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Kinetics of Reactions of Antioxidants from Some Food and Medicinal Plants with the Stable Radical 2,2-Diphenyl-1-Picrylhydrazyl

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Abstract—The run of kinetic curves and the changes in the rate of the reaction of the stable radical 2,2-diphenyl-1-picrylhydrazyl with antioxidants present in extracts from some food and medicinal plants and with some individual natural phenolic antioxidants in ethanol have been investigated spectrophotometrically at different hydrogen chloride concentrations. The contributions from different mechanisms to the kinetics of these processes have been evaluated. The choice of optimal hydrogen chloride concentrations facilitating a comparison of the kinetic parameters obtained for various objects (3–10 mmol/L) has been substantiated. Comparative antiradical activity has been measured for complex samples obtained from food and medicinal plants.

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INTRODUCTION

The stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is often used in research practice for the determination of amounts of antiradical antioxidants (AOs) that are present in various objects [1–6]. A high reproducibility of results, selectivity toward antiradical AOs, and a high sensitivity and availability of the necessary equipment are advantages of the method.

It was observed in some works [7–10] that quantitative AO content data obtained for various objects by the method based on the AO–DPPH reaction and those obtained by other methods coincide closely.

Different mechanisms of the reactions of the AO with DPPH and other radicals were discussed [11–16]. These are a radical mechanism, namely, hydrogen atom transfer (HAT), its variant called proton coupling electron transfer (PCET), and ionic mechanisms, such as sequential proton loss–electron transfer (SPLET) and electron transfer–proton transfer (ET–PT). Some researchers accept the SPLET mechanism and deny ET–PT, and vice versa.

The reaction occurring via the HAT radical mechanism based on homolytic hydrogen atom abstraction from the AO molecule,

$$ArOH + DPPH^{\bullet} \rightarrow ArO^{\bullet} + DPPH\text{-}H, \qquad (I)$$

proceeds at the highest rate in nonpolar solvents.

The ionic mechanisms and their relative contributions to the reaction rates of the radicals with AOs in various reaction media will be considered in detail below.

A kinetic method for the determination of the comparative reactivity of the AOs present in multicomponent systems of plant origin toward DPPH was proposed in an earlier work [17]. In this method, the reaction is carried out in a 0.1 mM HCl solution in ethanol to significantly retard the process by suppressing the SPLET mechanism [14], which does not occur in the chain peroxidation of lipids in a hydrophobic phase. The addition of an acid to the reaction system excludes the unpredicted influence of organic acids present in objects of plant origin. In addition, the influence of the difference in pK_a between phenolic AOs on the kinetics of the process is excluded, so the reaction rate depends mainly on the dissociation energy of the OH bond in phenols. It was experimentally proved in later studies that the SPLET mechanism is not completely suppressed in reactions of DPPH with extractable substances of some food plants at a hydrogen chloride concentration of 0.1–1 mmol/L.

The purpose of the present study was to carry out a detailed analysis of the kinetics of the reactions considered here for the interaction of AOs from some food and medicinal plants with the stable radical DPPH and to interpret kinetic data for this interaction taking into account different reaction mechanisms.

EXPERIMENTAL

The objects of this study were aqueous and ethanolic extracts of food and medicinal plants (Tables 1, 2) and individual phenolic AOs that are abundant in the vegetable world, namely, rutin (Roth) and gallic acid (Serva). Ethanol (azeotropic ethanol–water mixture) was distilled with a reflux condenser. Extraction with ethanol, water, or their mixture was carried out for 30 min in a shaker. The objects studied (apples, onions, and garlic bulbs) were minced. Juice was squeezed out

Table 1. Antiradical activity of antioxidants in ethanolic extracts from some food and medicinal plants at an HCl concentration of 3.3 mmol/L

AO source	$w_0 \times 10^8$, mol $L^{-1} s^{-1}$	k_{eff}, L $mol^{-1} s^{-1}$
Lemon (fruit pulp)	13.0	260
Orange (fruit pulp)	11.0	220
Grapefruit (fruit pulp)	9.1	190
Onion (bulbs)	10.0	210
Garlic (bulbs)	4.0	81
Common tansy (leaves)	5.0	100
Hibiscus tea	24.0	500
Apple (Golden cultivar)	11.5	230
Red grapes (fruits)	7.5	150

$[DPPH]_0 = 6.5 \times 10^{-5}$ mol/L, $[AO]_0 = IC_{50}$, $T = 293$ K.

Table 2. Antiradical activity of antioxidants in ethanolic extracts from some food and medicinal plants at an HCl concentration of 0.1 mmol/L

AO source	$w_0 \times 10^8$, mol $L^{-1} s^{-1}$	k_{eff}, L $mol^{-1} s^{-1}$
Peppermint	2.0	41
St. John's wort	2.1	43
Lemon balm	2.4	49
Yarrow	2.3	48
Chamomile	2.6	53
Common tansy	2.0	41
Hibiscus tea	12.0	250
Apple (Golden cultivar)	5.0	100
Red grapes	4.3	87

$[DPPH]_0 = 6.5 \times 10^{-5}$ mol/L, $[AO]_0 = IC_{50}$, $T = 293$ K.

of citrus pulp, and AOs were additionally extracted from the remainder.

The reactions of the stable radical DPPH with the extractable substances present in the analyzed objects were carried out in ethanol [18] to record kinetic curves and to determine the initial reaction rate under the chosen standard conditions. The initial reactant concentrations in the mixture were $[DPPH]_0 = 6.5 \times 10^{-5}$ mol/L and $[AO]_0 = IC_{50}$, where IC_{50} is the initial concentration of the AO at which the conversion of the radical in the absence of the acid reaches 50% 30 min after mixing the reactants. This is equivalent to 7.5×10^{-6} mol/L of quercetin [19]. The necessary amount of the hydrogen chloride solution in 96% ethanol was added to the, after which the DPPH solution was added. The temperature of the reaction mixture in the spectrophotometric cell was maintained at 20 ± 0.5°C using a temperature-controlled cell holder. The recording of the absorbance decay curve for the DPPH solution at $\lambda = 517$ nm was begun immediately after reactant mixing. The initial reaction rate w_0, which was the kinetic parameter of interest, was calculated using the Microcal Origin 6.0 program, with account taken of the time interval between reactant mixing and the beginning of signal detection. All kinetic curves

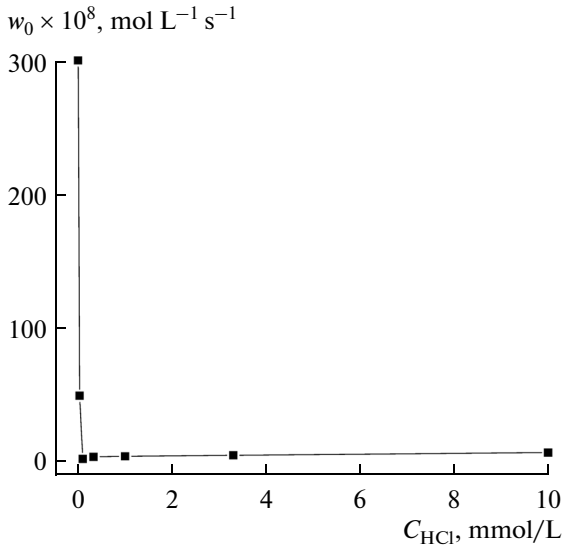

Fig. 1. Initial rate of the reaction of the DPPH radical with antioxidants from the extracts of common tansy leaves in ethanol (azeotropic ethanol–water mixture) versus HCl concentration. $[DPPH]_0 = 6.5 \times 10^{-5}$ mol/L, $[AO]_0 = IC_{50}$, $T = 293$ K.

were fitted with a high accuracy ($r^2 = 99.9$) to the equation

$$D_{DPPH} = D_\infty + A_1\exp(-t/a_1) + A_2\exp(-t/a_2), \quad (1)$$

where D_{DPPH} is the absorbance of the solution at $\lambda = 517$ nm, t is the time elapsed from the onset of the reaction, and D_∞, A_1, A_2, a_1, and a_2 are the parameters selected by the program (letter designations were changed according to their physical meaning). Therefore,

$$w_0 = (A_1/a_1 + A_2/a_2)\varepsilon_{DPPH}^{-1}, \quad (2)$$

where $\varepsilon_{DPPH} = (1.5 \pm 0.02) \times 10^4$ mol^{-1} cm^{-1} is the molar extinction coefficient of DPPH in ethanol [20]. The preexponential factor ratio A_1/A_2 characterizes the quantitative ratio between more and less reactive AOs.

At the initial moment, the apparent rate constant for the reaction of the AOs of the object with DPPH converted to the quercetin stoichiometry is

$$k_{eff} = \frac{w_0}{(7.515 \times 10^{-6})[DPPH]}. \quad (3)$$

RESULTS AND DISCUSSION

Figures 1–4 present kinetic data for the reactions of the stable radical DPPH with the antiradical AOs from some food and medicinal plants and with individual phenolic AOs (rutin and gallic acid) in various reaction media. In some media, as the hydrogen chloride concentration is increased, the reaction rate first decreases sharply and then increases. The minimum initial rate of the reaction between the AO extract from

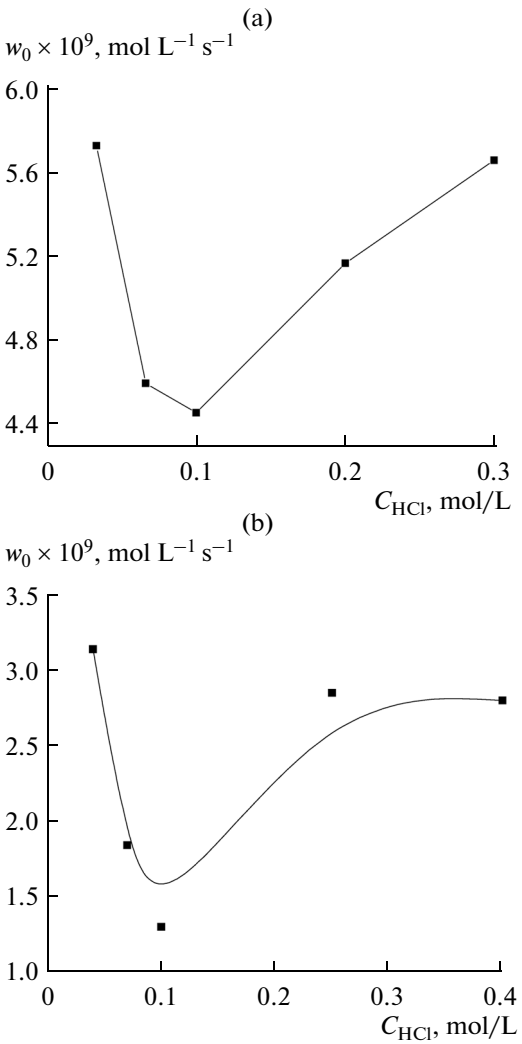

Fig. 2. Initial rate constant of the reaction of the DPPH radical with (a) rutin and (b) gallic acid (10^{-5} mol/L) in ethanol (azeotropic ethanol–water mixture) versus HCl concentration. $[DPPH]_0 = 6.5 \times 10^{-5}$ mol/L, $[AO]_0 = IC_{50}$, $T = 293$ K.

common tansy leaves and DPPH is observed at a hydrogen chloride concentration of 0.1 mmol/L (Fig. 1). The minimum rate of the reaction of rutin and gallic acid with DPPH is attained at the same HCl concentration (Fig. 2). However, the AOs of grapefruit, orange, garlic (Figs. 3, 4), and other representatives of citrus and onion families (lemon, onion) react with DPPH with a minimum rate at a hydrogen chloride concentration in ethanol from 3 to 10 mmol/L. For the reaction of DPPH with the AOs of orange fruits, the 0.1 mM HCl solution in ethanol inhibits the reaction proceeding via the SPLET mechanism so weakly that the initial segment of the kinetic curve (Fig. 4, curve *1*) cannot be recorded.

As can be seen from Figs. 1–4, in spite of the different positions of the reaction rate minima, the general character of reaction rate variation remains

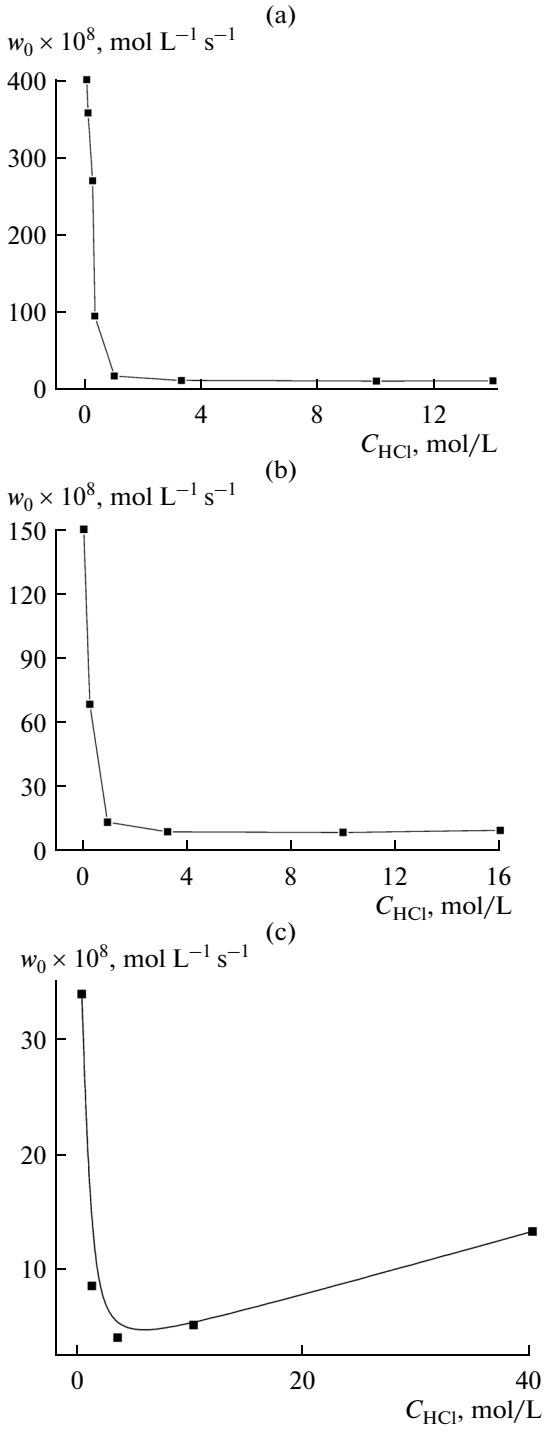

Fig. 3. Initial rate of the reaction of the DPPH radical with antioxidants from the extracts of (a) orange fruits, (b) grapefruit fruits, and (c) garlic bulbs in ethanol (azeotropic ethanol–water mixture) versus HCl concentration. $[DPPH]_0 = 6.5 \times 10^{-}$ mol/L, $[AO]_0 = IC_{50}$, $T = 293$ K.

unchanged with an increasing acid concentration. Due to the lone electron pairs of the oxygen atom of ethanol, molecules of the phenolic AOs can dissociate, donating a proton to the solvent molecule and

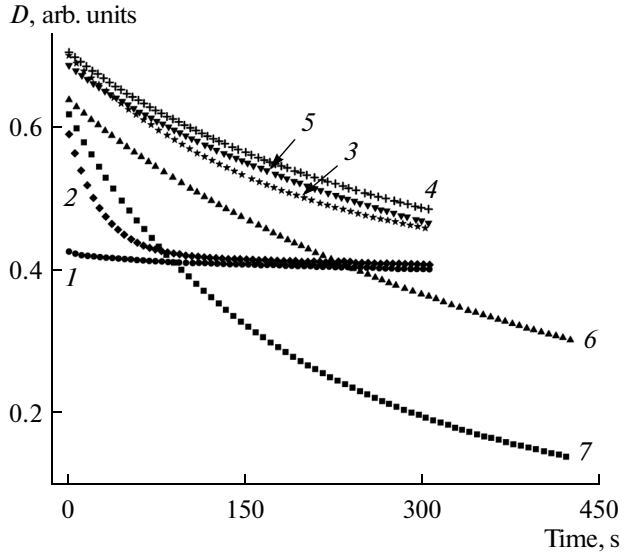

Fig. 4. Decrease in the absorbance of the DPPH solution during its interaction with antioxidants from orange fruits in ethanol (azeotropic ethanol–water mixture) in the presence of hydrochloric acid at a concentration of (*1*) 0.1, (*2*) 0.33, (*3*) 1, (*4*) 3.3, (*5*) 10, (*6*) 30, and (*7*) 100 mmol/L. $[DPPH]_0 = 6.5 \times 10^{-5}$ mol/L, $[AO]_0 = IC_{50}$, $T = 293$ K.

turning into phenolate anions. Therefore, the probability of the reaction proceeding via the SPLET mechanism is high in this reaction medium.

$$\mathrm{ArOH} \overset{-\mathrm{H}^+}{\rightleftharpoons} \mathrm{ArO}^- \xrightarrow{+\mathrm{DPPH}} \mathrm{ArO}^{\bullet} \longrightarrow \mathrm{ArO}^{\bullet} + \mathrm{DPPH}^- \xrightarrow{+\mathrm{H}^+} \mathrm{ArO}^{\bullet} + \mathrm{DPPH{-}H}. \quad \text{(II)}$$

Hydrogen chloride strongly retards the reaction proceeding via the SPLET mechanism, because the increase in the concentration of protonated solvent molecules ($solvH^+$) due to the introduction of the strong acid shifts the equilibrium toward nondissociated molecules of the phenolic AO:

$$\mathrm{ArO}^- + \mathrm{solvH}^+ \rightleftharpoons \mathrm{ArOH} + \mathrm{solv}. \quad \text{(III)}$$

The increase in the reaction rate due to a further increase in the HCl concentration indicates that the process occurs simultaneously via the SPLET mechanism and via at least one more mechanism sensitive to the acid concentration. In the HAT mechanism (purely radical one, involving no ion formation or charge transfer), the rate of DPPH consumption can decrease but cannot increase as the HCl concentration is increased, which is accompanied by an increase in the dielectric constant of the medium. The latter can favor the separation of ion pairs when the reaction follows the ET–PT mechanism [15], inhibiting the reverse process and increasing the rate of DPPH consumption:

$$\mathrm{ArOH} + \mathrm{DPPH}^{\bullet} \rightleftharpoons \mathrm{ArOH}^{+\bullet} + \mathrm{DPPH}^- \longrightarrow \mathrm{ArO}^{\bullet} + \mathrm{DPPH{-}H}. \quad \text{(IV)}$$

Thus, the dependence of the rate of the reaction of the DPPH radical with the extractable substances of

plants is described by the sum of two functions, one decreasing and the other increasing, which reflect the dependences of the rates of the SPLET and ET–PT processes, respectively, on the acid concentration. For the minimum overall reaction rate, the decrement of its SPLET component due to a change in the acid concentration is equal to the increment of the ET–PT component. As can be seen from Figs. 1–4, the minimum of the reaction rate in various multicomponent systems of vegetable origin falls in the HCl concentration range from 0.1 to 10 mmol/L. Thus, the reaction should be carried out at an HCl concentration of 3–10 mmol/L in the reaction system in order to reduce the rate of the SPLET process to a negligible value. It seems unreasonable to exceed this concentration because of the decreasing relative contribution from the radical HAT mechanism to the overall reaction rate. This mechanism of the reaction of the AOs with the radicals dominates in hydrophobic media. The mechanism is based on the homolytic cleavage of the OH bond, so the rate of the reaction proceeding via this mechanism can characterize the strength of this bond. In addition, at HCl concentrations of 30 and 100 mmol/L, DPPH degrades rapidly and this is accompanied by the discoloration of the solution (Fig. 4, curves *6*, *7*). The value of *D* shifts rapidly to absorbances that are well below the base line level of curve *1*, which corresponds to an HCl concentration of 0.1 mmol/L when curve *1* reaches a plateau. It seems inappropriate to select an HCl concentration for each particular object to minimize the reaction rate. The rate of the process occurring via the ET–PT mechanism depends on the hydrogen chloride concentration, whose variation does not allow one to compare the results. In addition, it is very difficult and takes a lot of time to find the position of this minimum for each object.

The kinetic parameters for the reactions of the DPPH radical with the AOs of some food and medicinal plants are presented in Table 1 as examples of determining the AO activity from experimental kinetic curves. As can be seen, the AOs of hibiscus tea, citrus fruits, apples, and *Allium cepa L* onions show the highest antiradical activity.

The antiradical activity of the extractable substances from many plants can also be determined at an HCl concentration of 0.1 mmol/L (Table 2). However, for the AOs reacting with DPPH at a minimum rate near this concentration, the observed kinetic parameters are 1.75–2.5 times lower than those measured at a concentration of 3.3 mmol/L. As a consequence, the relative contribution from the radical HAT process to the overall rate of the process is higher. Unfortunately, these data cannot be compared with the results of kinetic analysis of the antiradical activity of the AOs for which the minimum rate of their reaction with DPPH loccurs at hydrogen chloride concentrations higher than 0.1 mmol/L.

Thus, the reactions of the individual natural phenolic AOs with the stable radical DPPH in ethanol and the same reactions of complicated multicomponent systems of AOs extracted from food and medicinal plants show similar regularities as the pH of the reaction medium is varied. When the pH shifts to smaller values from the neutral value, the reaction rate first decreases sharply and then begins to increase. This is explained by the existence of several parallel mechanisms of the process and by the prevailing contribution from phenols to the total antiradical activity of the objects of vegetable origin. In the case of hydrochloric acid used as the acidifying agent, the minimum reaction rate is observed at an HCl concentration of 0.1 to 10 mmol/L, depending on the object. To facilitate a comparison of the results, the determination of the antiradical activity of AOs in the objects of vegetable origin by the DPPH method should be carried out in ethanol containing 3–10 mmol/L HCl. A comparative analysis is also possible at lower HCl concentrations, but it is necessary to determine the concentration minimizing the reaction rate for each of the objects and to choose the largest value. If the purpose is to analyze the activity of AOs in different samples of the same product, it is reasonable to choose such an HCl concentration in ethanol that would ensure the minimum rate of the reaction of DPPH with all AOs contained in the product.

CONCLUSIONS

(1) Similar regularities of in the variation of the initial rate of the process are observed for the reactions of DPPH in ethanol with individual phenolic AOs of vegetable origin and AOs contained in extracts from plants and food products. The minimum reaction rate is observed at hydrogen chloride concentrations in the range from 0.1 to 10 mmol/L.

(2) The pH effect on the rate of the reaction of the stable DPPH radical with the AOs of vegetable origin in ethanol is explained in terms of different contributions from the HAT, SPLET, and ET–PT mechanisms at different HCl concentrations in the reaction system.

(3) It is recommended that the reaction be carried out at an HCl concentration of 3–10 mmol/L in order to facilitate a comparison of the results.

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