Hybrid macromolecular antioxidants based on hydrophilic polymers and sterically hindered phenols

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A novel class of biologically active substances was created. These are hybrid macromolecular antioxidants (HMAO) based on hydrophilic polymers with chemically grafted sterically hindered phenols with different structural parameters. The antiradical activities of HMAO were assessed in reactions with 2,2-diphenyl-1-picrylhydrazyl and the corresponding sodium sulfonate in various solvents. The mechanism that explains the substantially enhanced activities of HMAO in water was proposed. The state of HMAO in solutions was studied by viscosimetry and photon correlation spectroscopy. HMAO were assayed in biological models.

Key words: hybrid bioantioxidants, sterically hindered phenols, hydrophilic polymers, antiradical activity, mechanism, aggregates, hydrodynamic properties, plasma substitutes.

Sterically hindered phenols (SHP) are the most representative and popular class of synthetic antioxidants used to solve problems of both applied and fundamental character. The first-generation phenolic antioxidants (ionol, simple substituted 2,6-di(*tert*-butyl)phenols, phenozans) have been widely employed as inhibitors of the thermooxidative degradation of polymers, oils, fats, fuels, *etc.*, as well as correctors of oxidant pathologies in living biological systems. However, a modern approach to creation of bioantioxidants requires narrower specialization and combination of the antioxidant properties with the capability for target delivery and structural interactions with the area to be protected within a biosystem. This can be reached in hybrid molecules whose separate fragments provide the desired polyfunctionality.[1] Examples of such hybrids include "floating" SHP containing a charged onium group (anchor) bound to a lipophilic long-chain alkyl substituent (float).[2] Such a structure enables the antioxidant to efficiently interact with the charged lipid bilayer of cell membranes that need antioxidant protection for maintenance of a normal level of lipid peroxidation.

A novel class of hybrid macromolecular antioxidants (HMAO) developed and studied over the last few years is based on hydrophilic biopolymers and synthetic polymers designed for medical purposes with SHP fragments chemically grafted to the polymer chain. This approach (called biomimetic) to the design of biologically active compounds (BAC) was successfully applied in the 1950—1960s for creation of a number of polymeric forms of BAC. Both biopolymers (cellulose, starch, and chitosan) and water-soluble synthetic polymers (polyvinylpyrrolidone, polyacrylic acid, polyvinyl alcohol, *etc.*) were used as the base polymers[3] to develop highly efficient biocatalysts, immunoactive drugs, polymeric derivatives of various low-molecular bioregulators, biocides, *etc.*[4—8]

Such macromolecular systems have some advantages, *e.g.*, adjustable solubility, prolonged action, and the enhanced stability and reduced toxicity of BAC. For instance, artificial biocatalysts are usually more resistant to denaturing effects and can be used repeatedly.[6]

In the structure of polymeric hybrids, the nature of linkage between the polymer and BAC is a substantial element.[5,9] An ether bond is stable in aqueous solutions over a wide pH range (as well as in biological media). This makes it possible to create compounds in which the attached BAC is active in the hybrid form, not as the liberated BAC molecule. This can be used to estimate how much the biological activity of BAC has changed upon its irreversible immobilization. When the molecule contains a labile covalent (acetal, aldimine, or ester) bond linking

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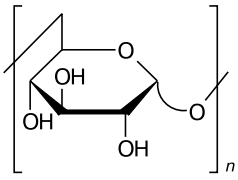
BAC with the polymer, the active substance can be liberated from the hybrid at different rates depending on the conditions (pH and temperature). It is the possibility of gradual hydrolysis that makes polymeric derivatives of plant growth regulators highly efficient. In contrast, hybrids acting as immunomodulators are effective in the polymer form only.[5]

Despite a sufficiently wide range of relevant publications and achievements in this field, so far many problems associated with the effect of the structural parameters on the activity of hybrid BAC are being solved empirically. With HMAO as an example, we implement, probably for the first time, a comprehensive study that includes development of methods of synthesis, structural identification, physicochemical characteristics and their dependence on the medium, assay with biological models, and estimation of the most promising ways of practical applications. Such a study is possible because of a structural variety of HMAO that differ in the nature and molecular weight (M) of the base polymer, the number of grafted SHP fragments, the type of the covalent bond between SHP and the polymer chain, and the nature and the length of the spacer between the SHP core and the polymer.

Results and Discussion

Hydroxy-containing hydrophilic polymers such as dextran (**1**) with M = 6000, 10 000, 18 000, 40 000, 70 000, and 200 000, hydroxyethylated starch (**2**) with M = 200 000, and polyvinyl alcohol (**3**) with M = 10 000 were employed as macromolecular bases in the synthesis of HMAO. These polymers are widely used in biology and medicine (*e.g.*, for preparation of colloidal plasma substitutes).[10]



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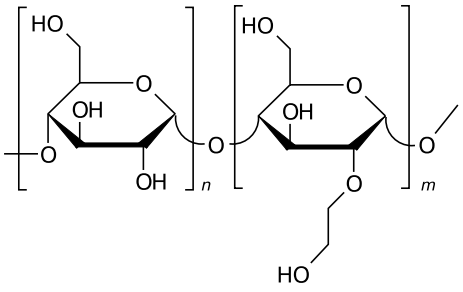

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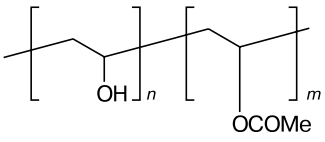
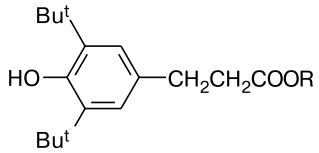


3

For chemical modification, we used functionalized 2,6- and 2,4-di(*tert*-butyl)phenols capable of interacting with the hydroxy groups of the polymers: β-[3,5-di(*tert*-butyl)-4-hydroxyphenyl]propionic acid (phenozan (**4a**)), 3,5-di(*tert*-butyl)-4-hydroxycinnamic acid, 4-bromo- or 4-acetoxymethyl-2,6-di(*tert*-butyl)phenol (BP), 4-[3,5-di(*tert*-butyl)-4-hydroxybenzylidene]-5-oxo-2-phenyl-4,5-dihydrooxazole, 2,6-di(*tert*-butyl)-4-(2-tosyloxy)ethoxymethylphenol, and 2,6-di(*tert*-butyl)-4-(4-tosyloxy)butoxymethylphenol.



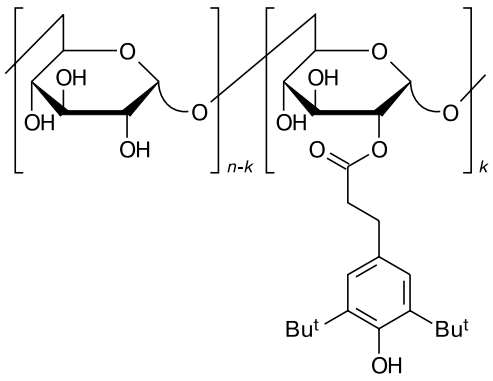
4a,**b**

R = H (**4a**), K (**4b**)

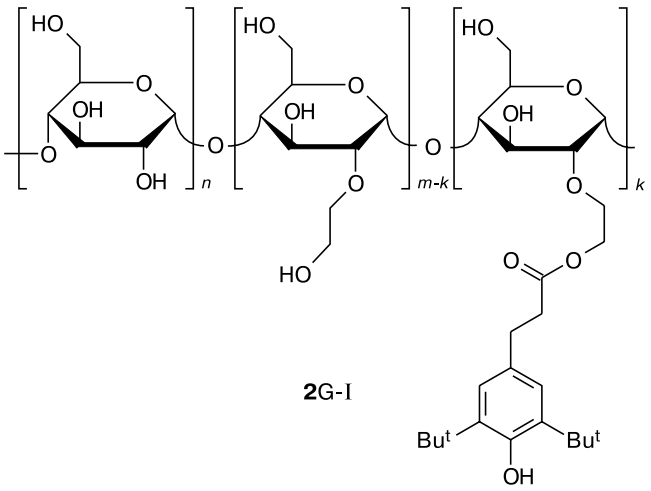
Several series of HMAO with different structural parameters were synthesized. Hybrids of the first series (HMAO-I) with ester bonds SHP—polymer were prepared by condensation of the polymer with phenozan (**4a**) in the presence of DCC and DMAP. By varying the ratio and concentrations of the reagents and the esterification time, we obtained hybrids based on dextran (**1**G-I), hydroxyethylated starch (**2**G-I), and polyvinyl alcohol (**3**G-I) with different degrees of substitution of the polymer units with SHP fragments (γ, mol.%). Hybrids **1**G-I were synthesized from dextrans with different molecular masses.[11–13] The γ values were determined by UV spectrophotometry (aromatic chromophore) and ^{1}H NMR spectroscopy that gave identical results.

We determined the solubilities of HMAO in water, which depend on the polymer and γ. The maximum degree of replacement at which HMAO dissolved completely (threshold water solubility) was 10 mol.% for **1**G-I, 6 mol.% for **2**G-I, and 2 mol.% for **3**G-I. With a further increase in γ, HMAO was dissolved in aqueous organic solvents (water—ethanol and water—dioxane); at the highest γ value obtained (for **1**G-I with M = 40 000, γ_{max} = 47.7 mol.%), the polymeric product was dissolved in dioxane, chloroform, and benzene. All HMAO were well soluble in DMSO.

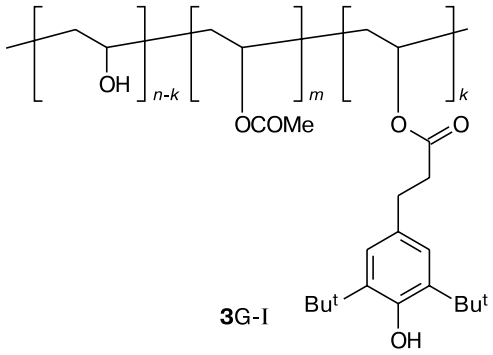
The second series of macromolecular antioxidants with ether bonds SHP—polymer (HMAO-II) was synthesized by reactions of polymers with 4-bromo- or 4-acetoxymethyl-2,6-di(*tert*-butyl)phenol. The resulting polymeric products contained different numbers of chemically bound



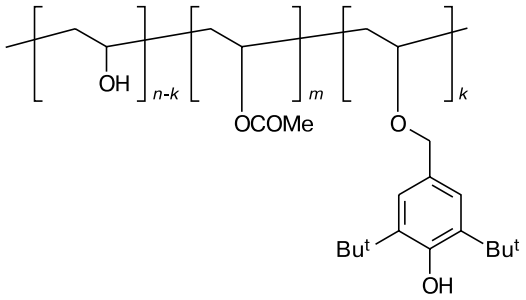
1G-I



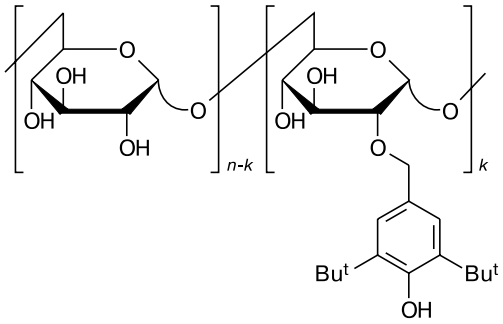
2G-I



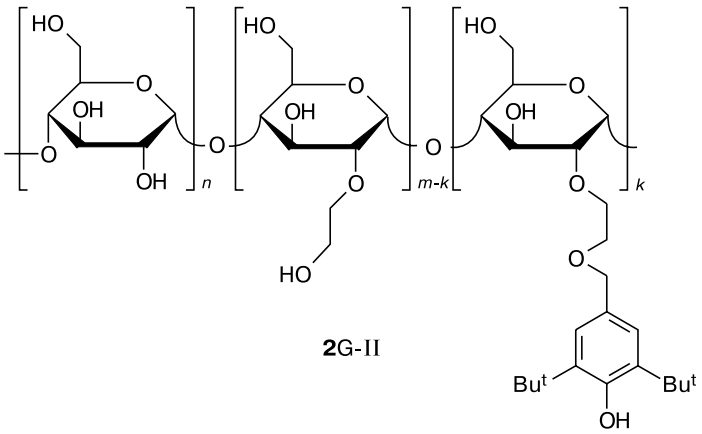
3G-I



3G-II



1G-II



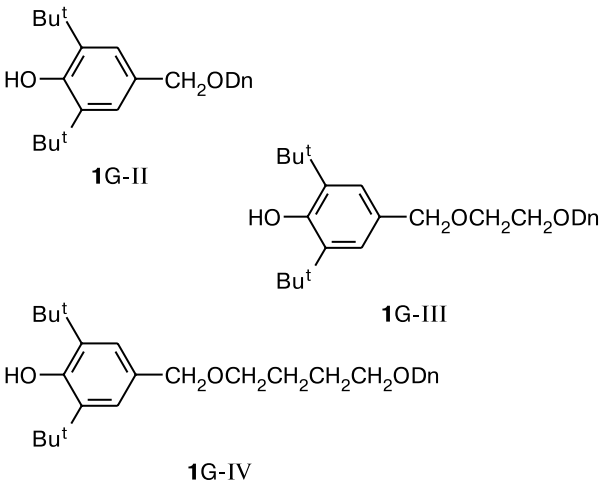
2G-II

SHP fragments and, consequently, were differently soluble in water.

A variant of HMAO-II are compounds obtained from 4-bromomethyl-2,6-di(*tert*-butyl)phenol and ethylene and butylene glycols. The synthesis of compounds of this series involved isolation of intermediate toluenesulfonates, which react with the hydroxy groups of the polymer (Scheme 1).

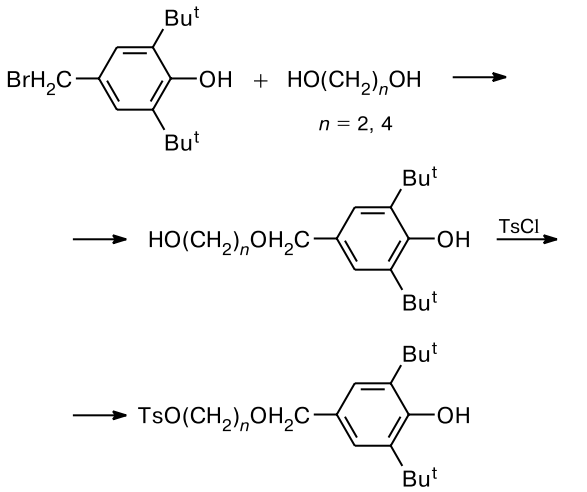
This method of synthesis of HMAO allowed us to vary the length of the spacer between the SHP core and the backbone chain of the polymer (dextrans (Dn) **1** with M = 1500, 6000, 10 000, 18 000, 40 000, 70 000, and 200 000).

To estimate the antioxidant activity of the HMAO obtained, we studied the kinetics of their reactions with the free radical 2,2-diphenyl-1-picrylhydrazyl (**5a**$^{\bullet}$) (Scheme 2). A correlation between the observed antiradical and antioxidant activities has been confirmed[14,15] with reactions of low-molecular SHP with **5a**$^{\bullet}$ as examples. Therefore, reactions of HMAO with **5a**$^{\bullet}$ can serve as



a test for quantitative estimation of their antioxidant properties.

Scheme 1



Scheme 2

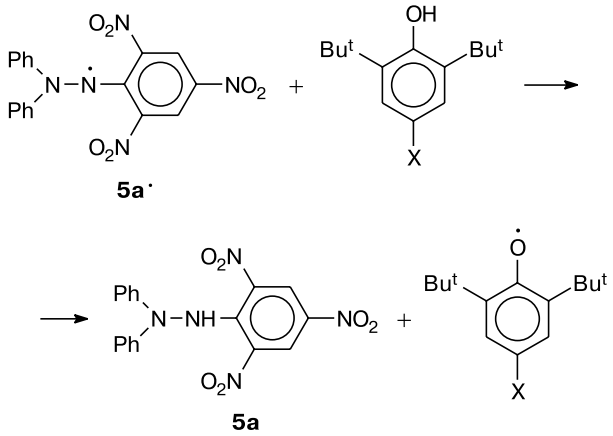


Table 1. Rate constants of the reactions of **5a**• with the antioxidants in various solvents

Antioxidant	K/L (mol s)$^{-1}$		
	Chloroform	Benzene	Dioxane
Phenozan	0.188±0.009	0.142±0.008	0.056±0.004
1G-I (γ = 47.7%, M = 40 000)	0.153±0.008	0.083±0.005	0.135±0.007

The reactions of polymeric antioxidants with **5a**• were carried out at 20 °C, showing the pseudofirst order with respect to the radical. The rate constants of the reactions with **5a**• were measured in both organic solvents (benzene, chloroform, and dioxane) and dioxane—water mixtures with different ratios of the components.

The antiradical effect with respect to **5a**• is usually tested in nonpolar solvents such as benzene, hexane, carbon tetrachloride, and dioxane.[5,16]

We found that the rate constants of the reactions of low-molecular SHP with **5a**• in chloroform and benzene are higher than those for HMAO, while in dioxane (Table 1) and in aqueous dioxane (Fig. 1), the pattern was opposite.

With an increase in the water content in aqueous dioxane (see Fig. 1), the rate constants of the reactions of both low-molecular SHP and **1**G-I with **5a**• increase. Interestingly, the higher the water content of the solvent the larger the difference between the rate constants of the reactions of **5a**• with both antioxidants.

Thus, the composition of the medium strongly influences the rate of the reactions of the antioxidants with **5a**•, **1**G-I being much more sensitive to variations in its composition. For instance, at the highest water content of aqueous dioxane, **1**G-I have the highest rate constants of the reactions with **5a**• compared to low-molecular SHP.

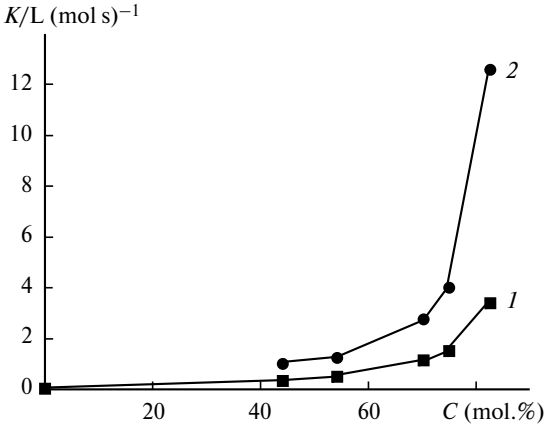
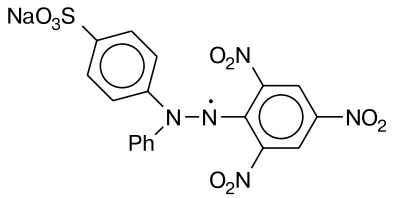


Fig. 1. Plots of the rate constants of the reactions of **5a**• with low-molecular SHP (*1*) and **1**G-I (γ = 10.4%, M = 40 000) (*2*) *vs.* the composition of the mixed solvent (C is the water content in aqueous dioxane).

In connection with this, we found it expedient to study the antiradical activity in water and other solvents (dioxane and aqueous dioxane) and establish a correlation between the activity and the medium polarity. To this end, we synthesized sodium 2,2-diphenyl-1-picrylhydrazylsulfonate (**5b**$^{\bullet}$), which is a water-soluble analog of **5a**$^{\bullet}$.[16]



5b$^{\bullet}$

In aqueous dioxane, radical **5b**$^{\bullet}$ proved to be less active than **5a**$^{\bullet}$, regardless of the antioxidant (Table 2). As in the reactions with **5a**$^{\bullet}$, a mixture of chemically nonbound dextran and phenoxan (potassium β-[3,5-di(*tert*-butyl)-4-hydroxyphenyl]propionate (**4b**)) reacted with **5b**$^{\bullet}$ at the same rate constant as did the reference low-molecular antioxidant **4b**. Thus, the comparison of the antiradical effects on both the free radicals showed the correctness of using **5b**$^{\bullet}$ in such investigations.

The influences of the molecular mass of the starting dextran and the degree of substitution γ of the glycoside units with the SHP fragments on the antiradical activity of the resulting hybrid compound were studied with **5b**$^{\bullet}$ in aqueous dioxane (1 : 1) (Fig. 2). It can be seen that the rate constants for **1**G-I are virtually γ-independent, varying within the experimental error (3.5±0.2 L (mol s)$^{-1}$). Another pattern was observed for **1**G-II. Up to γ = 12%, these hybrid compounds were more active than **4b**. However, with an increase in γ, they became as active as or even less active than compound **4b**. An increase in the molecular weight of HMAO from 10 000 to 200 000 lowered the rate constants by half.

In water alone, HMAO was more active than low-molecular antioxidant **4b** (see Fig. 1, Table 3).

It is worth noting that the difference between the rate constants for **1**G is determined by the nature and length of the spacer. The highest rate constant was obtained for **1**G-I with an ester bond. One can infer from the presented data that it is the hybrid structure of the polymeric antioxidant that determines the observed substantially higher antiradical effects of HMAO with respect to both free radicals in water and an aqueous organic solvent.

Table 2. Rate constants of the reactions of the antioxidants with the free radicals in aqueous dioxane (1 : 1)

Antioxidant	Solubility in water	K/L (mol s)$^{-1}$	
		5a$^{\bullet}$	**5b**$^{\bullet}$
Dextran (M = 40000)	+	0	0
Phenoxan (**4b**)	+	10.0±0.5	3.0±0.2
Phenozan (**4a**)	—	3.4±0.2	1.2±0.05
Phenoxan—dextran (1 : 10)	+	10.0±0.5	3.0±0.2
Phenozan—dextran (1 : 10)	—	3.4±0.2	1.2±0.05
1G-I (γ = 10.0 mol.%)	+	12.5±0.8	3.5±0.2

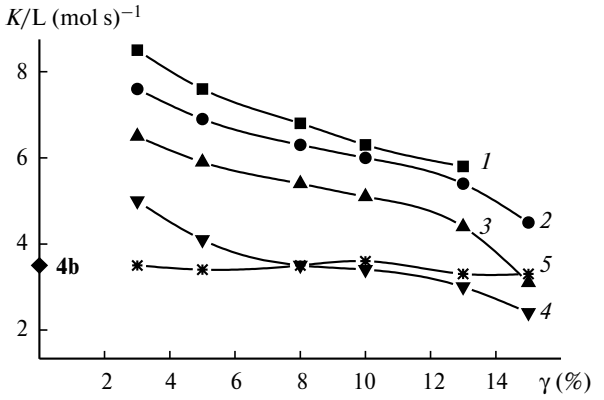


Fig. 2. Plots of the rate constants of the reactions of **5b**$^{\bullet}$ with **1**G-II (*1*—*4*) and **1**G-I (*5*) in aqueous dioxane (1 : 1 v/v) *vs*. the degree of substitution γ of glucose units in dextrans with M = 10 000 (*1*), 40 000 (*2*), 70 000 (*3*), 200 000 (*4*), and 40 000 (*5*).

It is known that the nature of the medium usually has a weak influence on the rates of radical reactions. However, such an influence was revealed in our study of the antiradical activity of HMAO in media with different polarities. This is evident from the plot of the rate constant *vs*. the composition of the solvent (Fig. 3).

With a decrease in the water content (the minimum water content is 30%, which is associated with the solubility of **1**G), the rate constants diminished, being close for all the antioxidants at equal water : dioxane ratios.

Such a pronounced dependence on the medium composition contradicts the generally accepted radical mechanism of hydrogen transfer from the SHP molecule to compound **5b**$^{\bullet}$ (see Ref. 16) (Scheme 3, pathway *a*).[5]

Table 3. Rate constants of the reactions of HMAO and phenoxan (**4b**) with **5b**$^{\bullet}$ in water

Antioxidant	K/L (mol s)$^{-1}$
1G-I	1100±50
1G-II	270±13
1G-III	356±18
1G-IV	28.5±1.5
2G-I	544±18
2G-II	226±10
3G-I	95±5
3G-II	70±3
4b	35.7±1.2

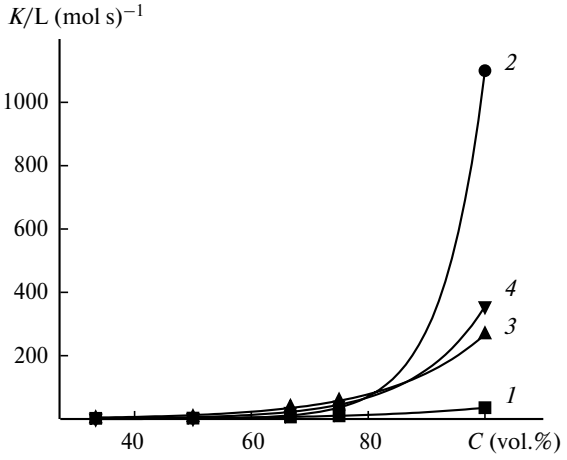


Fig. 3. Plots of the rate constants of the reactions of **5b**• with phenoxan **4b** (*1*) and various dextran-based HMAO (M = 40 000) (*2—4*) *vs.* the composition of the mixed solvent: (*2*) **1**G-I (γ = 9.1%), (*3*) **1**G-II (γ = 9.1%), and (*4*) **1**G-III (γ = 9.1%).

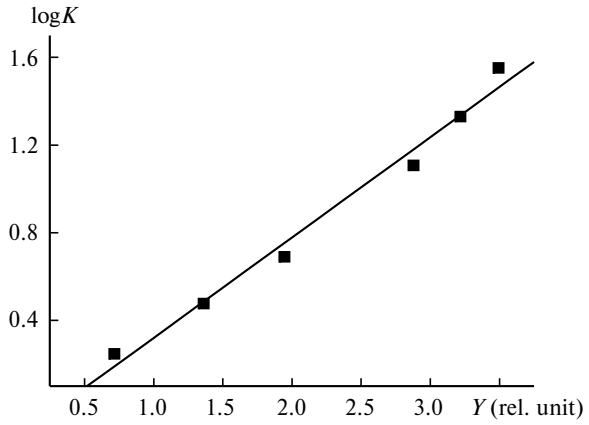


Fig. 4. Correlation between the logarithms of the rate constants log$K$ of the reaction of **5b**• with phenoxan **4b** and the ionizing capacity of aqueous dioxane.

One can assume that the mechanism of this reaction in aqueous media involves the intermediate formation of a radical cation (Scheme 3, pathway *b*).

It is known that the kinetics of the process involving radical cations is described by the Grünwald—Winstein equation:[17]

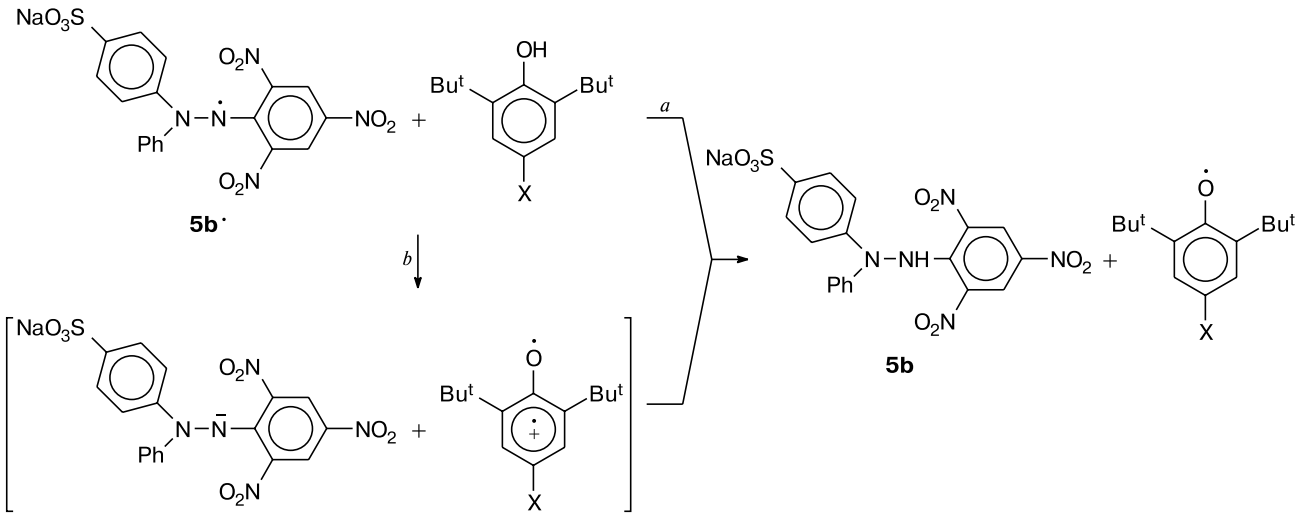
$$\log K/K_0 = m^*Y,$$

where Y is a measure of the ionizing capacity of the solvent, m is a factor that reflects the sensitivity of the reaction to a change in the ionizing capacity of the solvent, K is the reaction rate constant, and K_0 is the reaction rate constant in a standard solvent (80% ethanol).

This criterion was used to confirm the radical-cation mechanism of the reactions under study. For this purpose, we investigated the kinetics of the reaction of **5b**• with phenoxan (**4b**) in mixed solvents with known ionizing capacities (Y) and plotted the logarithm of its rate constant versus Y; the correlation coefficient was 0.992 (Fig. 4). The resulting linear plot indicates that the reaction kinetics obeys the Grünwald—Winstein equation and, consequently, confirms the radical-cation mechanism of the reaction.

The enhanced antiradical activity of HMAO compared to the low-molecular antioxidant in aqueous media may be due to the solvent shell of the macromolecule. The properties of water in the solvent shell of hydrophilic polymers differ from those of ordinary (bulk) water in increased ionizing capacity because of different orientations and mobility of water molecules and breaking of hydrogen bonds between them.[18,19] This water destructurization reduces the energy required to break hydrogen bonds in the solvation of radical cations.

Scheme 3



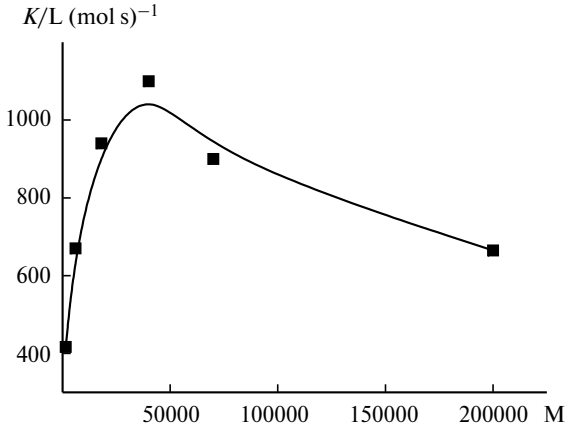


Fig. 5. Plot of the rate constants of the reaction of **1**G-I (γ = 9 mol.%) with **5b**$^{\bullet}$ in water (T = 20 °C) *vs.* the molecular mass of dextran.

In aqueous solutions, SHP fragments covalently bound to the polymer are most likely surrounded by solvent shells in which they seem to interact with the radical. The higher ionizing capacity of the solvent shell of the macromolecule favors better stabilization of the radical-ion transition state; this explains why hybrid compounds are highly active in water but become less active when the water content of the mixed solvent decreases.

The influence of the solvent shell on the reaction of HMAO with **5b**$^{\bullet}$ can be traced in the series of HMAO with different molecular masses and different spacer lengths.

The highest rate constants for the reaction with **5b**$^{\bullet}$ were obtained for **1**G-I (see Table 3). The rate constants for **1**G-II and **1**G-III were lower, while **1**G-IV with the longest spacer is as active as low-molecular SHP **4b**. This can be explained by the escape of the SHP core with this spacer from the solvent shell.

The influence of the solvent shell on the reaction of HMAO with **5b**$^{\bullet}$ is responsible for the appearance of a maximum on the plot of the rate constant *vs.* the molecular mass of the base polymer (Fig. 5). According to the literature data,[18,19] an increase in M of hydrophilic polymers enlarges the solvent shell and hence the reaction of **5b**$^{\bullet}$ with HMAO should be accelerated. However, above a certain M value, interactions of radicals with phenol fragments are sterically hindered, which results in the lowering of the rate constants.

Therefore, the nature of the spacer is one of the most significant factors that influence the antiradical activity of HMAO. Interpretation of this fact was substantially contributed by a study of the hydrodynamic properties of HMAO by viscosimetry and photon correlation spectroscopy (PCS)*.

* The investigations were carried out by S. K. Filippov and supervised by A. V. Lezov at the Department of Physics of the St. Petersburg State University.

Intrinsic viscosity is related to the speed of motion of macromolecules in a solvent and depends on the mass and shape of a polymer species in motion. Using photon correlation spectroscopy, one can determine the hydrodynamic radius of a light-scattering species (R_h). A combination of these two methods allows a qualitative description of the behavior of macromolecules in liquids. Dextran-based HMAO are studied best, because these compounds have a wider solubility range. The data from both methods were obtained for aqueous solutions and the aprotic solvent DMSO. In DMSO, in which hydrophobic interactions are weakened, the intrinsic viscosity η changes only slightly compared to the starting dextran, is virtually independent of γ, and equals 0.21—0.23 dL g^{-1}. In water, the intrinsic viscosities of the same products monotonically decrease from 0.13 dL g^{-1} for γ = 4% to 0.09 for γ = 9.4% (for the starting dextran, η = 0.16 dL g^{-1}). Such a shape of the dependence is explained by compactification of the macromolecules of modified dextran, which becomes more hydrophobic as the number of SHP fragments in the polymer chain increases.

This is supported by PCS data on dynamic light scattering. The spectrum of dextran shows two peaks due to species with very different sizes (Fig. 6). The left-hand peak corresponds to molecular sizes, while the right-hand peak corresponds to macromolecular aggregates. The same pattern was observed in DMSO, except that the aggregates are smaller than those in water.

Modification of dextran with SHP fragments dramatically changes the size distribution of species, lowers the polydispersity index, and gives rise to a pronounced peak that is narrower than the peak of the starting dextran. The species that correspond to this peak are much larger, for all HMAO, than their molecules. This suggests aggregation of modified dextran in aqueous solutions into species with R_h = 0.23—0.43 μm. In DMSO, the pattern is qualitatively the same, but the hydrodynamic radii are lower (R_h = 0.09—0.16 μm). Apparently, this accounts for the aforementioned fact that the antiradical activity of HMAO in DMSO and other organic solvents differs only slightly from that of the low-molecular analog SHP, being strongly dependent on the length of the spacer in aqueous solutions.

The hydrodynamic properties make HMAO well biocompatible; because of this, we estimated its antioxidant activity with several biological models. The range of optimum HMAO concentrations for biosystems was determined in the model of the hypotonic hemolysis of erythrocytes (Fig. 7).

The structural relation of HMAO to hydrophilic polymers used in medical practice as components of plasma substitutes prompted investigations of the properties of HMAO in the model of hemorrhagic shock (acute massive blood loss). Medicobiological tests were carried out at the V. A. Almazov Research Institute of Cardiology of

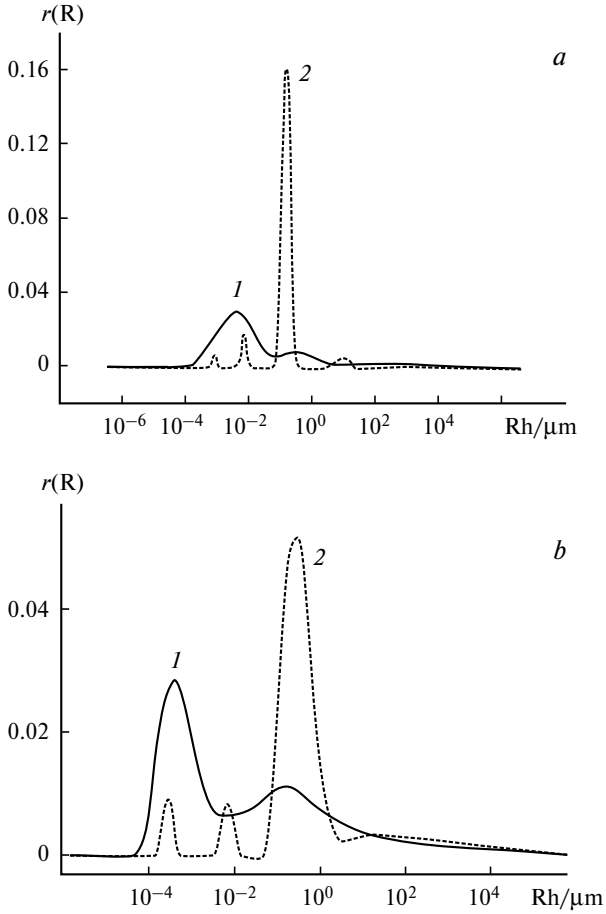


Fig. 6. Distribution of aggregates over the hydrodynamic radii for dextran (*1*) and dextran-based HMAO (γ = 8.2%) (*2*) in water (*a*) and DMSO (*b*) (C = 0.6 wt.%), $\theta = 90°$.

the Ministry of Public Health and Social Development of the RF.

Development of new means and methods for transfusional therapy belong to the most topical problems of modern medicine. Currently available plasma substitutes do not protect an organism that has experienced blood loss from post-transfusional complications (ischemia of organs and tissues, edemas, and encephalopathy) associated with an avalanche increase of oxidative processes after the restoration of bloodstream (oxygen paradox). The use of HMAO in plasma substitutes may be a solution to this problem. Laboratory tests in rats showed that in contrast to nonmodified plasma substitutes, compensation of blood loss with HMAO-containing ones ($2 \cdot 10^{-6}$ mol L^{-1} with γ taken into account) stabilizes the hemodynamic parameters 12—15 min after the beginning of infusion and keeps them constant throughout the observation time (~1 h). Infusion of required amounts of Reopolyglukin caused only a short-time hypertensive effect (Fig. 8), with edemas of tissues in 72% of rats.

A series of special experiments with massive blood loss preceded by infusion of HMAO-containing plasma substitutes revealed a 52% increase in the rat survival compared to a reference group. Thus, by using HMAO-containing plasma substitutes for compensation of blood loss, one can restore and maintain the hemodynamic parameters in animals at nearly the initial level, suppress free radical oxidation, and, consequently, prevent damage due to perfusion upon restoration of bloodstream.

The use of HMAO in plasma substitutes is an important but not unique line of their practical application. By varying the structural parameters, one can adapt HMAO to solution of various problems in biology, medicine, and agriculture. The latest data on the immunostabilizing and cytoprotective properties of HMAO[21] and on possible use of HMAO in formulations for low-temperature preservation of genetic materials provide convincing evidence for the necessity of further investigations of this class of bioantioxidants.

Thus, we created a novel class of phenolic antioxidants based on hydrophilic polymers with SHP fragments chemically grafted to the polymer chain and studied the

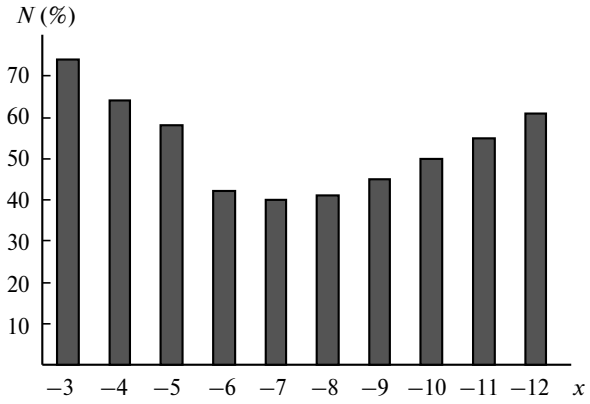


Fig. 7. Determination of the optimum concentration of HMAO (C_{HMAO}/mol L^{-1}) from the osmotic resistance of erythrocytes (upon blood loss); $C_{HMAO} = (1 \cdot 10^{x})$,where $x = -3 \div -12$ and N (%) is the number of lysated erythrocytes.

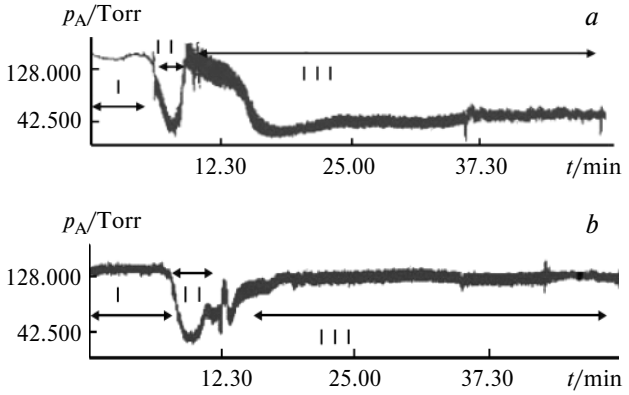


Fig. 8. Dynamics of the blood pressure level (P_A) on compensation of the blood loss with Reopolyglukin (*a*) and dextran-based HMAO (*b*).

structural factors of the high antiradical activity of HMAO in aqueous media. Using the kinetic method, we demonstrated that the mechanism of redox processes with HMAO in water involves the intermediate formation of radical ions. We considered an important role of supramolecular structures resulting from HMAO aggregation in aqueous media. Based on the PCS data on the structural features of solutions of HMAO, we explained why HMAO is differently active in organic and aqueous media. The biocompatibility and high antioxidant activity of HMAO was illustrated with living biological models. We outlined the main trends of practical application of HMAO.

Experimental

Commercial hydrophilic polymers with different molecular weights were used; their weights were additionally refined by viscosimetry on an Ubbelohde viscosimeter in water and DMSO at 25 °C. Polymers were purified by dialysis against water followed by freeze-drying.

Functionalized SHP that can etherify and esterify the OH groups of the polymer were used for the synthesis of HMAO. Typical procedures for the synthesis, isolation, and purification of HMAO are described in Ref. 12. The number of SHP fragments grafted to the polymer was determined by spectrophotometry from the absorption of the aromatic chromophore (λ_{max} = 275 nm, EtOH — water (1 : 1)) and expressed in terms of γ (mol.%) defined as the fraction of substituted units in the total number of the monomer units in the polymer. Phenozan was used as a reference compound. The reproducibility of the results was repeatedly confirmed by performing syntheses under identical conditions. The absence of low-molecular SHP in the reaction products was confirmed by gel permeation chromatography (Sephadex LH-20, EtOH—water (1 : 1)). The localization of SHP in the glucose unit of dextran followed from previous data[22,23] and was not studied further.

The kinetics of the reaction of HMAO with **5b**$^{\bullet}$ was monitored with an SF-56 spectrophotometer (LOMO) by recording changes in the optical density of **5b**$^{\bullet}$ at 520 nm (absorption peak). The molar extinction coefficient in water was 10 200±100 L (mol cm)$^{-1}$. The initial concentration of **5b**$^{\bullet}$ was $2 \cdot 10^{-5}$ mol L^{-1}; the initial concentration of HMAO was $4 \cdot 10^{-4}$ mol L^{-1} (with γ taken into account). The reaction was stopped when the conversion of **5b**$^{\bullet}$ reached 20%. In a control experiment, the concentration of the individual radical in aqueous dioxane remained unchanged upon 1-h irradiation in the cell at λ = 520 nm.

Dynamic light scattering experiments were carried out by PCS at 25, 38.8, and 50 °C on a Photo Cor Complex setup fitted with an automatic goniometer, a PhotoCor-FC real-time correlator, and a He—Ne laser (λ = 632.8 nm, ouput power 25 mW) as a light source. Scattering angles were 20—90°. The concentration of the polymer in solution was 0.6 wt.%. The signal acquisition time was 200—300 s. Autocorrelation functions were processed by the regulation method with the DyneLS program.

The properties of HMAO were studied with biological models of the hypotonic hemolysis of erythrocytes and hemorrhagic shock.

Model of the hypotonic hemolysis of erythrocytes. Hemolysis was carried out at 20 °C in a spectrophotometer cell containing 0.5% NaCl (2.5 mL) or a solution of HMAO (2.5 mL) in 0.5% NaCl. A suspension of erythrocytes (0.02 mL, ~$150 \cdot 10^7$ cells/mL) in saline was added with a micropipette. The kinetics of hypotonic hemolysis (yield of hemoglobin) was estimated from the diminished turbidity of the solution at λ = 800 nm. The efficiency of HMAO was calculated as a ratio of the degree of hemolysis with and without the antioxidant.

Model of hemorrhagic shock. Tests were carried out with male rats of the Sprague Dawley line (420—470 g) narcotized with Nembutal (intraperitoneal injection, 25 mg kg^{-1}). Blood pressure was measured directly in the femoral artery with a Baxter transducer and analyzed with the KardioPlus program. The heart rate was estimated from the intersystolic interval; the other femoral artery was used for bloodletting (specific blood volume 30 mL kg^{-1}, rate 0.8—1.0 mL (kg min)$^{-1}$). To compensate blood losses, the plasma substitutes Reopolyglukin and Hemodez ("Biokhimik", Saransk), 0.9% NaCl, the Locke—Ringer solution, and Infukoll (Serum-Werk Bernburg AG, Germany) were injected into rats of a reference group. Blood losses in rats of a test group were compensated with solutions of HMAO.

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