers formed uponthe oxidation of hydroquinone with hydrogen peroxide remains relevant. Numerous attempts to determine their structure have been unsuccessful. In this work, we have attempted to address this problem using MALDI mass spectrometry.

EXPERIMENTAL

Test samples. We had the opportunity to compare data for samples of products resulting from the interaction of hydroquinone with hydrogen peroxide, which varied in their reagent ratios.

Samples **I** (molar ratio of hydrogen peroxide to hydroquinone of 5 : 1) were synthesized under laboratory conditions according to the semi-industrial synthesis description in TU 9154-001-54773261-02. Samples **II** (molar ratio of hydrogen peroxide to hydroquinone of 3 : 1) were prepared according to the conditions outlined in patent [34]. For sample **I**, we used some data from report [35][1]. At various stages of this work, chemists N.M. Faustova and A.Yu. Eshchenko (Interregional Centre "Adaptogen", St. Petersburg) were involved. MALDI mass spectra were recorded for sample **II**.

Reagents and materials. The study used hydroquinone (analytical grade, Shostka Chemical Reagents Plant, Russia) without further purification and *p*-benzoquinone (analytical grade, Shostka Chemical Reagents Plant, Russia), which was recrystallized from heptane.

For HPLC analysis, acetonitrile (grade 1, Kriokhrom, Russia) was used as an eluent. For MALDI mass spectrometry, acetonitrile (HPLC grade, J.T. Baker), trifluoroacetic acid (TFA, 99%, Merck), 2,5-dihydroxybenzoic acid (DHB) and 3-hydroxypicolinic acid (HPA, >99%, Bruker Daltonics), and isopropanol (analytical grade, Vekton, Russia) were utilized.

Procedure for obtaining a mixture of hydroquinone oxidation products under laboratory conditions. Samples **II** were prepared according to the patent [34], with the loading volume reduced by approximately 500 times. For the synthesis of samples **I**, the procedure from [34] was modified in accordance with TU 9154-001-54773261-02 and report [35].

[1] The reference to the report by the Interregional Centre "Adaptogen" (St. Petersburg) is given with the permission of V.P. Tikhonov, Chief Executive Officer of Diod PJSC).

Table 1. Results of elemental analysis of various samples of hydroquinone oxidation products with hydrogen peroxide

Sample	C, wt %	H, wt %	O, wt %
I. According to TU 9154-001-54773261-02 (for one of the samples) [35]	42.9	2.9	54.1
Calculated for the molecular formula $(C_6H_4O_6)_n$	41.9	2.3	55.8
II. According to patent [34] (average values for nine samples)	51.5 ± 1.1	3.1 ± 0.1	45.4 ± 0.4
Calculated for the molecular formula $(C_6H_4O_4)_n$	51.4	2.9	45.7

We placed 40 mL of distilled water in a 150- to 200-mL beaker and heated to 50°C. Then, 10 g (91 mM) of hydroquinone was dissolved, and a solution of 50 mg of iron(II) sulfate in 1 mL of water was added. To this mixture, 30 mL of 30% hydrogen peroxide (density 1.13, 0.3 M) was added dropwise using a dropping funnel over 1.5 h upon vigorous stirring with a magnetic stirrer and cooling with water, ensuring that the temperature of the reaction mixture did not exceed 60°C. For sample **I**, the amount of hydrogen peroxide was proportionally increased, and the reaction mixture was further neutralized with a 20% aqueous solution of sodium hydroxide (at a ratio of 0.5 M to 1 M of hydroquinone) to a pH of 6.5–7.5. Water was then evaporated at 60°C, and the residue was dried over $CaCl_2$ in a vacuum desiccator until a constant weight was achieved. A total of 11.5 g of a product was obtained, consisting of shiny, brittle black crystals that were easily ground into a powder. The product was completely (sample **I**) or partially (sample **II**) soluble in water, forming a brown solution; it was fully soluble in alkaline aqueous solutions, methanol, and dimethylformamide (1%). The aqueous solution of sample **II** had pH 3–4. Heating it with conc. nitric acid produced a yellow color, typical of phenols and polyphenols. When aqueous solutions of the product were treated with metallic magnesium and conc. hydrochloric acid, they became completely decolorized. Additionally, treatment with a sodium sulfite solution significantly diminished the intensity of the color, which confirmed the presence of quinonoid chromophores in the sample components. The residual hydroquinone concentration varied from traces up to 1–1.5%, and iron concentration ranged from 0.1 to 0.2%. The role of iron(II) sulfate was to generate hydroxyl radicals,

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + OH\cdot.$$

The elemental analysis of the samples was performed using an HP-185B automatic CHN analyzer (Hewlett-Packard). Before an analysis, all samples were additionally dried in a vacuum with a water jet pump, and sample **I** was further converted into its H-form. The average mass fractions of C, H, and O for the samples prepared according to the conditions of TU 9154-001-54773261-02 and patent [34] are listed in Table 1. The molecular formulas of both samples, $(C_6H_4O_6)_n$ (**I**) and $(C_6H_4O_4)_n$ (**II**), are consistent with the reagent ratios used during their preparation (1 : 5) and (1 : 3), respectively,

$$nC_6H_6O_2 + 5nH_2O_2 = (C_6H_4O_6)_n + 6nH_2O, \quad (1)$$

$$nC_6H_6O_2 + 3nH_2O_2 = (C_6H_4O_4)_n + 4nH_2O. \quad (2)$$

A somewhat poorer correspondence between the composition of the products and the amount of oxidizer in reaction (1) is because, to increase the solubility of the products in water, their synthesis according to TU 9154-001-54773261-02 was conducted with the addition of NaOH. Analyzing the reaction products required either converting them into their H-form beforehand or applying appropriate corrections during calculations. Both options introduced additional errors in the results. Nevertheless, the data in Table 1 indicate that the composition of the products and the oxidation state of the hydroquinone fragments are clearly determined by the amount of oxidizer used.

Quinhydrone was synthesized from equimolar amounts of hydroquinone and *p*-benzoquinone according to the procedure described in [36], and its subsequent oxidation was carried out with a quinhydrone to H_2O_2 ratio of 1 : 2.5. This was done to ensure that the total oxidation state of the resulting product corresponded to sample **II** and Eq. (2).

The average molecular masses of the hydroquinone oxidation products were estimated (with low accuracy) by measuring the depression of the freezing point of their aqueous solutions and by titration with an alkali [35]. The low accuracy of the results is due to the interfering effect of sodium ions (sample **I**) or the partial solubility of the oligomer mixture in water (sample **II**). For samples of series **I**, the average molecular masses are approximately 470 ± 50. The ratio of this value to the mass of a basic unit (140 for $C_6H_4O_4$, 172 for $C_6H_4O_6$) gives the average number of fragments in the oligomer molecule, i.e., from 2.7 to 3.4, with an average of about 3.0.

Chromatographic and spectral properties of the products. An analysis of the hydroquinone oxidation products using hydrogen peroxide was conducted via HPLC using a Beckman System Gold chromatograph with a UV detector (detection wavelengths of 254 and 200 nm). The chromatographic column used was a

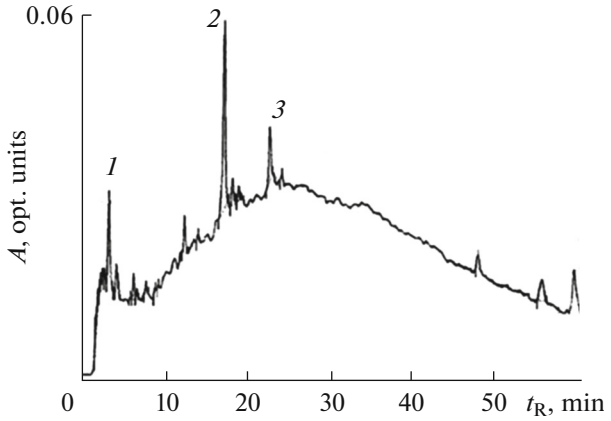


Fig. 1. Typical chromatogram of hydroquinone interaction products with hydrogen peroxide using gradient elution mode (sample from series **I**), detection wavelength 254 nm; (*1*) hydroquinone and (*2*, *3*) unidentified compounds.

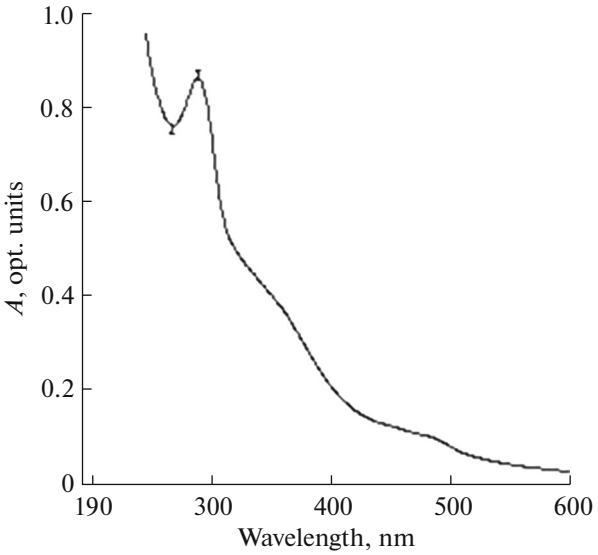


Fig. 2. Typical UV spectrum of water-soluble components from one of the samples in series **I**. The peak at 290 nm corresponds to the UV spectrum maximum of hydroquinone [37].

Luna C18, 150 mm in length, with an internal diameter of 4.6 mm (particle size of 5 μm), and it was equipped with a precolumn packed with the same type of the stationary phase. The eluents employed were acetonitrile and a 0.03% aqueous solution of trifluoroacetic acid (pH 2.8–3.0).The initial acetonitrile concentration was 10%, with a gradient elution rate of 1% min^{-1}, an eluent flow rate of 1.0 mL min^{-1}, and a sample volume of 20 μL. The chromatographic peak parameters were recorded using the GOLD V 402 software. A typical chromatogram for samples of series (**I**) is shown in Fig. 1.

The presence of a characteristic "bump"in the chromatogram indicates a significant number of unresolved components within a broad range of their hydrophilic/hydrophobic properties.

Infrared spectra of the hydroquinone oxidation products were recorded in KBr tablets or vaseline oil using an IR Affinity-1 Fourier-transform IR spectrometer (Shimadzu). The spectra show broad intense bands corresponding to stretching vibrations of OH groups, confirming the presence of intramolecular and intermolecular hydrogen bonding. For samples **I**, this regionis from 3600 to 2400 cm^{-1}, while for samples (II), it is from 3700 to 2800 cm^{-1}. Bands in the region 1100–1350 cm^{-1} (samples **I**) can be assigned to stretching vibrations of C–O bonds. The stretching vibrations of carbonyl fragments are evidenced by characteristic bands at 1625–1630 and 1700–1710 cm^{-1} (series **I**) and around 1680 cm^{-1} (series **II**). In the latter case, the intensity is low, indicating a relatively small amount of benzoquinone fragments in the structure.

Ultraviolet spectra were recorded on a UV-1800 spectrometer (Shimadzu). In the spectra of all samples (series **I** and **II**), moderately intense bands are observed in the region 285–290 nm. The intensities of these bands are maximal for the fraction of water-soluble components of samples **I**, which suggests that they correspond to residual amounts of hydroquinone. A typical UV spectrum of this fraction (Fig. 2) closely matches the spectrum of the Hypoxen preparation [6].

The ^1H NMR spectra (400.13 MHz) and ^{13}C NMR spectra (100.61 MHz) were recorded using a Bruker AVANCE III-400 spectrometer for solutions in DMSO-d_6, using the residual proton signal of the solvent (at 2.50 ppm) as an internal standard in the ^1H NMR spectra, and the carbon signal of the solvent (at 39.50 ppm) as an internal standard in the ^{13}C NMR spectra. The ^1H NMR spectra are not sufficiently informative because of the presence of trace amounts of Fe^{3+} in the samples, which causes signal broadening. However, these spectra for the solutions of samples from series **I** in D_2O confirm the presence of residual amounts of hydroquinone, as evidenced by a single discrete peak at 6.7 ppm, corresponding to the aromatic protons of hydroquinone, while other signals are poorly defined.Thus, hydroquinone remains in the composition of the reaction products even ata molar excess of hydrogen peroxide in a 5 : 1 ratio. This persistence is likely due to oligomeric reaction products being more readily oxidized compared to the original hydroquinone.

Mass spectra of samples **II**, detected for both positively and negatively charged ions, were recorded independently in two laboratories.

Series no. 1 of MALDI mass spectrometric analyses was performed using a Bruker Daltonics Ultraflex II mass spectrometer (Bruker, Germany), equipped with a nitrogen laser (λ = 337 nm) and a time-of-flight mass analyzer. A solution of a sample with DHB

Table 2. Values of m/z for protonated molecules of compounds used for mass spectrometer calibration

No.	Compound	m/z $[M + H]^+_{monoisot.}$	m/z $[M + H]^+_{average}$
1	Angiotensin II	1046.542	1047.189
2	Angiotensin I	1296.685	1297.486
3	Substance P	1347.735	1348.642
4	Bombesin	1619.822	1620.860
5	Renin Substrate	1758.933	1760.026
6	ACTH_clip (1-17)	2093.086	2094.427
7	ACTH_clip (18-39)	2465.198	2466.681
8	Somatostatin (28)	3147.471	3149.574

(matrix) was applied to the surface of a standard stainless steel substrate (Bruker, Germany). After the sample had completely dried at room temperature in air, the substrate was placed into the mass spectrometer (pressure up to 10^{-9} atm). Mass spectra were recorded in the reflectron mode within the m/z range 20–2000 Da, detecting both positively and negatively charged ions under the following conditions (positive/negative ions): extraction electrode voltage 25/20 kV; post-acceleration voltage 21.5/17.5 kV; voltage on the focusing system 10/7.5 kV; retarding voltage on the ion mirror 26.4/21 kV; reflecting voltage on the ion mirror 14.2/11 kV. To obtain the most informative mass spectra, the laser operated in the following mode: 50 pulses at a frequency of 20 Hz, laser pulse energy of 60–80 μJ.

Series no. 2 of the MALDI mass spectra were recorded using an UltrafleXtreme instrument (Bruker Daltonics). Mass spectra were obtained in both linear and reflectron modes within the m/z range 900–3300 Da, detecting both positively and negatively charged ions. For the linear mode, a solution of a DHB matrix (10 mg mL^{-1}) in 50% aqueous acetonitrile with 0.1% TFA was used. For the reflectron mode, a solution of an HPA matrix (10 mg mL^{-1}) in 50% aqueous acetonitrile with 0.1% TFA was employed. A 0.7-μL aliquot portion of a corresponding matrix solution was applied to a stainless steel MALDI target spot, followed by the addition of 0.7 μL of a sample solution in isopropanol or methanol (1 mg mL^{-1}). The target was then left at room temperature until the complete evaporation of the solvent occurred. Calibration of the mass spectrometer within the selected m/z range was performed using the Peptide Calibration Standard II (Bruker Daltonics), based on m/z values corresponding to the monoisotopic (reflectron mode) or average molecular masses (linear mode) of the protonated molecules of the calibration mixture components (Table 2).

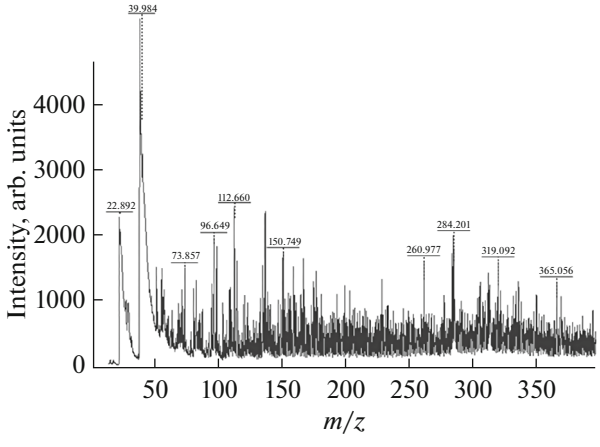


Fig. 3. Fragment of a MALDI mass spectrum of hydroquinone interaction products with hydrogen peroxide (sample from series **II**); positive ions detection, matrix DHB.

RESULTS AND DISCUSSION

MALDI mass spectra of the samples from series **II** were recorded using different instruments over various mass ranges.

Figure 3 shows a typical MALDI mass spectrum (with detection of positively charged ions) for one of the samples from series **II** in the m/z range 15–400 Da (using the DHB matrix). In this spectrum, the most intense signals correspond to the ions Na^+ (m/z 23) and K^+ (m/z 39). As for the collection of all other peaks, there is low selectivity in the fragmentation of the sample (no dominant peaks in terms of intensity) and no periodicity in the mass numbers of the detected signals. The need in recording the mass spectrum in this specific mass range was driven by the necessity of a check for the potential presence of relatively low-molecular-weight carboxylic acids among the products of hydroquinone oxidation [24].

In the MALDI mass spectrum of the same sample, using the same matrix and in the same m/z range, but recorded for negatively charged ions (Fig. 4), the most intense signal at m/z 153 correspondingly belongs to the $[M–H]^-$ ions of dihydroxybenzoic acid ($C_7H_5O_4$, molecular weight 154). Among the remaining signals in this range, there is no predominance of intensity peaks or any elements of periodicity in the mass numbers.

The periodicity of mass numbers in MALDI mass spectra is often considered one of the most characteristic features of polymer analysis. For example, the MALDI mass spectrum of polystyrene (as shown in a brochure by Bruker Daltonics, see Fig. 5) demonstrates a distinctive set of oligomer signals in the range from 1000 to over 3000 Da. These signals exhibit a characteristic interval of 104.0624 Da, corresponding to the fragment C_8H_8. Furthermore, signals that do not fit this sequence are virtually absent from the spectrum.

No signs of such periodicity in the mass numbers were observed in the MALDI mass spectra of the

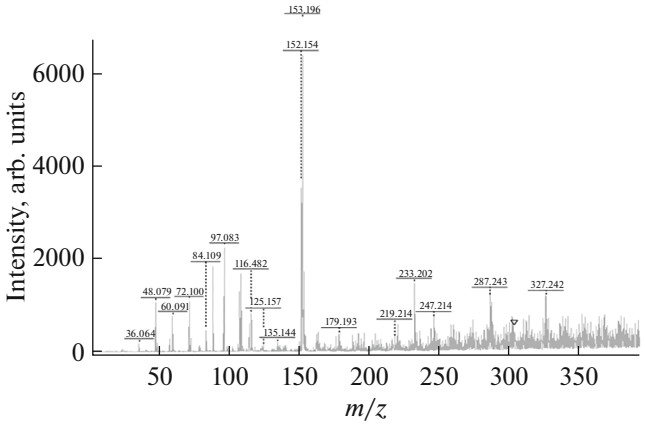


Fig. 4. Fragment of a MALDI mass spectrum of hydroquinone interaction products with hydrogen peroxide (sample from series **II**); negative ions detection, matrix DHB.

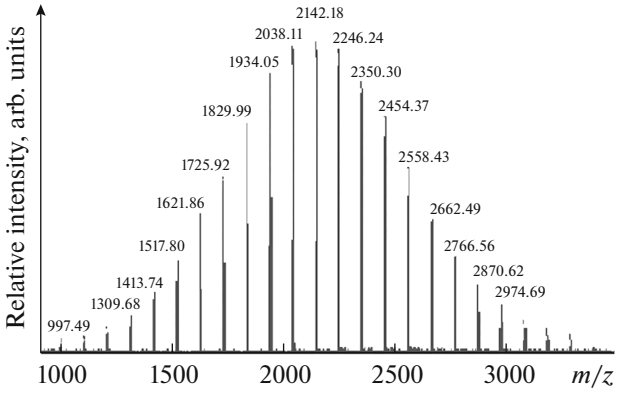


Fig. 5. MALDI mass spectrum of polystyrene sample (from Bruker Daltonics promotional brochure, presented for comparison). The average mass differences between all signals are 104.0624 Da (for fragment C_8H_8, calculated M = 104.0626 Da).

products formed in the reaction of hydroquinone with hydrogen peroxide in the region of low m/z values (<1000 Da). This characteristic is consistently reproduced in the spectra recorded in different laboratories, suggesting it reflects an inherent property of the characterized oligomer mixture rather than errors in the measurement. The absence of periodic elements in the lower m/z range confirms the lack of a regular sequence of uniform units within the oligomer chain, making it practically impossible to find a definitive structure.

The absence of periodicity in MALDI mass spectra, combined with their low reproducibility both on different instruments and within the same instrument for different samples, can be attributed to several factors. The primary factor appears to be an irregular alternation and ease of mutual transformations between the hydroquinone and benzoquinone structural fragments. In conjugated oligomer chains, it is challenging to assess the specific manifestations of such monomeric units in their mass spectra. However, in a first approximation, the ionization and fragmentation of polymers should be affected by hydroquinone elements in the structure, given that the ionization energy of hydroquinone is 2 eV lower than that of *p*-benzoquinone [7.94(1) and 9.9(2) eV, respectively]. At least three other factors can further complicate the structure of oligomers and, consequently, their mass spectra.

Firstly, the reaction of hydrogen peroxide with carbonyl compounds (in this case, benzoquinone fragments of the oligomer structure) can lead to the formation of peroxides. A particularly illustrative example is the reaction of acetone with H_2O_2, which results in the formation of acyclic dimer of acetone peroxide (3,3,6,6-tetramethyl-1,2,4,5-tetroxane, CAS no. 1073-91-2) and the trimer (3,3,6,6,9,9-hexamethyl-1,2,4,5,7,8-hexoxane, CAS no. 56990-20-6). Both the dimer and the trimer of acetone peroxide are quite stable and have been characterized not only by mass spectrometry but also by gas chromatographic retention indices [37].

Another factor complicating the fragmentation patterns of the products of the reaction of hydroquinone with hydrogen peroxide is the formation of stable hydrates due to the addition of water to carbonyl groups, which is typical for quinones containing multiple hydroxyl groups. For instance, while the hydrate of unsubstituted *p*-benzoquinone is unknown, there occur both an anhydrous form (CAS no. 123334-16-7) and a monohydrate (CAS no. 1215458-51-7) as well as a dihydrate (CAS no. 567648-2) of tetrahydroxy-*p*-benzoquinone. A possibility of hydrate formation is indicated by the abnormally high intensity of O–H stretching bands in the infrared spectra of the samples. A combination of these factors prevents assigning any single, definitive structure to the oligomers of the reaction between hydroquinone and hydrogen peroxide.

Finally, one cannot disregard the third factor—the formation of charge-transfer molecular complexes, such as the aforementioned quinhydrone. A similar complex is formed between *p*-benzoquinone and pyrocatechol [38]. It is plausible that analogous complexes can form between polyhydroxy-substituted benzoquinones and polyhydroxybenzenes, including segments of oligomer chains. The formation of a quinhydrone dimer [39] gives evidence supporting this possibility.

Despite the combined effects of all the aforementioned factors, unexpected periodic elements have been identified in the region of relatively weak signals in the MALDI mass spectra with higher mass numbers. For instance, Fig. 6 shows fragments of a MALDI mass spectrum for one of the samples in series **II** within the m/z range from 800 to 1600. This spectrum reveals a sequence of signals: 942→1016→1090→1164→1238→1312→ and so on, with a mass difference of 74 Da. Additionally, a second sequence of lower intensity signals is observed, with m/z values reduced by Da: 928→1002→1076→1150→1224→

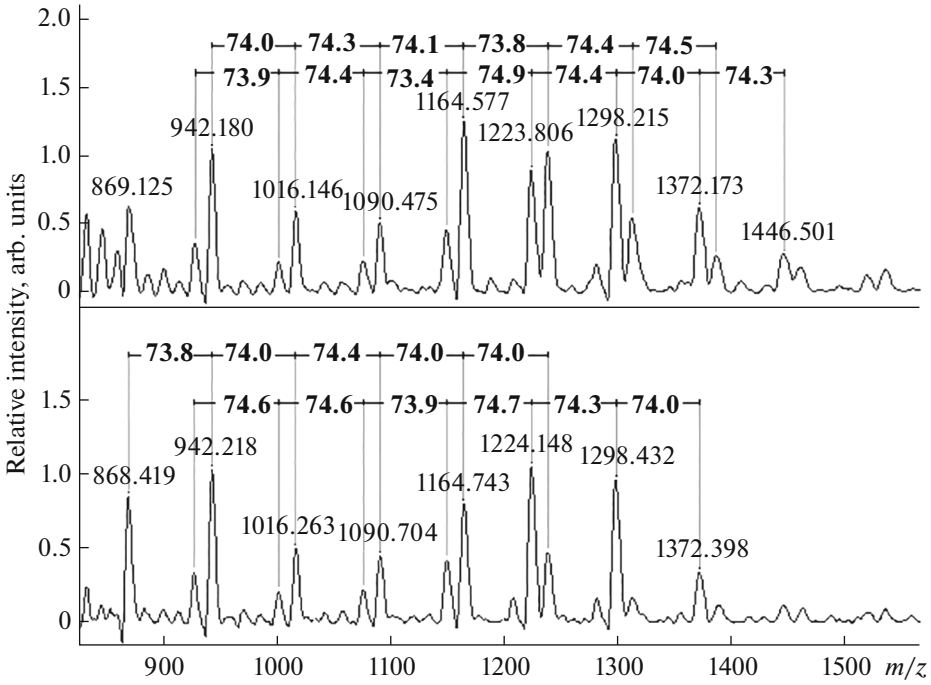


Fig. 6. Fragments of MALDI mass spectra of hydroquinone interaction products with hydrogen peroxide (sample from series **II** in isopropanol) in the range of 800–1600 Da (positive ions detection, matrix DHB). Two mass spectra were recorded from two different spots for reproducibility check.

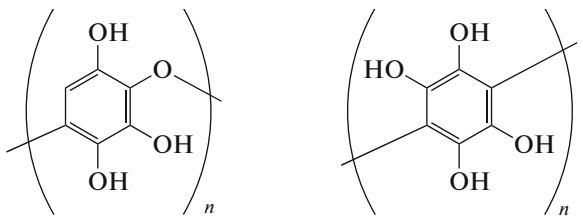
1298→1372→ and so on. To verify reproducibility, mass spectra were recorded for two spots obtained by applying the sample solution in isopropanol onto the substrate.

Figure 7 displays fragments of MALDI mass spectra for the same sample in a different solvent (methanol) within the m/z range of approximately 850 to 2600. Even with the naked eye, one can discern the same periodic elements in the signals, with a periodicity of 74 Da.

In the region of $m/z < 1100$ Da, the periodicity of the signals becomes less pronounced, which is particularly evident in the left part of Fig. 7. As noted earlier, the main reason for this is the lack of the regular alternation of identical structural fragments in the oligomers.

The difference in mass-to-charge ratios (m/z) of 74 does not correspond to either hydroquinone or benzoquinone structural fragments of the oligomer. To explain this mass difference $\Delta(m/z)$, it is useful to examine the electron ionization mass spectrum of tetrahydroxy-*p*-benzoquinone ($C_6H_4O_6$) [36] (Fig. 8). The primary fragmentation process of the molecular ions (m/z 172) of this compound involves the loss of a CO fragment, resulting in ions with m/z 144. However, subsequent fragmentation of $[M-CO]^{+\bullet}$ ions leads to ions with m/z 70, which corresponds to the loss of a fragment with a mass of 74, likely composed of $C_2H_2O_3$. We assume that similar ions in MALDI mass spectra also arise from the loss of 74 mass units from fragment ions of various oligomers rather than from molecular ions. There remains a small possibility that these ions result from the loss of not a single entity, but the sequential or the simultaneous loss of several fragments, such as $[X - 2CO - H_2O] = [X - 74]$.

The detection of periodic signals with mass differences of 74 Da in the MALDI mass spectra of products of the reaction of hydroquinone with hydrogen peroxide suggests the presence of polyhydroxybenzoquinone fragments in the oligomer chains. However, the question of how the rings are connected (via C–C or C–O–C bonds) remains unresolved. Unfortunately, the available spectral and chemical information does not allow a definitive answer to this question. Nevertheless, it is interesting to note the differences in the composition of the two available samples of products of the reaction of hydroquinone with hydrogen peroxide **I** and **II**. For the second sample (with an elementary fragment composition of $C_6H_4O_4$), two types of connection of polyhydroxyphenylene units can be considered. However, neither of these structures explains the appearance of signals with $\Delta(m/z) = 74$ in the MALDI mass spectra,



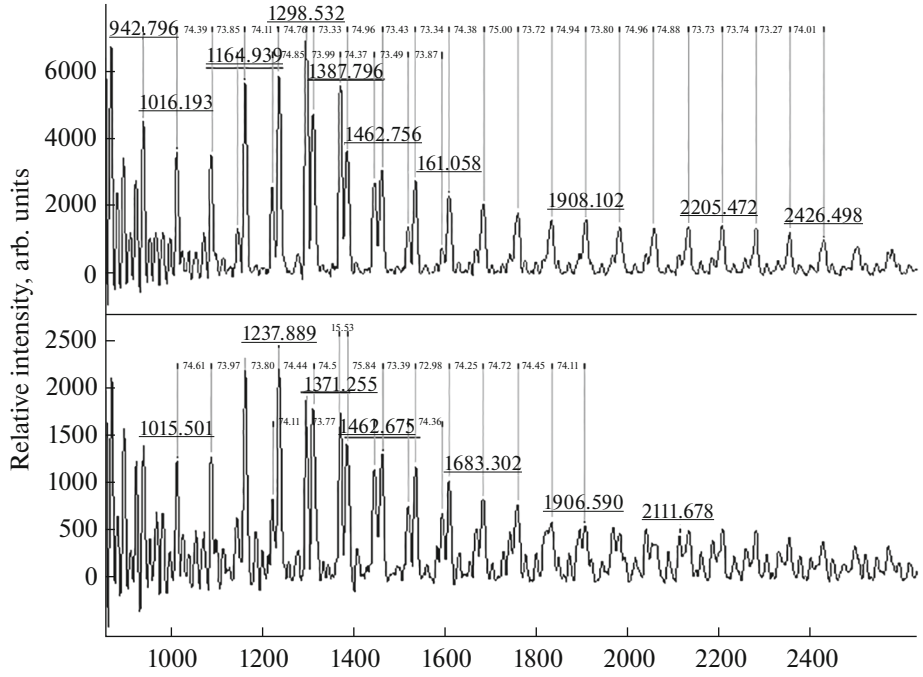


Fig. 7. Fragments of MALDI mass spectra of hydroquinone interaction products with hydrogen peroxide (sample from series **II** in methanol) in the range 850–2600 Da (positive ions detection, matrix DHB). Two mass spectra were recorded from two different spots for reproducibility check.

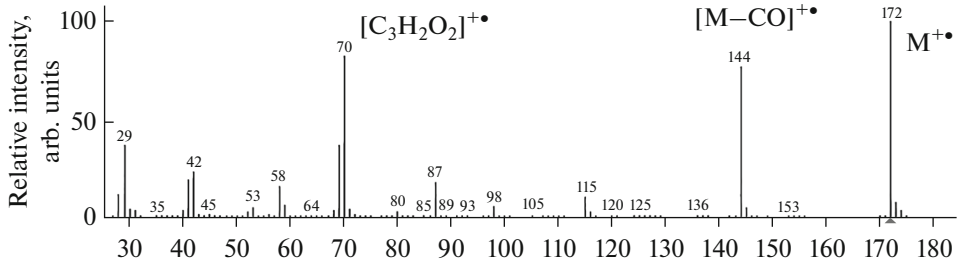
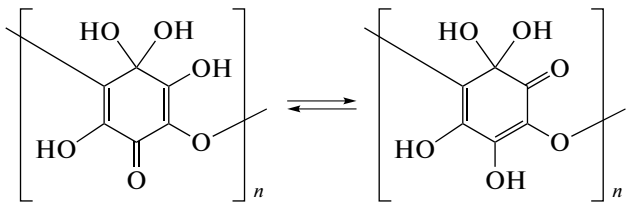
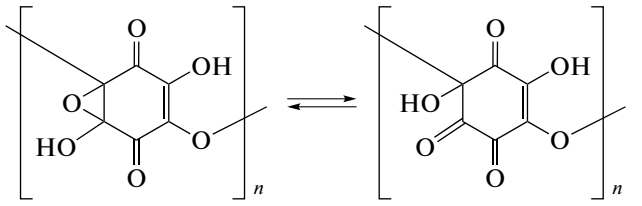


Fig. 8. Electron ionization mass spectrum of tetrahydroxy-*p*-benzoquinone [37].

We note that the elementary unit of sample **I** (according to TU 9154-001-54773261-02) [35] has a higher oxidation state, specifically $[C_6H_4O_6]$. This could be due not only to the presence of one of carbonyl groups in a hydrated form (which is typical for polyhydroxybenzoquinones) but also to the linking of benzoquinone fragments via an oxygen atom,



In other words, the presence of six oxygen atoms in the elementary unit of an oligomer can be rationally explained only if one of these atoms forms a bridge between the units. Such a structure includes a collection of atoms that closely matches the composition of the fragment $C_2H_2O_3$ with an m/z of 74 (cyclic fragment $-C(OH)_2-CO-$), which aligns with the mass spectrometry data. However, this structure also retains an element of uncertainty. One of the known mechanisms for the interaction of benzoquinone with hydrogen peroxide involves the epoxidation of the quinone at the C=C bond [25, 26], which might also lead to elementary units of the composition $C_6H_4O_6$, where the composition remains unchanged even after the potential opening of the epoxide ring due to tautomeric transformations,



An additional conclusion about the mechanism of the oxidation of hydroquinone by hydrogen peroxide was also drawn based on MALDI mass spectra. This conclusion does not require a detailed interpretation of the spectra. It was established that the primary oxidation product is indeed quinhydrone, not hydroquinone itself but a preformed molecular complex of hydroquinone with benzoquinone—quinhydrone—under a lower molar excess of hydrogen peroxide (1 : 2.5). The mass spectra of the resulting products were found to be identical, confirming this outcome,

$$(n/2)[(C_6H_6O_2)(C_6H_4O_2) + 2.5nH_2O_2 = (C_6H_4O_4)_n + 3nH_2O. \quad (3)$$

CONCLUSIONS

Oxidation of hydroquinone with hydrogen peroxide in the presence of iron(II) sulfate leads to complex mixtures of oligomers. The composition of these mixtures depends on the reagent ratio and varies from $(C_6H_4O_4)_n$ at a molar ratio of hydroquinone to hydrogen peroxide of 1 : 3, to $(C_6H_4O_6)_n$ at a ratio of 1 : 5. MALDI mass spectra of these mixtures in the range m/z below 1000 show no discernible patterns or periodicity in the mass-to-charge ratios of the signals. This indicates the absence of a regular sequence of uniform units in the oligomer chains, primarily due to the indeterminate ratio and irregular alternation of polyhydroxyphenylene and polyhydroxybenzoquinone fragments. The lack of periodicity in the mass-to-charge ratios of the signals can also result from several additional factors, including the formation of stable hydrates, peroxides, and, ultimately, molecular complexes during the interaction of hydroxyphenylene and benzoquinone fragments within the oligomer chains.

Nevertheless, mass spectra of the products of the reaction of hydroquinone with hydrogen peroxide in the $m/z > 1000$ range revealed signals characterized by a periodicity with a mass difference of $\Delta(m/z) = 74$. This difference corresponds to neither hydroquinone nor benzoquinone fragments and can only be attributed to a particle of a composition of $C_2H_2O_3$. Given that a similar mass difference is observed in the electron ionization spectrum of tetrahydroxy-*p*-benzoquinone, the detection of such a periodic element can be considered an indirect confirmation of the presence of polyhydroxy-*p*-benzoquinone fragments in the structure.

The available spectral information does not allow for a direct determination of the type of a linkage between the elementary units in the oligomers: whether it is C–C or C–O–C between the polyhydroxyphenylene and polyhydroxybenzoquinone fragments. However, the formation of an oligomer of a composition of $(C_6H_4O_6)_n$, which contains six oxygen atoms in the elementary unit, is most rationally explained if one of the oxygen atoms forms a bridge between the units.

Overall, it can be concluded that the high lability of the oligomeric products formed by the oxidation of hydroquinone with hydrogen peroxide—characterized by their ease of both oxidation and reduction—makes it impossible to determine their structure definitively. This highlights the unique nature of these products.

Moreover, it is worth noting the somewhat unconventional interpretation of MALDI mass spectra in the low m/z range. The absence of interpretable signals and periodicity elements has, nevertheless, proven to be highly informative for drawing important conclusions about the chemical nature of the oligomeric mixtures.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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