References

1 Matsuura, T., Kawai, M., Nakashima, R., Butsugan, Y.: J. Chem. Soc. (C), 664 (1970)
2 Kawai, M.; Matsumoto, A.; Makino, B.; Mori, H.; Ogura, T.; Butsugan, Y.; Ogawa, K.; Hayashi, M.: Bull. Chem. Soc. Jpn. **66**, 1299 (1993)
3 Makino, B.; Kawai, M.; Kito, K., Yamamura, H.; Butsugan, Y.: Tetrahedron **51**, 12529 (1995)
4 Kawai, M.; Matsuura, T.: Tetrahedron **26**, 1743 (1970)
5 Kawai, M.; Matsuura, T.; Kyuno, S.; Matsuki, H.; Takenaka, M.; Katsuoka, T.; Butsugan, Y.; Saito, K.: Phytochemistry **26**, 3313 (1987)
6 Kawai, M.; Ogura, T.; Nakanishi, M.; Matsuura, T.; Butsugan, Y.; Mori, Y.; Harada, K.; Suzuki, M.: Bull. Chem. Soc. Jpn. **61**, 2696 (1988)
7 Kawai, M.; Ogura, T.; Makino, B., Matsumoto, A.; Yamamura, H.; Butsugan, Y.; Hayashi, M.: Phytochemistry **31**, 4299 (1992)
8 Row, L. R.; Sarma, N. S.; Reddy, K. S.; Matsuura, T.; Nakashima, R.: Phytochemistry **17**, 1647 (1978)
9 Row, L. R.; Reddy, K. S.; Sarma, N. S.; Matsuura, T.; Nakashima, R.: Phytochemistry **19**, 1175 (1980)
10 Antoun, M. D.; Abramson, D.; Tyson, R. L.; Chang, C.-J.; McLaughlin, J. L.; Peck, G.; Cassady, J. M.: J. Nat. Prod. **44**, 579 (1981)
11 Luis, J. G.; Echeverri, F.; Quiñones, W.; González, A. G.; Torres, F.; Cardona, G.; Archbold, R.; Perales, A.: Tetrahedron **50**, 1217 (1994)
12 Ripperger, H.; Kamperdick, C.: Pharmazie **53**, 144 (1998)
13 Makino, B.; Kawai, M.; Ogura, T.; Nakanishi, M.; Yamamura, H.; Butsugan, Y.: J. Nat. Prod. **58**, 1668 (1995)
14 Kawai, M.; Makino, B;.Yamamura, H.; Araki, S.; Butsugan, Y.; Ohya, J.: Pharmazie, in print

Received August 16, 2001
Accepted September 15, 2001

Professor Masao Kawai (Ph.D)
Department of Applied Chemistry
Nagoya Institute of Technology
Gokiso-cho, Showa-ku
Nagoya 466–8555
Japan
kawai@ach.nitech.ac.jp

Faculty of Pharmacy, Belgrade University, Yugoslavia

Potassium titanyloxalate as analytical reagent for micro-quantitative determination of quercetin

N. Pejić, V. Kuntić and D. Malešev

Quercetin ($C_{15}H_{10}O_7$; 3,3′,4′,5,7-pentaxydroxyflavone) is the aglycon of the flavonol type for a great number of different glycosides; one of them is rutin which has therapeutic action and is mostly applied as a drug for curing blood vessel diseases.

Quercetin as well as rutin has been determinated spectrophotometrically *via* a complexing reaction with many metal ions [1–6]. In our previous paper [7], we investigated the complexation reaction of rutin with the potassium-titanyloxalate, $K_2[TiO(C_2O_4)_2]$ (PTOx) which significantly lowered the detection limit for rutin determination.

Thus, the aim of the present study was to investigate the titanyloxalato-quercetin complex in order to use this particular reaction of complexation to improve detection limits for the determination of quercetin.

PTOx and quercetin form a complex of distinctive yellow-orange color. The intensity and hue of the color are strongly dependent on the pH, the concentration of the reactants, the ionic strength and temperature. The complex formation was investigated in a wide pH range from 3.6 to 10.0 (Fig. 1).

In the pH range from 3.6 to 5.6, the complex spectra shows the absorption maximum at $\lambda = 420$ nm. At higher pH values, the absorption maxima are batochromly shifted. Since the observed batochromic shift may be caused either by formation of complexes with different stoichiometric composition or by dissociation of the already existing complex, the composition of complex was determined at several pH values: in the pH range without any shifts (pH = 3.6 and pH = 4.3) and in the pH range where the shift is of about 10 nm (pH = 7.2). Examining on higher pH values is useless, since hydrolysis of the titaniloxalate ion implies decreasing of liberate metal ion.

The stoichiometric composition of the complex was investigated by the method of continual variations of equimolar solutions [8] and by the molar ratio method [9]. According to the former method, mixed solutions of PTOx and

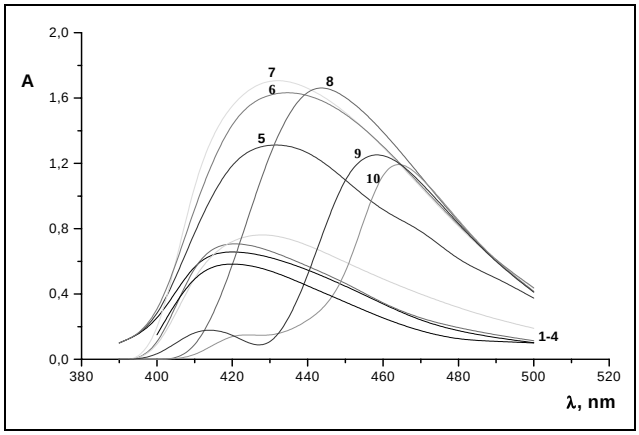

Fig. 1: Absorption spectra of the complex of quercetin, $c_{Querc} = 5.0 \times 10^{-4}$ M and potassium titanyloxalate, $c_{PTOx} = 2.5 \times 10^{-5}$ M, obtained for different values of pH (curves 1–10): 3.6 (1), 4.3 (2), 5.0 (3), 5.6 (4), 6.4 (5), 6.9 (6), 7.2 (7), 8.2 (8), 9.2 (9) and 10.0 (10). The ionic strength is $I = 7.5 \times 10^{-5}$ M, and the temperature T = 296 K. Blank for all cases is quercetin in the same concentration, pH, ionic strength and temperature as in the mixture.

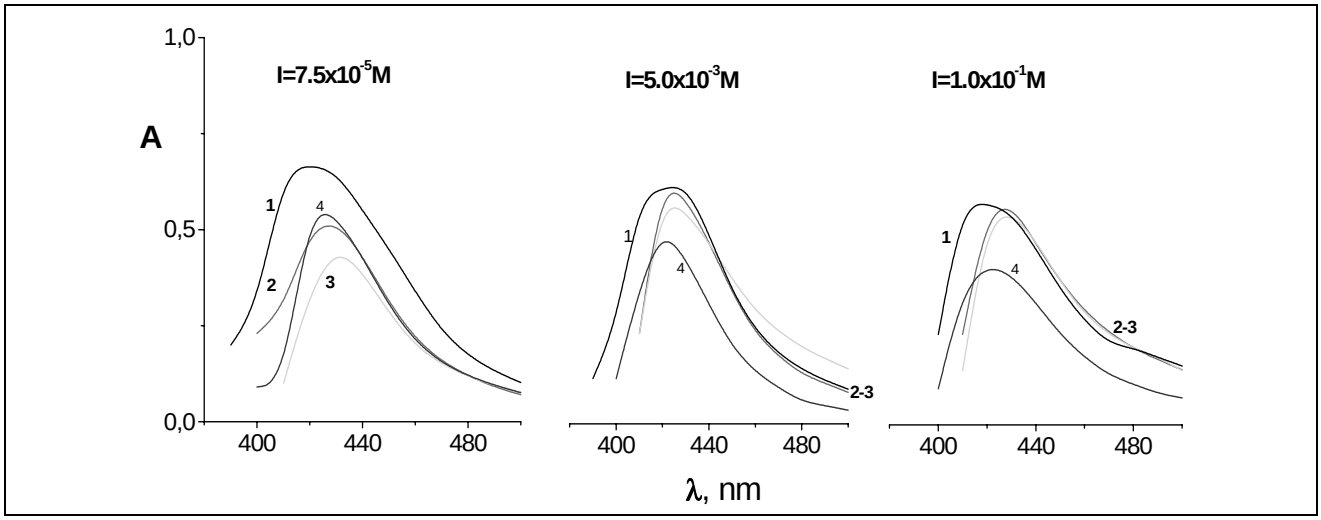

Fig. 2: Absorption spectra of the complex of quercetin $c_{Querc} = 5.0 \times 10^{-4}$M and potassium titanyloxalate, $c_{PTOx} = 2.5 \times 10^{-5}$ M, at different temperatures (curves 1–4): 1 – 296 K, 2 – 301 K, 3 – 307 K and 4 – 312 K; the ionic strengths $I = 7.5 \times 10^{-5}$ M, $I = 5.0 \times 10^{-3}$ M and $I = 1.0 \times 10^{-1}$ M. Blank is quercetin in the same concentration, temperature, pH and ionic strength as in the mixture.

quercetin having total concentration $c_o = 3.0 \times 10^{-4}$ M (at all pH values) were used. The *Job's* curves obtained at both pH values had a maximum at $x_{PTOx} = 0.33$ denoting the formation of a PTOx : quercetin = 1 : 2 complex. According to the latter method, solutions containing a constant PTOx concentration (5.00×10^{-5} M) and varied quercetin concentrations (from 2.50×10^{-5} M to 2.25×10^{-4} M) were used. The straight line $A = f(c_{Querc}/c_{PTOx})$ with interceptions at $c_{Querc}/c_{PTOx} = 2$ was obtained. Thus, both methods confirm unambiguously that the stoichiometric ratio of PTOx to quercetin in the complex is 1 : 2 throughout the investigated pH range, and the observed batochromic shifts do not originate from complexes with different stoichiometric composition.

The influence of the ionic strength (7.5×10^{-5} M, 5.0×10^{-3} M and 1.0×10^{-1} M), and temperature (296 K, 301 K, 307 K and 312 K) on complex formation was investigated. As shown in Fig. 2, the ionic strength as well as temperature influences complexation and with increasing of both, the mentioned complexation is slightly reduced.

On the basis of previously described examination of the complexation reaction, we selected the best conditions for quercetin determination, which corresponds to pH = 7.2, $\lambda = 430$ nm, (with molar absorption coefficient $a_{430} = (66 \pm 2) \cdot 10^{-3}$ dm^3 mol^{-1} cm^{-1}), the ionic strength, $I = 7.5 \times 10^{-5}$ M and the temperature T = 296 K.

The calibration curve for quercetin was obtained from a series of standard solutions of the complex, in which the amount of quercetin varied, whereas the concentration of PTOx was kept constant (1.0×10^{-3} M). A linear dependence of the absorbency on the quercetin concentration was obtained in the concentration range of quercetin from 2.5×10^{-6} M to 5.0×10^{-5} M. The detection limit, defined as the concentration of the tested species which produces signal-to noise ratio of 3, is determined to be 1.2×10^{-6} M. By applying the least square method, we calculated the regression equation, $A = 15000c + 0.0011$, having a correlation coefficient r = 0.9997 which indicates excellent linearity. Compared to spectrophotometric methods [1–3], the detection limit of quercetin about one order of magnitude is lowered.

Experimental

1. 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.

2. Reagents

Potassium titanyloxalate, $K_2[TiO(C_2O_4)_2]$, by Anala® (Poole, England); Quercetin; absolute Ethanol, $NaNO_3$, by Merck (Darmstad, Germany); All reagents were of p.a. grade.

3. General procedure

The standard stock solution of quercetin was prepared by dissolving it in absolute ethanol, and the standard stock solution of $K_2[TiO(C_2O_4)_2]$ was prepared by dissolving it in water; all standard solutions were 50% ethanol. The pH of all solutions was adjusted by succinate buffer solution, suitable for etanol-water mixtures [10]. 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 M $NaNO_3$ instead of water.

References

1 Radović, Z.; Malešev, D.: Arch. Pharm. **320**, 188 (1987)
2 Malešev, D.; Radović, Z.: Pharmazie **42**, 59 (1987)
3 Kuntić, V.; Blagojević, S.; Malešev, D.; Radović, Z.; Bogavac, M.: Monatsh. Chem. **129**, 41 (1998)
4 Malešev, D.; Radović, Z.; Jelikić-Stankov, M.; Bogavac, M.: Anal. Lett. **24**, 1159 (1991)
5 Kuntić, V.; Kosanić, M.; Malešev, D.; Radović, Z.: Pharmazie **53**, 724 (1998)
6 Kuntić, V.; Malešev, D.; Radović, Z.; Kosanić, M.; Mioč, U.; Vukojević, V.: J. Agric. Food Chem. **46**, 5139 (1998)
7 Kuntić, V.; Malešev, D.; Radović, Z.; Vukojević, V.: Monatsh. Chem. **131**, 769 (2000)
8 Irving, H.; Pierce, T.: J. Chem. Soc. **16**, 111 (1959)
9 Yoe, J. H.; Jones, A. L.: Ind. Engl. Chem. An. Ed. **16**, 111 (1994)
10 Perin, D. D.; Dempsy, B.; in: Wiley, D.; Wiley, J. (Eds.): Buffers for pH and Metal Ion Control, p. 89, Chapman and Hall, London (1973)

Received June 22, 2001
Accepted August 1, 2001

Nataša Pejić
Faculty of Pharmacy
Vojvode Stepe 450
11000 Belgrade
Yugoslavia
bimesel@eunet.yu