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Determination of dissociation constants of quercetin

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Quercetin ($C_{15}H_{10}O_7$, 3,3′,4′,5,7-pentaxydroxyflavone) is a flavonoid typically found in red wine, green tea, onions, apples and leafy vegetables. Quercetin has potent antioxidant properties due to free radical scavenging, which protects cellular structures and blood vessels from damaging [1].

For absorption, distribution and elimination of medical substances, the pK values are of great importance. Values for quercetin pK_a found in the literature are given in Table 1. Differences between reported values of pK_a, as well as the possibility to determine two or more pK_a values for quercetin, are caused by experimental conditions and applied methods [2–4].

Here, we determined the dissociation constants of quercetin in 50% ethanol solution by potentiometric titrations with NaOH. The determination of the quercetin dissociation constants is based on the dependence between the function of formation (the average number of bound protons), $\bar{n}$, and acidity of a solution [5]. From experimental data, $\bar{n}$ was calculated by the Rossotti-Rossotti method [6], where $\bar{n}$ was determined at each point of the titration curve according to the following equation:

$$\bar{n} = \frac{c_Q + c_{HCl} - [H_3O^+] + [OH^-] - c_{NaOH}}{c_Q} \qquad (1)$$

where c_Q represents the total concentration of quercetin; $[H_3O^+]$ is the concentration of free hydronium ions, obtained at each point of the titration curve from pH readings corrected for the activity coefficient; $[OH^-]$ represents the concentration of hydroxyl ions originating from water dissociation; c_{NaOH} is the concentration of added NaOH and c_{HCl} the concentration of HCl which was added to the quercetin solution before titration; c denotes the stoichiometric, and brackets denote the equilibrium concentrations.

The characteristic curve, $\bar{n} = f(pH)$, is presented in the Fig. Maximal value of $\bar{n}$ is two, indicating that two protons from one quercetin molecule can participate in an acid-base reaction. The obtained curve has two well resolved parts suggesting that values of pK_{d1} and pK_{d2} are sufficiently different (about four orders of magnitude). Thus, the dissociation of quercetin, with the corresponding dissociation constants can be presented as:

$$H_2Q \overset{K_{d_1}}{\longleftrightarrow} H^+ + HQ^- \qquad K_{d1} = \frac{[H^+][HQ^-]}{[H_2Q]} \qquad (2)$$

$$HQ^- \overset{K_{d_2}}{\longleftrightarrow} H^+ + Q^{2-} \qquad K_{d2} = \frac{[H^+][Q^{2-}]}{[HQ^-]} \qquad (3)$$

From the general expression for $\bar{n}$:

$$\bar{n} = \frac{\sum_{1}^{n} n[H_nQ]}{\sum_{0}^{n} [H_nQ]} \qquad (4)$$

and eqs. (2) and (3), the constants for a stepwise dissociation, K_{d1} and K_{d2}, were calculated according to eqs. (5) and (6):

$$\frac{2-\bar{n}}{\bar{n}-1} = \frac{K_{d1}}{[H_3O^+]} \qquad (5)$$

$$\frac{1-\bar{n}}{\bar{n}} = \frac{K_{d2}}{[H_3O^+]} \qquad (6)$$

and obtained as the slope of the straight lines,

$$\frac{2-\bar{n}}{\bar{n}-1} = f\left(\frac{1}{[H_3O^+]}\right) \quad \text{and} \quad \frac{1-\bar{n}}{\bar{n}} = f\left(\frac{1}{[H_3O^+]}\right).$$

These calculations are repeated for all temperatures and all values of the ionic strengths. The dissociation constants of quercetin are given in Table 2.

The low value for pK_1 is caused by HCl addition, owing to the method applied [6]; in that way the dissociation of quercetin is completely prevented and quercetin exists entirely in the protonic form H_2Q. If one would start the

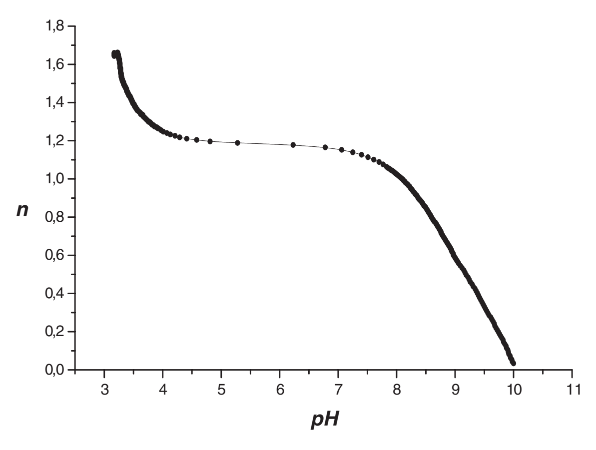

Fig.: Dependence of the formation function ($\bar{n}$) on the pH; T = 34.0 °C, I = 0.0024 M

Table 1: Values of pK_a for quercetin found in literature

pK_1	pK_2	pK_3	pK_4	pK_5	References
8.64	7.47	/	/	/	[2]
11.0	9.9	8.0	7.1	5.7	[3]
/	9.77	8.21	6.95	5.54	[3]
7.03	9.15	/	/	/	[4]

Table 2: pK_a values of quercetin determined at different temperatures and ionic strengths

T/°C	I = 0.0024 M		I = 0.034 M		I = 0.062 M		I = 0.000 M*	
	pK_{d1}	pK_{d2}	pK_{d1}	pK_{d2}	pK_{d1}	pK_{d2}	pK^0_{d1}	pK^0_{d2}
28	3.47	9.24	3.36	8.99	3.29	8.78	3.52	9.36
34	3.24	9.05	2.94	8.72	2.92	8.69	3.30	9.13
39	3.07	8.82	2.94	8.65	2.89	8.61	3.11	8.87

* Obtained as intercept from a straight line $pK_d = f(I^{1/2})$

titration with a nonacidic quercetin solution, only one equivalent point (pK_2) would be attainable.

Thermodynamic parameters for the dissociation of quercetin were calculated from the slopes of the straight lines $\log K_d^0 = f\left(\frac{1}{T}\right)$: $\Delta H = 72.5$ kJ/mol, $\Delta S = 0.17$ kJ/K mol and $\Delta G = 20.4$ kJ/mol, for the first dissociation constant, and $\Delta H = 86.3$ kJ/mol, $\Delta S = 0.11$ kJ/Kmol and $\Delta G = 54.1$ kJ/mol, for the second dissociation constant of quercetin.

Experimental

1. Apparatus

A pH-meter (pHM-82 Radiometer Copenhagen), accuracy of ±0.001 pH, equipped with the combined electrode (No. CW. 733 Serial No. 35162, Russel) was used. The temperature was controlled within ±0.2 K by a circulating water thermostat (Serie U, MLW Freital, Germany).

2. Reagents

Quercetin; absolute ethanol, NaOH, $NaNO_3$, by Merck (Darmstad, Germany); all of *p.a.* grade. The solution of NaOH (carbonate free) was standardized by potentiometric titration against A.R. potassium hydrogen phthalate (dried 1^h at 120 °C). The solution of HCl, added to the quercetin solution before titration, was standardized by potentiometic titration with 0.514 M NaOH.

3. General procedure

Titrations of 0.0025 M quercetin solution with 0.514 M NaOH were carried out in a 51 ml glass vessel closed by a cover with five holes. Through the holes on the lid, the thermometer, the combined electrode, and the nitrogen inlet tubes were inserted. One of the holes was closed with a glass stopper, while the titrant was successively introduced trough the remaining hole. The measurements were performed at three temperatures (28, 34 and 39 °C) and at three values of ionic strength (0.0024, 0.034 and 0.062 M). The ionic strength was adjusted by 2 M $NaNO_3$. Each potentiometric titration, with about 180 experimental points, was done in triplicate.

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Thermodynamic study of local anesthetics based on heptacainium chloride derivatives

Study of local anesthetics. Part 163*

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We have previously studied the critical micellar concentration (CMC) of the derivatives of piperidinoethylesters of 2-alkoxyphenylcarbamic acid. We found that in a homologous series of alkyloxy substituents, the CMC decreases with the number of the carbon atoms up to the heptyloxy (n = 7) and then increases [1].

The aim of the present paper was to study the thermodynamic parameters of local anesthetics of heptacainium chloride derivatives in the medium of distilled water, using UV spectrophotometry at a temperature range T = 294–318 K. Based on our results (Table 1), the thermodynamic magnitudes were calculated ($-\Delta H^\circ$, $-\Delta G^\circ$, $-\Delta S^\circ$) according to the "phase separation (PS_1 and PS_2)" model [2]. The equation $CMC = f(T) = A + BT + CT^2$ represents the dependence of the critical micellar concentration upon temperature at $pH \approx 4.5{-}5$, where (A, B, C) = constants of the second degree polynomial and T = absolute temperature. Gibbs energy change can be estimated according to the equation;

$$\Delta G^\circ = \gamma\, RT \ln (CMC) \tag{1}$$

where R = gas constant and γ = degree of counterion binding (if $\gamma = 1$ the anti-ions are completely ionized, if $\gamma = 2$ all the anti-ions are bound to micelles). The enthalpy of micellization is defined by the equation

$$\Delta H^\circ = -\gamma\, RT^2\, [\partial \ln (CMC)/\partial T] \tag{2}$$

and the entropy contribution of micellization can be calculated as follows:

$$\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T \tag{3}$$

ΔG°, ΔH°, ΔS° values are listed in Table 2.

Table 1: CMC (mol/l) values in relation to temperature T (K)

T (K)	CMC (mol/l)			
	n = 5	n = 7	n = 8	n = 9
294	3.990×10^{-4}	3.151×10^{-4}	3.914×10^{-4}	4.763×10^{-4}
298	4.016×10^{-4}	3.199×10^{-4}	4.122×10^{-4}	4.841×10^{-4}
303	4.145×10^{-4}	3.212×10^{-4}	4.198×10^{-4}	4.906×10^{-4}
308	4.311×10^{-4}	3.258×10^{-4}	4.288×10^{-4}	5.029×10^{-4}
313	4.399×10^{-4}	3.332×10^{-4}	4.419×10^{-4}	5.084×10^{-4}
318	4.424×10^{-4}	3.545×10^{-4}	4.539×10^{-4}	5.243×10^{-4}