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Indicator Properties of Oligoethylene Glycol Hybrids with Sterically Hindered Phenols

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Abstract—Aqueous solutions of hybrid compounds (conjugates) formed by polyethylene glycols modified at the terminal hydroxy groups with sterically hindered phenol lose their phase stability at a certain temperature which depends on the molecular weight of polyethylene glycol, structure of sterically hindered phenol, conjugate concentration, and composition of the medium. This property may be used to estimate the effect of structural factors on the hydrophobic–hydrophilic balance in the conjugate–water system.

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Synthesis of hybrid compounds whose molecules contain fragments with different functionalities constitutes one of the most promising lines in the design of modern biologically active substances. Hybrid structures are advantageous due to enhanced efficiency, prolonged action, extended solubility range, and reduced toxicity [1].

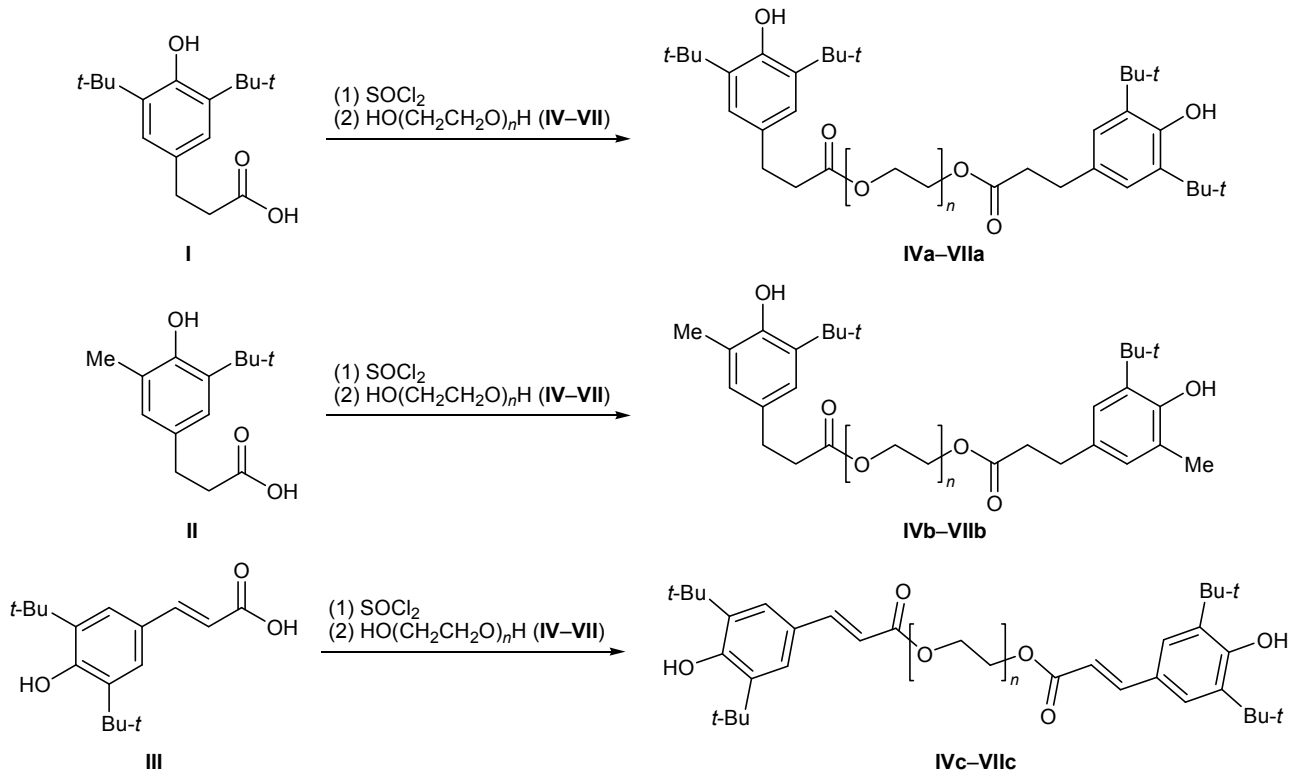
Hybrid macromolecular antioxidants derived from hydrophilic natural and synthetic polymers and sterically hindered phenols (SHP) exhibit in aqueous solution antiradical activity which considerably exceeds the activity of mixtures of the corresponding unbound SHP and polymer [2]. Studies on such antioxidants based on dextran showed that their enhanced antiradical activity is determined by hydrated structures formed in aqueous solution, by the nature of the spacer connecting the phenolic fragment and polymer chain, and by the position of redox-active phenolic fragment inside or outside the polymer hydration shell, which depends on the spacer length [3]. The highest activity was found for conjugates with C_3 spacers, which were prepared from 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propanoic acid (**I**), 3-(5-*tert*-butyl-4-hydroxy-3-methylphenyl)propanoic acid (**II**), and 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)prop-2-enoic acid (**III**) [4].

Polyethylene glycols (PEGs) are of particular interest as base polymeric structure for the synthesis of hybrid macromolecular antioxidants. Polyether backbone of PEG is chemically inert; on the other hand, PEGs are characterized by high biocompatibility, the lack of toxicity and immunogenicity, and good solubility in both water and organic solvents. The presence of terminal hydroxy groups in PEG macromolecules ensures their covalent bonding with various biologically active compounds. This approach is widely used

Table 1. Characteristics of conjugates **IVa**, **IVb**, **Va–Vc**, **VIa–VIc**, and **VIIa–VIIc**

Conjugate no.	*M*	Solubility in water at 25°C, *c* = 3.0 g/dL	Concentration of SHP fragments in conjugate, wt %
IVa	3400	–	13.3
Va	3900	+	11.8
VIa	6800	+	7.1
VIIa	21600	+	2.4
IVb	3400	+	11.4
Vb	3900	+	10.0
VIb	6800	+	6.5
VIIb	21600	+	2.0
Vc	3900	+	11.5
VIc	6800	+	7.1
VIIc	21600	+	2.4

Scheme 1.



IV, $n = 77$; **V**, $n = 89$; **VI**, $n = 155$; **VII**, $n = 491$.

in the design of PEG-based dosage forms of various drugs, cosmetics, and perfumes [5].

Up to now, a number of conjugates have been synthesized via condensation of acid **I**–**III** chlorides with polyethylene glycols having molecular weights of 3400 (**IV**), 3900 (**V**), 6800 (**VI**), and 21600 (**VII**) [6, 7] (Scheme 1). The electronic absorption spectra of the obtained conjugates are shown in figure, and their other characteristics are given in Table 1. All conjugates displayed a high antiradical activity in aqueous–organic medium; their activity was assessed by measuring the rate constants k for their reaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Tables 2, 3). The antiradical activity of conjugates based on acid **II** was higher by a factor of ~1.5–2 than the activity of those based on acid **I**. We failed to evaluate the rate constants k for conjugates **Vc**–**VIIc**, for they reacted with DPPH almost instantaneously.

It is known that the system PEG–water is characterized by a critical mixing temperature; below that

Table 2. Rate constants for the reactions of sterically hindered phenol derivatives and conjugates with DPPH in aqueous ethanol (1 : 1 by volume) at 20°C

Antioxidant	k, l mol^{-1} s^{-1}
I	3.1±0.1
VIIa	45.4±2.8
VIIb	87.2±4.7
VIIc	–[a]

[a] The rate constant was not determined because of very fast reaction.

Table 3. Rate constants for the reactions of sterically hindered phenol derivatives and conjugates with DPPH in aqueous dioxane (1 : 1 by volume) at 20°C

Antioxidant	k, l mol^{-1} s^{-1}	Antioxidant	k, l mol^{-1} s^{-1}
I	1.7±0.05	**Vb**	38.1±1.50
IVa	18.4±0.90	**VIb**	41.4±1.70
Va	30.1±1.50	**VIIb**	40.3±1.7
VIa	24.6±1.20	**III**	–[a]
VIIa	21.1±1.20	**Vc**	–[a]
II	3.9±0.09	**VIc**	–[a]
IVb	44.2±1.90	**VIIc**	–[a]

[a] The rate constant was not determined because of very fast reaction.

Table 4. Phase separation temperatures (T_{PS}) of aqueous conjugate solutions

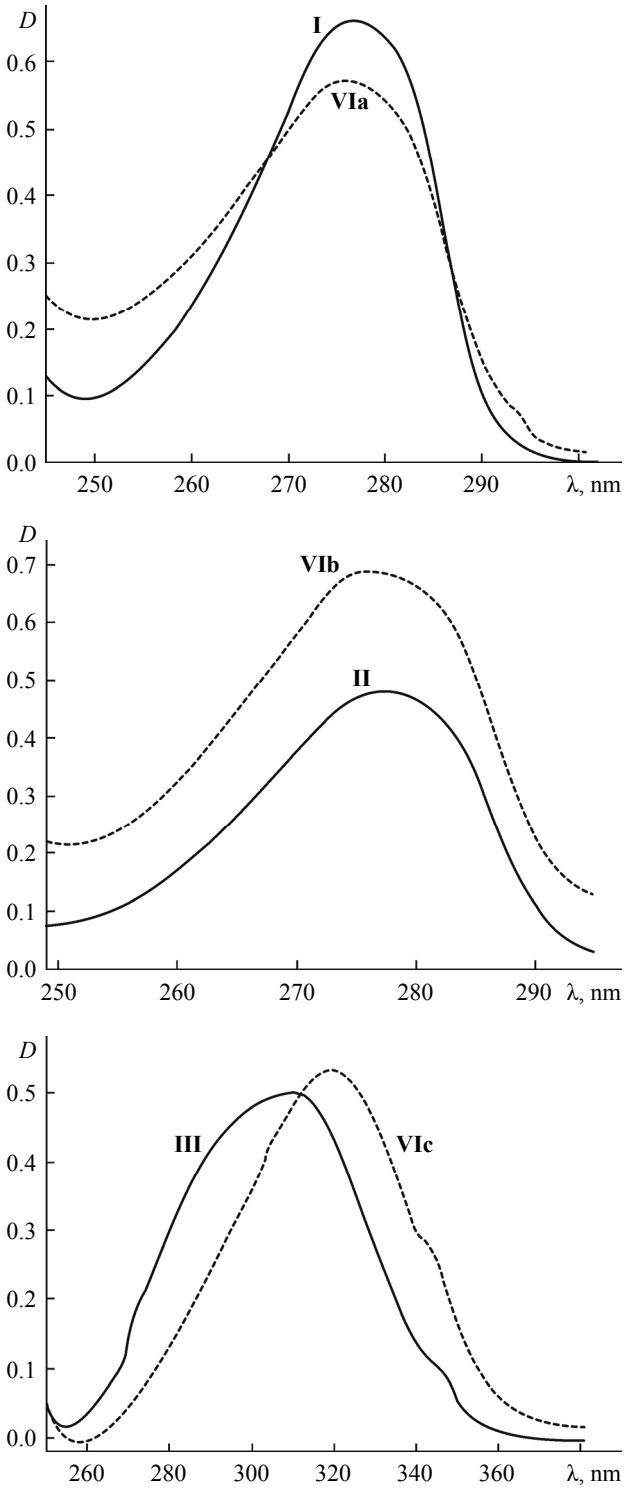
Conjugate no.	Phase separation temperature, °C
IVa	22
Va	38
VIa	64
VIIa	>100
IVb	43
Vb	54
VIb	75
VIIb	>100
Vc	49
VIc	76
VIIc	>100

Table 5. Phase separation temperatures of solutions of conjugate **Vc** (c = 3.41 g/100 g of water) at different NaCl concentrations

Concentration of NaCl, g/100 g of water	Phase separation temperature, °C
0	49
0.360	48.5
0.975	48
2.933	39.5

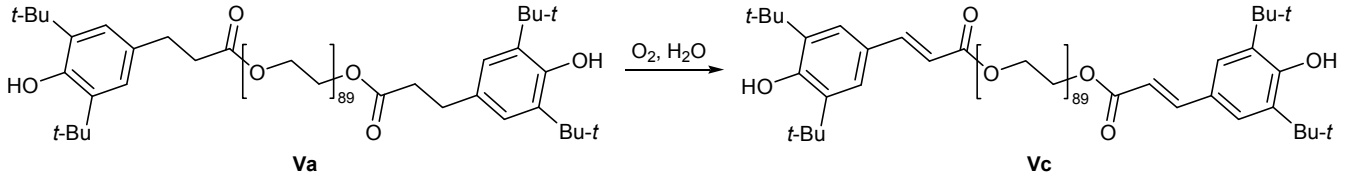
temperature no phase separation can be achieved regardless of the polymer concentration. If the temperature is higher, the system divides into layers, and polymer separates from the solution. The critical mixing temperature for PEG with M 5000 is above 100°C; as the molecular weight of PEG increased to 5 000 000, the critical mixing temperature decreased to 20°C [8]. Thus, the critical mixing temperature decreases as the molecular weight of PEG rises.

As expected, no phase separation was observed in aqueous solutions of PEG used for the synthesis of conjugates in the temperature range from 15 to 100°C. Aqueous solutions of the conjugates became turbid with subsequent phase separation and precipitation as the temperature rose. Table 4 contains the phase separation temperatures measured for a conjugate concentration of 3%. Increase of the phase separation temperature with rise in the molecular weight of conjugates based on the same SHP derivative should be noted. This is likely to be related to a shift of the hydrophobic–hydrophilic balance of conjugate toward higher hydrophilicity as the molecular weight increases. On the other hand, variation of the phase separation temperature for conjugates with the same molecular weight but different SHP fragments reflects the relative hydrophobicities of the SHP fragments,



Electronic absorption spectra of sterically hindered phenols **I**–**III** and conjugates **VIa**–**VIc**; c = 0.1 (**I**), 0.9 (**VIa**), 0.06 (**II**), 1.4 (**VIb**), 0.007 (**III**), 0.06 g/L ((**VIc**).

Scheme 2.



which change in the series **I** > **III** > **II**. The stronger the hydrophobic interaction, the lower the phase separation temperature.

Prokopov et al. [9] studied metabolism of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propanoic acid (**I**) in animals and found that this acid undergoes autooxidation to 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)prop-2-enoic acid (**III**). We also observed autooxidation of conjugate **Va** (phase separation temperature T_{PS} = 38°C) to compound **Vc** (T_{PS} = 49°C) on storage for several months on exposure to atmospheric moisture and oxygen. This process was accompanied by a red shift of the absorption maximum from λ 280 nm for **Va** to λ 340 nm (**Vc**) due to appearance of conjugated C=C bond in the latter (Scheme 2).

The phase separation temperature is sensitive to the electrolyte composition of conjugate solution, which may be of crucial importance for the use of hybrid antioxidants in biological systems. According to the data of photon correlation spectroscopy, addition of sodium chloride to a solution of **Vc** leads to monotonic reduction of the phase separation temperature as the electrolyte concentration increases (Table 5).

Thus, the results of our study have shown that polyethylene glycol–sterically hindered phenol conjugates in aqueous solution behave as temperature-sensitive systems. The phase separation temperature of these systems depends on the conjugate structure and electrolyte composition, which may be used to estimate hydrophobicity of SHP fragments linked to PEG. The highest sensitivity to the SHP structure is observed for series **V** conjugates.

EXPERIMENTAL

The UV spectra were measured in the range from λ 240 to 400 nm on a Shimadzu UV 1800 spectrophotometer using quartz cells with a cell path length of 1 cm; aqueous ethanol (1:1) was used as solvent. The ^{1}H NMR spectra were recorded from solutions in $CDCl_3$ on a Bruker DPX-300 instrument.

Hydrodynamic properties of solutions of unmodified PEGs and conjugates were studied by viscometry and photon correlation spectroscopy on a PhotoCor® spectrometer [7]. The kinetics of the reactions of antioxidants with DPPH were monitored by spectrophotometry (Shimadzu UV 1800) by measuring the optical density of solutions at λ 520 nm according to the procedure described in [3].

3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)propanoyl chloride. Thionyl chloride, 0.2 mL (21 mmol), was added to a solution of 0.5 g (18 mmol) of acid **I** in 5 mL of chloroform, and the mixture was heated for 3 h under reflux with protection from atmospheric moisture. When the reaction was complete, the solvent and excess thionyl chloride were distilled off under reduced pressure.

Conjugates IVa–VIIa (*general procedure*). A solution of 0.25 g of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propanoyl chloride in 1 mL of chloroform and 0.2 mL of triethylamine were added to a solution of 0.5 g of PEG in 2 mL of chloroform. The mixture was purged with argon over a period of 5 min, continuously stirred for 4–5 h at 40°C, and left overnight at room temperature. The product was precipitated by pouring the mixture into 60 mL of isopropyl alcohol–petroleum ether (2:1), separated on a centrifuge, washed twice with the precipitating mixture, filtered through a Schott filter, washed with diethyl ether, and dried in air. The product was additionally reprecipitated from chloroform into diethyl ether, filtered through a Schott filter, washed with diethyl ether, and dried under reduced pressure. The presence of low-molecular-weight impurities was checked by TLC and NMR. ^{1}H NMR spectrum of **Va**, δ, ppm: 1.39 s (18H, *t*-Bu), 2.59 br.t (2H, CH_2CO), 2.83 br.t (2H, $CH_2C_6H_2$), 3.61 d (178H, CH_2OCH_2), 4.20 br.t (2H, $COOCH_2$), 5.05 s (1H, OH), 6.95 br.s (2H, H_{arom}).

Conjugates **IVb–VIIb** and **IVc–VIIc** were synthesized in a similar way from polyethylene glycols **IV**–**VII** and sterically hindered phenols **II** and **III**.

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