ISSN 1061-9348, Journal of Analytical Chemistry, 2015, Vol. 70, No. 1, pp. 55–59. © Pleiades Publishing, Ltd., 2015.
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ARTICLES

Study of the Acid-Base Properties of Quercetin in Aqueous Solutions by Color Measurements

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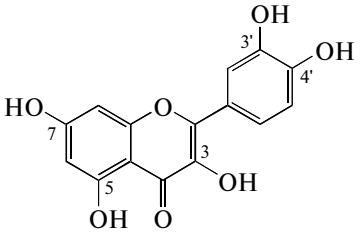
Received October 2, 2013; in final form, February 20, 2014

Abstract—Acid-base properties of quercetin in aqueous solutions are studied by the method chemical color measurements and dissociation constants (pK) are determined. The pK values are assigned to corresponding functional groups of quercetin and a probable scheme of their dissociation is proposed. A diagram of the distribution of ion-molecular forms of quercetin in the pH range 1–13 is built.

Keywords: quercetin, chemical color measurements, ionization constants, distribution diagram

DOI: 10.1134/S1061934815010062

Flavonoids form one of the most numerous and widespread groups of natural substances accumulated in plants in significant amounts [1, 2]. These polyphenolic compounds are strong antioxidants [3, 4]. The increasing interest in flavonoids is due to their wide pharmacological activity. The knowledge of the dissociation constants of flavonoids allows one to understand their pharmacological behavior and to assess the nature (direction, intensity) of their electrostatic interactions with the components of biological systems and also the transport properties of these compounds in living organisms [5–7]. Quercetin is a weak pentabasic acid H_5A and finds application not only as a component of dietary supplements and medicines but also as an analytical reagent [8]:



This compound was selected as an subject of research. We could expect that the physical and chemical properties, first of all, the pK of such abundant and widely used compound have been reliably determined. However, by now not all functional groups of quercetin have been characterized by corresponding pK values, and the available data are scattered, which complicates the choice of the results that well agree with each other. In addition, quercetin can occur in the cationic H_6A^+ form protonated by the carbonyl oxygen atom [9, 10]; the corresponding protonation constant (pK = 2.28) was determined by spectrophotometry [9]. The lack of reliable data on the acid-base properties of the functional groups of quercetin to a certain degree complicates the description of processes of their interaction with the components of chemical systems of both inorganic and organic nature. Zenkevich et al. in [11] summarized the data on the pK of quercetin obtained in 1954–2008 and noted that the pK values for some OH groups estimated by different researchers using independent methods differ by 2–4 pK units. This anomaly can be explained by at least two reasons: (1) the high oxidizability of quercetin, especially in alkaline solutions and (2) the similarity of the acid-base properties of some of its functional groups. The last problem presents the most difficulty in the determination of pK by classical physical and chemical methods. Different modifications of potentiometric and spectrophotometric methods were proposed for the diminution and possible elimination of interfering factors. Zenkevich with coauthors in [11] recommended the addition of a nonionic surfactant and bubbling the reaction mixture with nitrogen in the course of potentiometric titration. They obtained pK values 6.62 ± 0.04 and 9.69 ± 0.25, which were not assigned to corresponding functional groups. For the calculation of pK in aqueous solutions, the authors of the works [13, 14] used an extrapolation approach based on the potentiometeric determination of pK in the presence of different amounts of a surfactant (pK 6.51 ± 0.03, 3-OH group) [12] or different concentrations of methanol in the studied solution (pK 7.59 ± 0.06, 7-OH group, and 9.33 ± 0.04) [13]. The possibility of the spectrophotometric determination of the pK of quercetin in a nitrogen atmosphere was demonstrated in [14]. Sanmartin with coauthors in [15] used capillary electrophoresis (CE) and calculated pK values 7.19 (7-OH), 9.36, and 11.56 based on the depen-

dences of the migration times of the system components on the pH of the leading electrolyte. In this method the partial oxidation of quercetin did not affect the results, because the oxidation products were detected independently. However, CE did not allow the researchers to characterize all functional groups of quercetin by corresponding constants.

Note that in the above works the assignment of pK to specific functional groups was done only partially. Using modified methods, researchers could solve one of the above problems and determine pK of functional groups substantially differing in acidity. However, none of the proposed versions of modified potentiometric and spectrophotometric methods ensured the determination of pK characterizing equilibria between quercetin species with close acid-base properties. An alternative solution of the above problems can be provided by the method of chemical color measurements (CCM). The CCM method consists in the calculation of the chromaticity coordinates of equilibrium ion-molecular forms based on the available spectrophotometric characteristics of acid-base systems. The method allows the researcher to spectrally distinguish similar forms of the studied substances and thus to obtain new data about these forms [16, 17]. The prospects for using CCM for the determination of the pK of synthetic organic reagents in solutions were demonstrated in [17, 18]. The possibility of the determination of pK of natural dyes (based on extracts from vegetable raw materials) in solutions by color measurements on an example of anthocyanidine of pelargonidin and cyanidine we studied in [19, 20].

This work is devoted to the detailed study of protolytic equilibria in aqueous solutions of quercetin using the method of chemical color measurements and to the determination of the pK of its functional groups within the wide range of medium acidities.

EXPERIMENTAL

Solutions and reagents. We used reagents of now worse than analytical grade. The stock solution of quercetin with the concentration 5×10^{-4} M was prepared by dissolving a precisely weighed portion of the reagent in 50% ethanol immediately before use. The required acidity of the solution was created by adding sulfuric acid solutions and sodium hydroxide.

Instruments. Electronic absorption spectra were recorded on an SF-56 spectrophotometer (LOMO-Spektr experimental design bureau, St. Petersburg) in the wavelength range 380–780 nm using cells with the thickness of absorbing layer 1 cm. The acidity of the medium was controlled with an ESL-63-07 glass electrode coupled with an EVL-1M3 silver-silver chloride reference electrode on an I-130 potentiometer calibrated using standard buffer solutions.

Procedure. To determine pK, 10-mL portions of the stock solution of quercetin were put into a series of 50-mL volumetric flasks. Each solution was brought to the corresponding acidity, which was varied in the pH range 1–12 at steps 1 pH unit and diluted to the mark with water. To prevent the oxidation of quercetin with air oxygen, nitrogen was bubbled through the studied solutions at a rate of ~5 mL/min. When the maxima were not separated clearly and for the stronger differentiation of pK values, the step of acidity variation was reduced to 0.10 pH units. The ionic strength of solutions was maintained constant at a level of 0.05 by adding a calculated amount of KCl. Taking into account the dilution of the stock solution, the concentration of ethanol in the solution studied by photometry did not exceed 10%. It is known from [21] that alcohol concentrations up to 10% does not significantly affect the value of pK and that the obtained values of dissociation constants of quercetin can be referred to aqueous solutions. As an alternative to color measurements we used spectrophotometry under identical experimental conditions.

Calculations. We used the following color functions (CF): X, Y, Z, chromaticity coordinates in the CIEXYZ system; L, A, B, chromaticity coordinates in the CIELAB system; color saturation (S) and specific color distinction (SCD). The last named CF for the studied solutions were obtained from chromaticity coordinates X, Y, Z calculated on the basis of the recorded absorption spectra by the method of selected ordinates using the basic software of the spectrophotometer. The SCD values for quercetin solutions were determined by the equation

$$SCD = \frac{\Delta S}{\Delta \mathrm{pH}}, \quad (1)$$

where $\Delta \mathrm{pH} = \mathrm{pH}_1 - \mathrm{pH}_2$, $\Delta S = |S_1 - S_2|$, S_1 and S_2 are color saturations of the studied solutions at pH_1 and pH_2, respectively.

To determine pK by spectrophotometry, the recorded electronic absorption spectra were processed using the SpectroCalc-H5A software. The algorithm of the calculation of H_5A is based on methods of iteration and multiple linear regression analysis by the least-squares technique and is applicable to studies of substances even at the significant overlapping of absorption bands in the spectra of individual forms [22].

The pK values of quercetin were predicted theoretically based on the structures of its ion-molecular forms with the geometries optimized using software packages Marvin 5.9.1. and ACDLabs Professional 6.0. The calculations of pK were based on the possibilities of the QSPR (Quantitative Structure-Property Relationship) method, utilizing intermediate results of quantum-chemical calculations as descriptors [23].

RESULTS AND DISCUSSION

Using the results of performed spectrophotometric studies in the atmosphere of air and an inert atmo-

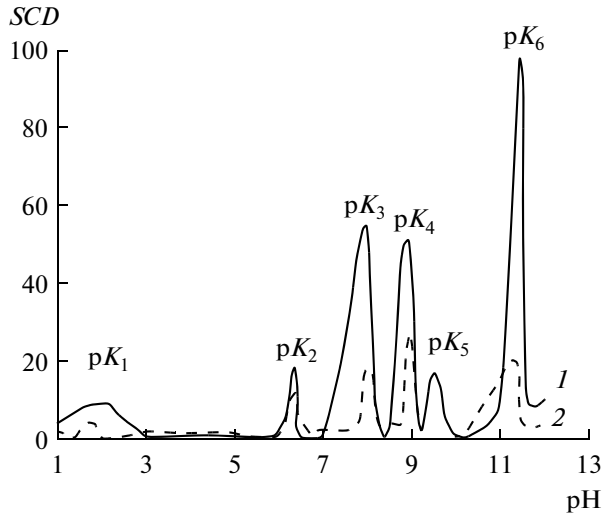


Fig. 1. Changes in the value of color function SCD of a solution of quercetin depending on the pH of the medium in the atmosphere of (*1*) nitrogen and (*2*) air.

sphere of nitrogen and also the results of corresponding calculations, we constructed curves of the dependence of the values the SCD color function of quercetin solutions on the acidity of the medium (Fig. 1, curves *1* and *2*). It can be seen that the shapes of the specified curves are identical and they exhibit equal numbers of maxima, differing in intensity. The values of pH corresponding to maxima in curve *1* coincide with the respective values in curve *2*. The presence of six maxima in the presented graphical dependency unambiguously points to the existence of seven ion-molecular forms of the dye in solution; these forms are in dynamic equilibrium, depending on the acidity of the medium. The values of pH corresponding to each maximum are numerically equal to the values of pK of the functional groups of quercetin in solution (see table).

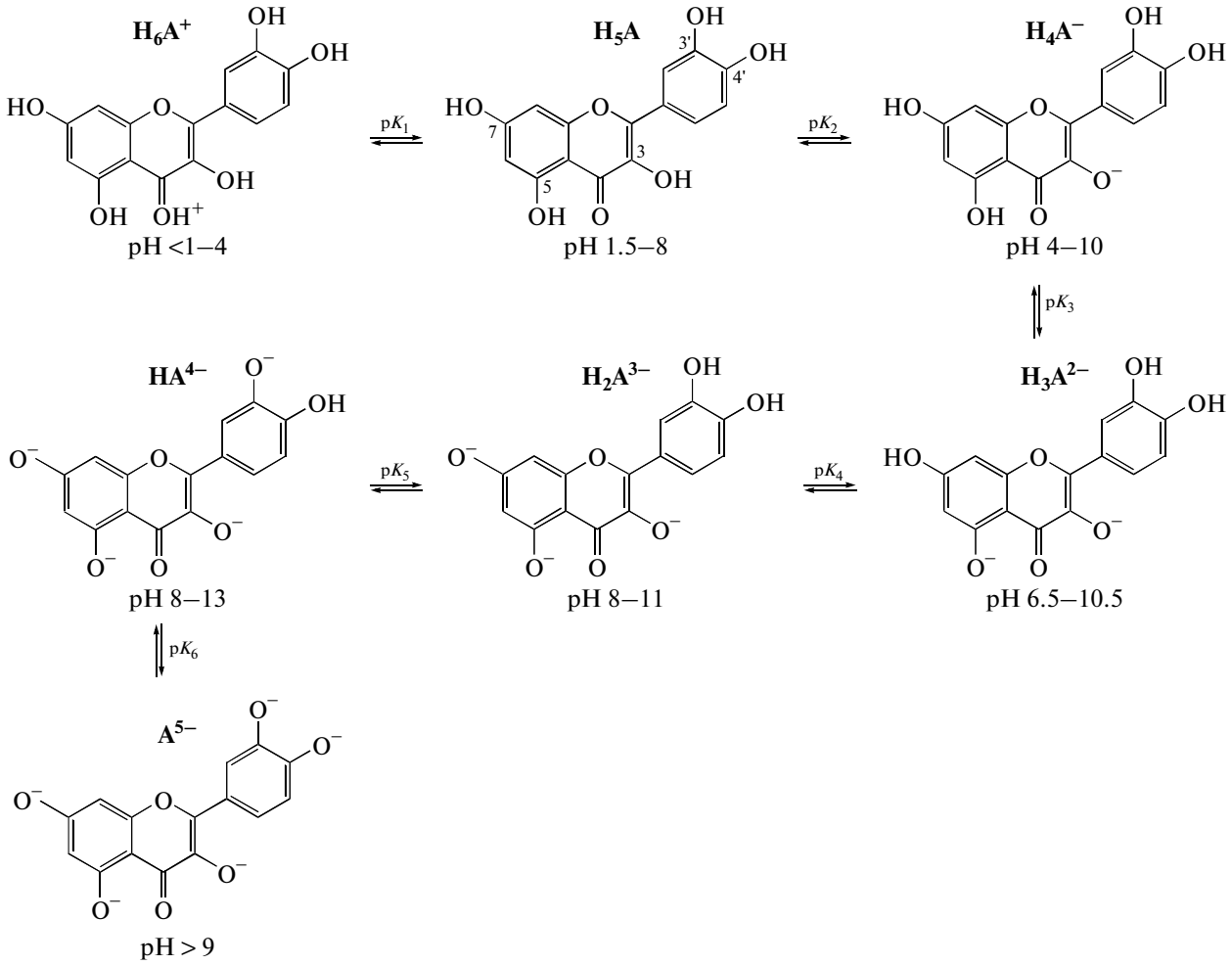
A noticeable decrease in the intensity of maxima in curve *2* is, probably, due to the reduction of the concentration of initial quercetin as a result of the oxidation decomposition of its molecules by air oxygen. It is known from [24] that quercetin is oxidized with air oxygen within a rather wide pH range and that the rate of this process increases with increasing pH. It was found that, in alkaline solutions, the [gamma]-pyrone fragment decomposes with the formation of phloroglucinol and 2,4,6-trihydrobenzoic and 3,4-dihydroxybenzoic acids [24]. The differences between curves *1* and *2* in intensity (Fig. 1) suggests that, as in CE [15], the products of quercetin decomposition were not detected in this version of CCM. This can be explained by the fact that the products of quercetin oxidation absorb in the UV region, whereas color functions are calculated using spectrophotometric data in the visible region (380–780 nm).

As can be seen in the table, using spectrophotometry, we could not determine the pK values of hydroxyl groups in positions 3' and 7 with similar acidities and also the protonation constant of the carbonyl oxygen atom. The use of the QSPR approach allowed us to estimate pK values of a number of functional groups of quercetin and note that they are close to pK values determined by color measurements. This is indicative of the reliability of the results obtained and of the possibility of using CCM for the study of protolytic equilibria in quercetin solutions. A comparative analysis of the experimental and calculated data presented in the table suggests that the CCM procedure is characterized by acceptable reproducibility and a rather narrow confidence interval. Using the Student teat [25] we found that the average values of pK of functional groups of quercetin obtained by CCM did not significantly differ from those calculated from the spectrophotometric data and on the basis of QSPR determination. This allowed us to conclude that CCM is free from systematic errors and is indicative of the accuracy of the results obtained.

Based on the sum of the results of studies by color measurements and spectrophotometry and also on the results of calculations, we proposed the following probable scheme of ion-molecular equilibria of quercetin in solutions in wide range of pH (1–13):

Dissociation constants of quercetin ($n = 3$, $P = 0.95$)

Method	$pK_{C=OH^+}$	$pK_{3\text{-}OH}$	$pK_{5\text{-}OH}$	$pK_{7\text{-}OH}$	$pK_{3'\text{-}OH}$	$pK_{4'\text{-}OH}$
Color measurements	1.8 ± 0.1	6.4 ± 0.1	8.1 ± 0.1	9.0 ± 0.1	9.6 ± 0.1	11.3 ± 0.1
Spectrophotometry	–	6.6 ± 0.1	8.1 ± 0.1	–	–	11.4 ± 0.1
QSPR	–	6.5 ± 0.6	8.1 ± 0.6	9.1 ± 0.3	9.5 ± 0.2	–



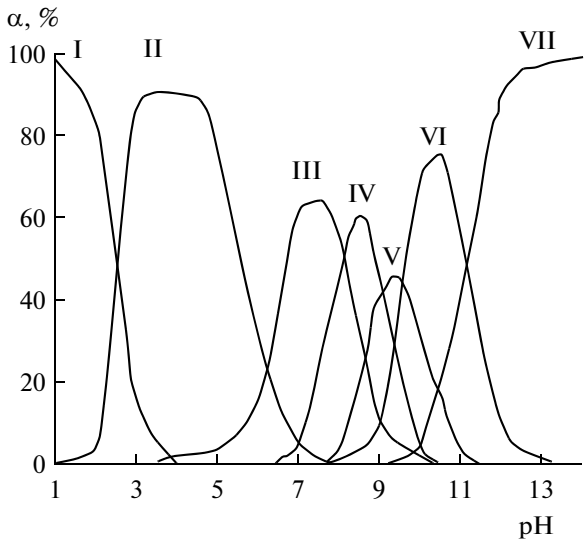


Fig. 2. Diagram of the distribution of ion-molecular forms of quercetin in solution: I, H_6A^+; II, H_5A; III, H_4A^-; IV, H_3A^{2-}; V, H_2A^{3-}; VI, HA^{4-}; VII, A^{5-}.

According to the proposed scheme, with an increase in the pH of the medium, proton is detached from the protonated carbonyl group (H_6A^+) with the formation of an electroneutral quercetin molecule (H_5A). An analysis of the structure–reactivity correlation allows a conclusion that the 3-OH group in the *o* position to the electron acceptor carbonyl group dissociates first, because of which the first anionic form (H_4A^-) forms in the solution. This is confirmed by the data on the values of the energies of the heterolytic decompositions of O–H bonds [12]. In turn, the ionization of the hydroxyl group in position 3 will lead to the strong mesomeric effect with the transfer of electron density to the pyrocatechol fragment, which hinders the dissociation of OH groups in positions 3' and 4'. We should expect that the acid properties of OH groups in position 5 are stronger than those of hydroxyl groups in positions 7, 3', and 4', which is attested by the possibility of complex formation of quercetin with metals ions with the participation of the OH group in position 5 and the carbonyl group in position 4 at the blocked 3-OH group [26]. We can suppose the dissociation of 5-OH groups with the formation of the H_3A^{2-} form, which, in turn, will hinder

the dissociation of hydroxyl groups in the pyrocatechol fragment. Based on the aforesaid, we suppose that the most probable is the dissociation of the 7-OH group and the appearance of the H_2A^{3-} form in the solution. Quantum-chemical calculations performed using the HyperChem Pro 6 software and the semiempirical CNDO method allowed us to suggest the subsequent stepwise dissociation of OH groups in positions 3' and 4' with the formation of anionic HA^{4-} and A^{5-} forms, respectively. The ion-molecular forms of quercetin occurring in the aqueous solution are in dynamic equilibrium, depending on the acidity of the medium, and can be presented by a corresponding distribution diagram (Fig. 2).

As can be seen in Fig. 2, in the narrow pH range 8–10, five or six of its acid or base species can simultaneously occur in an aqueous solution of quercetin, which considerably complicates the determination of pK values characterizing equilibria between these forms by optical and electrochemical methods. This is confirmed by the lack of published data on the pK of hydroxyl groups in positions 7 and 3' with similar acidities. However, the superposition of such number of protolytic equilibria does not prevent the determination of pK by color measurements (see table), which ensures recording fine differences in the spectral characteristics of individual acid-base forms of quercetin as corresponding values of color functions.

Noted that the use of color functions of ion-molecular forms of dyes of different nature as analytical signal expands the possibilities of the study of their acid-base properties and allows the researcher to obtain an overall picture of protolytic equilibria occurring in a wide range of pH. The advantages of CCM over classical physicochemical methods in the study of protolytic equilibria in dye solutions have been demonstrated.

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Translated by E. Rykova