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Spectrophotometric Investigation of the Complexing Reaction between Rutin and Titanyloxalate Anion in 50% Ethanol

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Summary. In the present work, rutin (3,3′,4′,5,7-pentahydrohyflavone-3-rhamnoglucoside) was determinated *via* a complexing reaction with a titanyloxalate anion. $K_2[TiO(C_2O_4)_2]$ and rutin react in 50% ethanol forming a 1:2 complex in a *pH* range from 4.00 to 11.50, in which the $TiO(C_2O_4)_2^{2-}$ ion is linked to rutin through the 4-carbonyl and 5-hydroxyl group. The thermodynamic stability constant $\log \beta_2^0$ of the complex is determined to 10.80 at $pH = 6.50$. The change of the standard *Gibbs* free energy ΔG^0 amounts to $-61\,\mathrm{kJ \cdot mol^{-1}}$, indicating that the process of complex formation is spontaneous. The optimal conditions for the spectrophotometric determination of microconcentrations of rutin are at $pH = 6.40$ and $\lambda = 430\,\mathrm{nm}$, where the complex shows an absorption maximum with a molar absorption coefficient $a_{430} = (60 \pm 2) \cdot 10^3\,\mathrm{dm^3 \cdot mol^{-1} \cdot cm^{-1}}$. The method is applied rutin determination from tablets.

Keywords. Rutin; Titanyloxalate anion; Complexes; UV/Vis spectroscopy.

Introduction

Rutin belongs to the group of flavonoids [1], very important phenolic plant constituents occurring in various kinds of foods (fruits and vegetables) and beverages (coffee, beer, tea, fruit juices). Among other flavonoid derivatives, which all display a remarkable array of biological and pharmacological activities (antioxidative, antiinflammatory, antimicrobial, anticancerogeneous, *etc.*) [2], rutin shows the most potent therapeutic action. Since it increases the strength of the capillaries, regulates their permeability, and helps hemorrhages and ruptures in the capillaries and the connective tissues, rutin is mostly applied as a drug for curing blood vessel diseases. Therefore, investigations of reactions that may be applied for quantitative determination of rutin are of considerable importance.

Currently, a direct spectrophotometric method for rutin determination is recommended [3]. Also, due to its structure (Scheme 1), rutin can be determinated *via* complexing reactions with metals and metal groups, many of which have been investigated by the authors [4–8]. However, by the particular complexing reaction,

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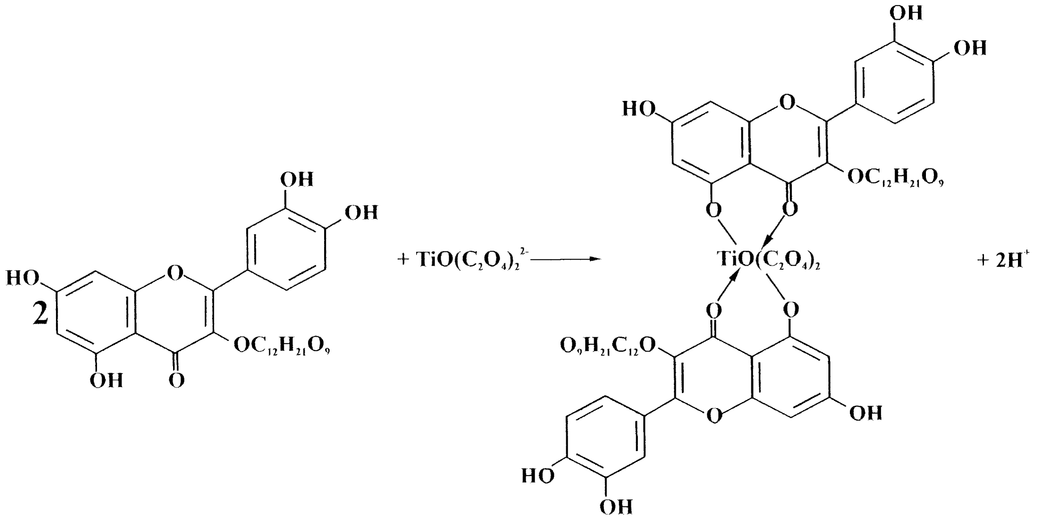

(a) (b)

Scheme 1

described in this paper, the limit for quantitative determination of rutin can be significantly improved. Therefore, the aim of the present work is to investigate the titanyloxalato-rutin complex and to introduce a new method for the determination of rutin *via* a complex forming reaction with titanyl oxalate anion.

Results and Discussion

The absorption spectrum of the complex and its pH dependence

$K_2[TiO(C_2O_4)_2]$ and rutin form a complex of distinctive yellow-orange color. The intensity and hue of the color depend strongly on the *pH* and the concentration of the reactants. We have investigated the complex formation over a wide *pH* range (from 2.40 to 11.50). The formation of the complex at *pH* values lower than 4.00 is difficult since rutin, being a weak acid, is predominantly present in its undissociated form. In the *pH* range from 4.00 to 6.40 the absorption spectrum of the complex show an absorption maximum at $\lambda = 429\,\text{nm}$. At higher *pH* values, however, the absorption maxima are bathochromically shifted. The molar absorption coefficients are also strongly *pH* dependent (Fig 1). The observed bathochromic shift may be caused either by formation of complexes with different stoichiometric composition or by dissociation of the already existing complex. Therefore, the complex composition was determined at several *pH* values.

Determination of the composition of the complex

The stoichiometric composition of the complex at three chosen *pH* values (4.20, 8.00, and 11.50) was investigated by the method of continuous variations of equimolar solutions [9] and by the molar ratios method [10]. For the first method,

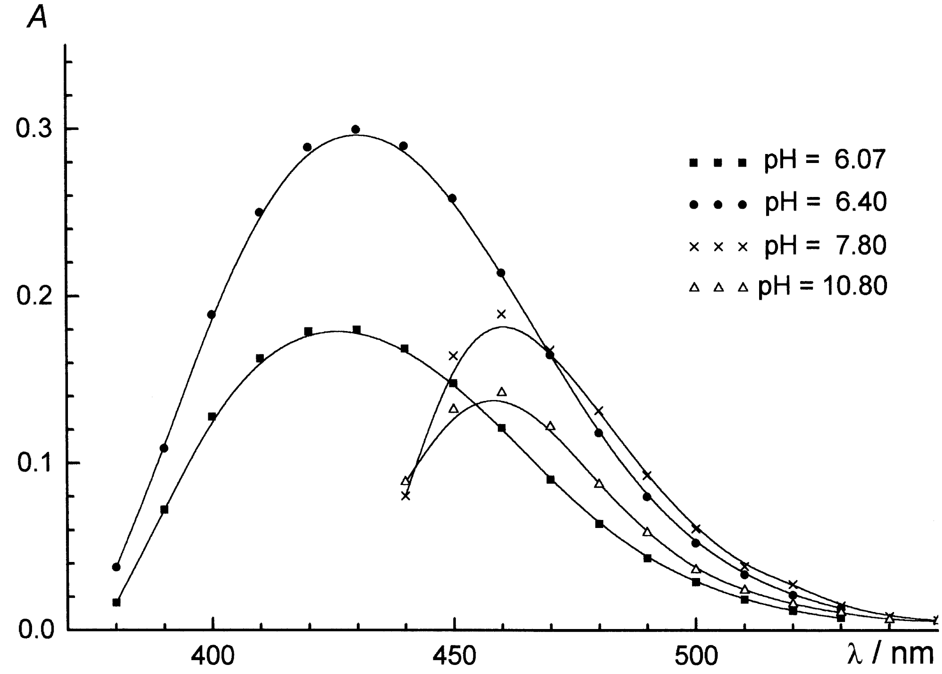

Fig. 1. Absorption spectra of the titanlyloxalate-rutin complex at different *pH* values; $c_{\text{rutin}} = 1.20 \cdot 10^{-4}\,\text{mol} \cdot \text{dm}^{-3}$, $c_{\text{titanyloxalate}} = 5.00 \cdot 10^{-6}\,\text{mol} \cdot \text{dm}^{-3}$

mixed solutions of $K_2[TiO(C_2O_4)_2]$ and rutin of total concentration $c_0 = 1.25 \cdot 10^{-3}\,\text{mol} \cdot \text{dm}^{-3}$ at $pH = 4.20$ and $c_0 = 1.25 \cdot 10^{-4}\,\text{mol} \cdot \text{dm}^{-3}$ at $pH = 8.00$ and 11.50 were used. The *Job* curves obtained at all *pH* values had a maximum at $x_{\text{titanyloxalate}} = 0.33$, denoting the formation of a titanyloxalate:rutin = 1:2 complex (Fig. 2). For the second method, solutions containing a constant $K_2[TiO(C_2O_4)_2]$ concentration ($5.00 \cdot 10^{-4}\,\text{mol} \cdot \text{dm}^{-3}$ at $pH = 4.20$ and $5.00 \cdot 10^{-5}\,\text{mol} \cdot \text{dm}^{-3}$ at $pH = 8.00$ and 11.50) and variable rutin concentrations (from $4.00 \cdot 10^{-4}$ to $1.60 \cdot 10^{-3}\,\text{mol} \cdot \text{dm}^{-3}$ at $pH = 4.20$ and from $4.00 \cdot 10^{-5}$ to $1.60 \cdot 10^{-4}\,\text{mol} \cdot \text{dm}^{-3}$ at $pH = 8.00$ and 11.50) were used. A straight line $A = f(c_{\text{rutin}}/c_{\text{titanyloxalate}})$ with interceptions at $c_{\text{rutin}}/c_{\text{titanyloxalate}} = 2$ was obtained (Fig 3). Thus, both methods confirm unambiguously that the stoichiometric ratio of titanyloxalate to rutin in the complex is 1:2 throughout the investigated *pH* range and the observed bathochromic shift does not originate from complexes with different stoichiometric composition.

Potentiometric titration

To shed light on the complex dissociation, potentiometric titrations of rutin and the complex with NaOH were performed. The titration curves obtained, together with the first derivative curves, are shown in Fig. 4.

In both cases, the shapes of the titration curves are typical for the titration of weak polybasic acids by a strong base. The titration curve for rutin (Fig. 4, a and c)

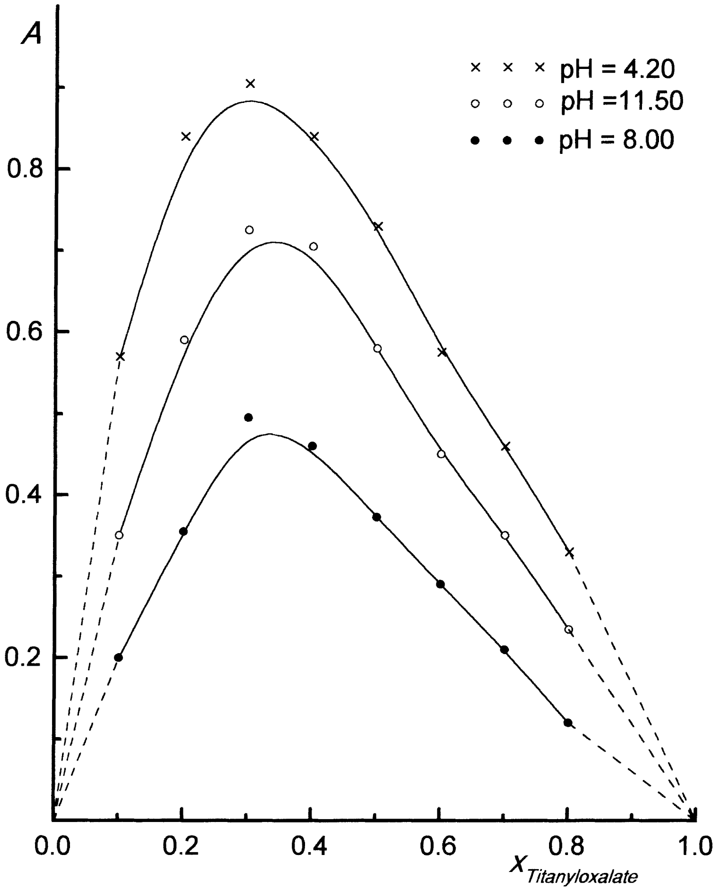

Fig. 2. Method of continuous variation of equimolar solutions; mixed solutions of $K_2[TiO(C_2O_4)_2]$ and rutin have total concentrations $c_0 = 1.25 \cdot 10^{-3}$ mol · dm^{-3} at $pH = 4.20$ and $c_0 = 1.25 \cdot 10^{-4}$ mol · dm^{-3} at $pH = 8.00$ and 11.50

shows two equivalent points at $pH = 5.17$ and 9.35, indicating dissociation of the hydroxyl groups in two steps. At lower *pH* values, the hydroxyl groups in positions 5 and 7 dissociate simultaneously, followed by the simultaneous dissociation of the hydroxyl groups at positions 3′ and 4′ at higher *pH* [6]. The titration curve of the complex (Fig. 4, b and d) shows two well resolved equivalent points at $pH = 4.32$ and 9.25. The shift of the first equivalent point towards lower *pH* values compared to rutin indicates that in the complex the titanyloxalate anion is linked to rutin *via* the 4-carbonyl and 5-hydroxyl group and is invoked by the inductive effect caused by the binding. Dissociation of the 3′- and 4′-hydroxyl groups in the complex occurs at the same *pH* as in rutin, indicating that they are not affected by the binding. At a *pH* value of about 6.5, a weakly manifested equivalent point occurs, maybe be due to the hydrolysis products.

Complex formation

The pH values of equimolar solutions of $K_2[TiO(C_2O_4)_2]$ and rutin ($c = 2.34 \cdot 10^{-3}$ mol · dm^{-3}) are 4.40 and 5.25. The *pH* of the mixture of $K_2[TiO(C_2O_4)_2]$ and

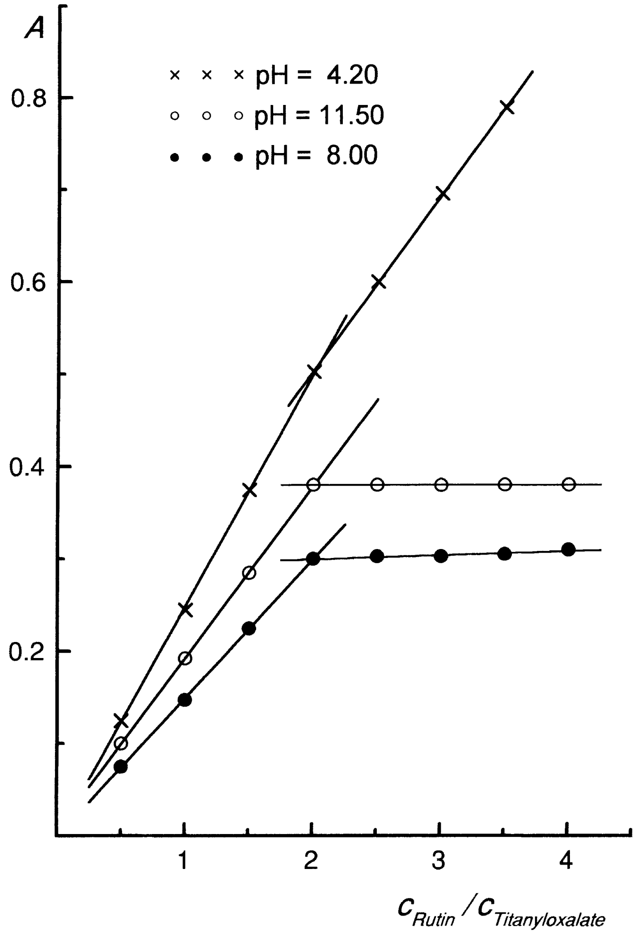

Fig. 3. Method of molar ratios; mixed solutions have constant $K_2[TiO(C_2O_4)_2]$ concentration ($5.00 \cdot 10^{-4}\,mol \cdot dm^{-3}$ at $pH = 4.20$ and $5.00 \cdot 10^{-5}\,mol \cdot dm^{-3}$ at $pH = 8.00$ and 11.50) and variable rutin concentrations (from $4.00 \cdot 10^{-4}$ to $1.60 \cdot 10^{-3}\,mol \cdot dm^{-3}$ at $pH = 4.20$ and from $4.00 \cdot 10^{-5}$ to $1.60 \cdot 10^{-4}\,mol \cdot dm^{-3}$ at $pH = 8.00$ and 11.50)

rutin (in which the concentrations are the same as in the individual solutions), however, is only 4.10, indicating that a proton is delivered. Since a 1:2 complex is formed, the reaction of complex formation may be represented as given in Scheme 1.

Determination of the thermodynamic stability constant of the complex

The concentration stability constants β_2 of the complex at different ionic strengths were determined by a modified version of *Bjerrum*'s method [11]. At different pH values, the absorbances of a mixture containing rutin and $K_2[TiO(C_2O_4)_2]$ and of a solution containing rutin alone were measured. From the two curves $\Delta A = f(pH)$ was calculated at $\lambda = 470\,nm$ (Fig. 5). The highest concentration of the complex was found at $pH = 7.50$, and it may be assumed that at that value the concentration of the complex is approximately equal to the total $K_2[TiO(C_2O_4)_2]$ concentration,

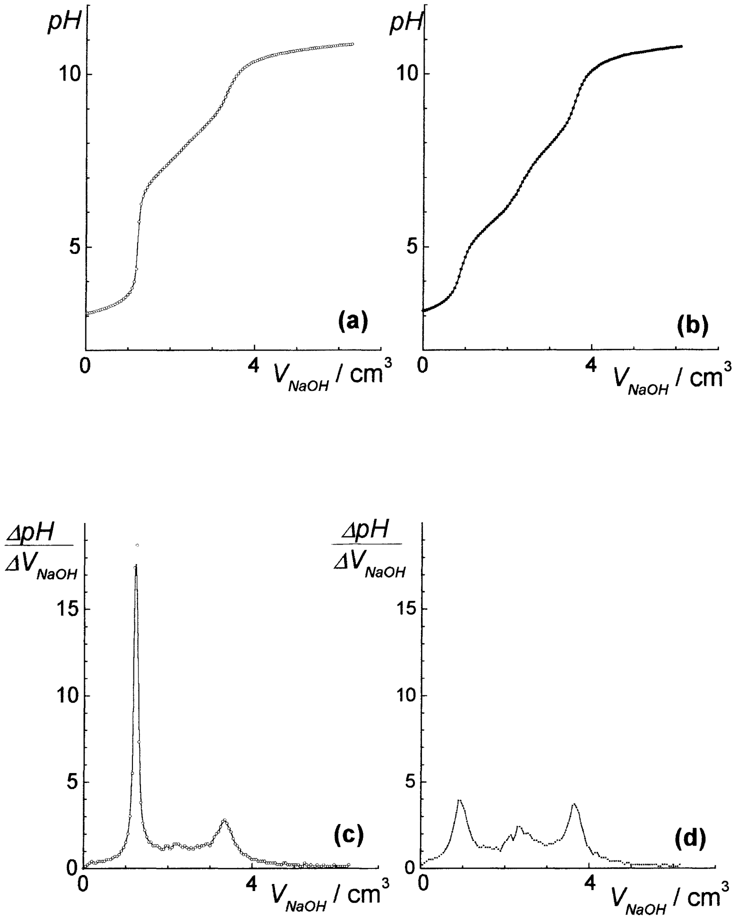

Fig. 4. Potentiometric titration curves of (a) rutin and (b) the titanyloxalate-rutin complex and their first derivatives curves (c and d); $c_{\text{rutin}} = 2.30 \cdot 10^{-3}\,\text{mol} \cdot \text{dm}^{-3}$, $c_{\text{titanyloxalate}} = 1.00 \cdot 10^{-3}\,\text{mol} \cdot \text{dm}^{-3}$; the titrations were performed with 0.05 M NaOH in 50% ethanol, adding volumes of 50 mm^3

[Complex] $\approx$ $[\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}]_0$, in view of the fact that the concentration of rutin in the solution is twenty times higher than the $\text{K}_2[\text{TiO}(\text{C}_2\text{O}_4)_2]$ concentration. Therefore, the molar absorption coefficient of the complex was calculated from $a = A_{\text{max}}/[\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}]_0$. The concentrations of complex, $\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}$, and $\text{C}_{27}\text{H}_{29}\text{O}_{16}^-$ (denoted $[R^-]$) were calculated from $[\text{Complex}] = A/a \cdot b$, $[\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}]_0 = [\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}] + [\text{Complex}]$, $[\text{H}R]_0 = [\text{H}R] + [R^-] + [\text{Complex}]$, and $K_{d_1} = [\text{H}^+][R^-]/[\text{H}R]$, where b is the optical pathlength, HR denotes $\text{C}_{27}\text{H}_{29}\text{O}_{16}\text{H}$, and $k_{d_1} = 1.32 \cdot 10^{-8}$ is the first dissociation constant of rutin in 50% ethanol as determined from the titration curve given in Fig. 4a. This value agrees within experimental error with the value calculated from pH-metric measurements of rutin alone as given above. The concentration stability constant, $\beta_2 = [\text{Complex}]/[\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}] \cdot [R^-]^2$ was calculated for four different ionic strengths: $8.85 \cdot 10^{-5}$, $1.00 \cdot 10^{-4}$, $1.00 \cdot 10^{-2}$, and $1.00 \cdot 10^{-1}\,\text{mol} \cdot \text{dm}^{-3}$ at $pH = 6.50$. A linear dependence of $\log\beta_2$ *vs.* the square root of the ionic strength was obtained: $\log\beta_2 = 10.80 - 0.457\sqrt{I}$. The value of the thermodynamic stability constant was determined from the interception with the ordinate: $\log\beta_2^0 = 10.80$.

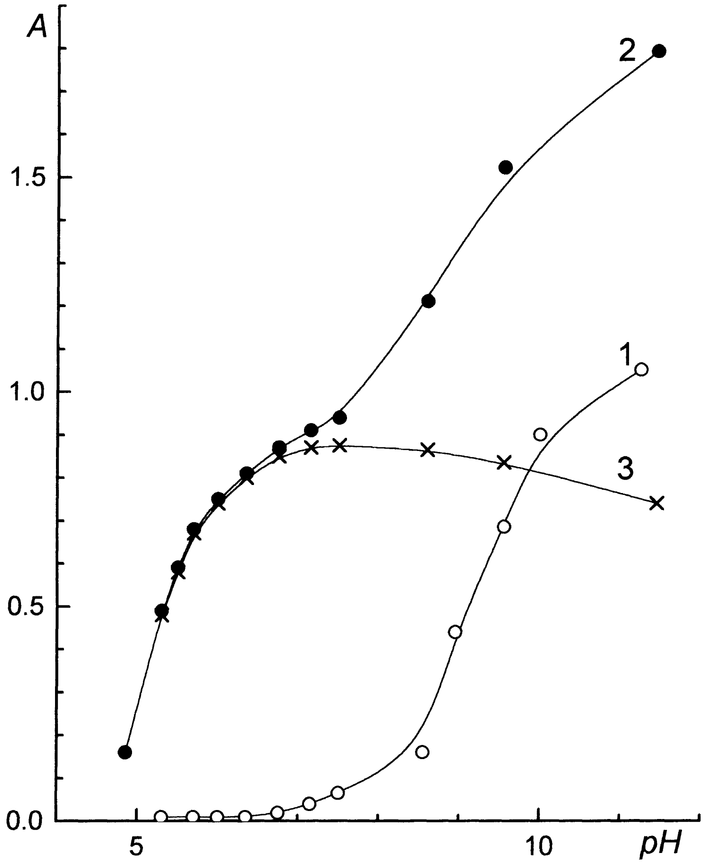

Fig. 5. Dependence of absorbance on *pH* at $\lambda = 470\,\text{nm}$; curve 1: rutin, $c_{\text{rutin}} = 5.90 \cdot 10^{-4}\,\text{mol} \cdot \text{dm}^{-3}$, curve 2: mixture of rutin ($c_{\text{rutin}} = 5.90 \cdot 10^{-4}\,\text{mol} \cdot \text{dm}^{-3}$) and $K_2[TiO(C_2O_4)_2]$ ($c_{\text{titanyloxalate}} = 2.90 \cdot 10^{-5}\,\text{mol} \cdot \text{dm}^{-3}$), curve 3: complex, (obtained by subtracting curve 1 from curve 2); $\lambda = 470\,\text{nm}$

From the thermodynamic stability constant the change of the standard *Gibbs* free energy of the complex formation was calculated: $\Delta G^0 = -61\,\text{kJ} \cdot \text{mol}^{-1}$ at $T = 296\,\text{K}$. The negative value indicates that the process of the complex formation is spontaneous.

Quantitative determination of rutin

The direct spectrophotometric determination of rutin is usually performed in absolute ethanol at $\lambda_{\text{max}} = 258$ or $362\,\text{nm}$, where rutin shows absorption maxima with molar absorption coefficients $a_{259} = (22 \pm 2) \cdot 10^3$ and $a_{362} = (18 \pm 2) \cdot 10^3\,\text{dm}^3 \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, respectively, at its natural *pH*. The sensitivity of the direct method can be somewhat improved (about 12%) by working at higher *pH*. However, formation of the complex between $TiO(C_2O_4)_2^{2-}$ ion and rutin enables a quantitative determination of microconcentrations of rutin. The optimal conditions are at $pH = 6.40$ and $\lambda = 430\,\text{nm}$, where the complex shows an absorption maximum with $a_{430} = (60 \pm 2) \cdot 10^3 \cdot \text{dm}^3 \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$. The calibration curve for rutin was obtained from a series of standard solutions of the complex in wich the amount of rutin was varying, whereas the concentration of $K_2[TiO(C_2O_4)_2]$ was kept constant ($2.0 \cdot 10^{-4}\,mol \cdot dm^{-3}$). The *pH* value of each solution was adjusted at $pH = 6.40$ with NaOH, and the absorbance was measured at $\lambda = 430\,nm$. A linear dependence of the absorbance on the rutin concentration was obtained in the concentration range of rutin from $1.50 \cdot 10^{-6}$ to $1.75 \cdot 10^{-5}\,mol \cdot dm^{-3}$. By application of the least squares method, a regression equation $A = 65900 \cdot c + 0.03$ with a correlation coefficient of $r = 0.997$ was established.

Determination of rutin in Rutinion® forte tablets

The stability of the complex between rutin and titanyloxalate anion allows to apply the method described above for the determination of rutin in tablets. Tablet diluents and excipients did not interfere with the complexing reaction. For expected 100 mg of rutin, 97.92 mg were found (7 measurements; recovery: 97.92%; *RSD*: 0.87%).

Experimental

Apparatus

All spectrophotometric measurements were performed on a Beckman DU-650 spectrophotometer (Fullerton, USA) using a 1 cm quartz quvette. For *pH* measurements an ordinary saturated calomel-glass electrode (Radiometer, Copenhagen, Denmark) and a *pH*-meter (Potentiometer MA 5730, Iskra, Hojrul, Slovenia) were used.

Reagents

$K_2[TiO(C_2O_4)_2]$: AnalaR (Poole, England); rutin: Fluka (Deisenhofen, Germany); absolute EtoH, $NaNO_3$, NaOH, HNO_3: Merck (Darmstadt, Germany). All reagents were of *p.a.* grade and were used without any further purification. Doubly distilled water was used as a solvent throughout. Rutinion® forte tablets where available from biomo Naturmedizin GmbH.

General procedure

The standard stock solution of rutin was prepared dissolving rutin in absolute EtOH, and the standard stock solution of $K_2[TiO(C_2O_4)_2]$ was prepared by dissolving the titanium compound in H_2O.

All standard solutions were prepared in $20\,cm^3$ volumetric flasks by mixing appropriate volumes of the rutin standard stock solution (V_1), absolute ethanol (V_2), H_2O (V_3), and the $K_2[TiO(C_2O_4)_2]$ standard stock solution (V_4) in this order. To obtain final solutions of the required molar concentrations in 50% ethanol, $V_1 + V_2 = 10\,cm^3$ and $V_3 + V_4 = 10\,cm^3$ is required. Because volumes are not strictly additive, the volumetric flasks were filled to the mark with 50% ethanol.

The *pH* of all solutions was adjusted by adding $2.0\,mol \cdot dm^{-3}$ NaOH and/or $2.0\,mol \cdot dm^{-3}$ HNO_3 from the very tip of a glass rod which was first dipped into NaOH or HNO_3, wiped with filter paper, and then immersed in the standard solution. This procedure was repeated until the required *pH* was achieved. In this way, the change in volume of the standard solution is insignificant. Potentiometric titrations were performed with $0.05\,mol \cdot dm^{-3}$ NaOH in 50% ethanol. For the determination of the thermodynamic stability constant, the ionic strength of the standard solutions was adjusted by addition of the required volume of $1.0\,mol \cdot dm^{-3}$ $NaNO_3$ instead of H_2O.

For the rutin determination in Rutinion® forte tablets, ten tablets were weighed, and the sample solution was prepared dissolving an amount equivalent to the average weight of one tablet in EtOH by shaking for 15 min in an ultrasonic bath and subsequent filtering. Rutin from the sample was afterwards complexed with the same concentration of $K_2[TiO(C_2O_4)_2]$ as in the calibration curve.

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