ISSN 1070-4272, Russian Journal of Applied Chemistry, 2014, Vol. 87, No. 1, pp. 36−41. © Pleiades Publishing, Ltd., 2014.
Original Russian Text © N.A. Lipkovskaya, V.N. Barvinchenko, T.V. Fedyanina, A.A. Rugal', 2014, published in Zhurnal Prikladnoi Khimii, 2014, Vol. 87, No. 1, pp. 40−45.

ORGANIC SYNTHESIS AND INDUSTRIAL ORGANIC CHEMISTRY

Physicochemical Properties of Quercetin and Rutin in Aqueous Solutions of Decamethoxin Antiseptic Drug

N. A. Lipkovskaya, V. N. Barvinchenko, T. V. Fedyanina, and A. A. Rugal'

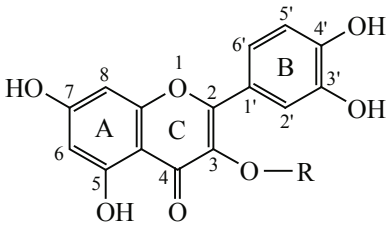
Institute of Surface Chemistry, National Academy of Sciences of Ukraine, ul. Generala Naumova, Kiev, Ukraine
e-mail: tfedyanina@gmail.com

Received June 17, 2013

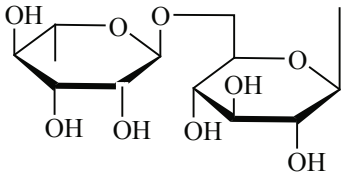
Abstract—The effect of a cationic surfactant, Decamethoxin, on the physicochemical properties of structurally related natural flavonoids, quercetin and rutin, was studied by spectrophotometry. The spectral and protolytic properties and the solubility of quercetin and rutin strongly depend on the Decamethoxin concentration in solution. The presence of Decamethoxin in the solution favors the tautomeric transition of the enol form of quercetin to the keto form.

DOI: 10.1134/S1070427214010054

Vegetable flavonoids rutin (5,7,3',4'-tetrahydroxyflavone-3-rutinoside, Rt) and quercetin (3,5,7,3',4'-pentahydroxyflavone, Q) [1] exhibit a wide spectrum of pharmacological properties, which are largely determined by their high antioxidant activity.



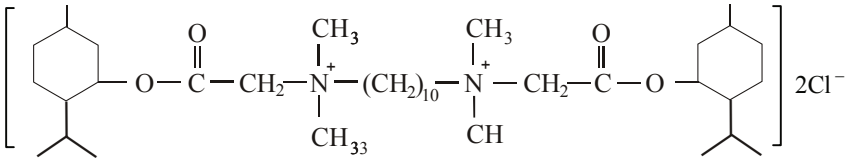
Quercetin: R = H
Rutin: R = rutinose



These bioactive polyphenols of vitamin P group are widely used in medicine owing to angioprotective, gastroprotective, diuretic, spasmolytic, antisclerotic, anti-inflammatory, and antiviral effects [2]. Extremely low solubility of rutin and especially quercetin in aqueous solutions and body fluids gives rise to certain problems in the development of highly effective drugs, because the solubility is one of the main biopharmaceutical characteristics largely determining the bioequivalence of a drug [3].

The following substances are used today for increasing the quercetin solubility: mono- and polysaccharides ("quercetin granules" and Kvertin drug forms), polyvinylpyrrolidone of molecular weight 12600 (Korvitin), and phospholipid liposomes (Lipoflavon) [4]. The solubility of quercetin and rutin also significantly increases in aqueous solutions of β-cyclodextrin [5], polyvinylpyrroilidone of molecular weight 8000 [6], and human serum albumin [6] and in micellar solutions of Miramistin cationic surfactant [7] owing to the formation of supramolecular complexes.

Surfactants are widely used in pharmaceutical technology as hydrophilizers, solubilizers, emulsifiers, and stabilizers [8]. Cationic surfactants combining surface activity with bactericidal properties are the most promising for use in pharmaceutical technology. Decamethoxin (DCM), an antiseptic drug with a wide spectrum of activity, is a cationic surfactant [2]:



Decamethoxin is a gemini surfactant, because it consists of two polar head groups linked by a spacer and of two hydrophobic tails. Quantitative data on the effect of decamethoxin on the solubility, spectra, acid–base properties, and structural characteristics of flavonoids are lacking.

The goal of this study was to examine the effect of a cationic gemini surfactant, Decamethoxin, in a wide concentration range on the physicochemical properties of quercetin and rutin in the region of physiological pH values, because such data should be taken into account in the development and standardization of liquid drug forms based on such binary systems.

EXPERIMENTAL

The initial solutions of quercetin and rutin (Sichuan Xieli Pharmaceutical Co. Ltd, Korea) of analytically pure grade and of Decamethoxin (Pilot Plant of the Institute of Organic Chemistry, National Academy of Sciences of Ukraine) of pharmacopoeial purity were prepared by dissolving accurately weighed portions of the corresponding substances. In all the experiments (except solubility studies), the concentration of flavonoids was 2×10^{-5} M, and that of ethanol, 4%; the ionic strength was $\mu = 0.2$ (NaCl). The electronic absorption spectra of the solutions were recorded with a Specord M-40 spectrophotometr (Carl Zeiss, Jena, Germany) in a cell with $l = 1$ cm. The effect of the background on the analytical signal obtained in measuring the absorption spectra of the solution was eliminated by using the method of heterochromatic extrapolation at two wavelengths [9]. The solution acidity was monitored with a glass electrode of a Hanna instruments HI 221 universal pH-meter.

The effect of Decamethoxin on the spectral characteristics of flavonoids was studied at pH 3.0 and 7.4. These values are within the physiological acidity range (pH 3–8.5). Figure 1 shows the visible absorption spectra of (a) quercetin and (b) rutin solutions in the presence of Decamethoxin and without it. In the spectra of both flavonoids, the absorption bands undergo bathochromic shift both with increasing pH of solution (curves *1*, *3*) and on introducing the cationic surfactant (curves *2*, *4*).

It is well known that the properties of surfactant solutions strongly depend on their concentration and sharply change on reaching the critical micelle concentration (CMC). Therefore, we examined the effect exerted by Decamethoxin on the spectral characteristics of quercetin and rutin (namely, on the positions of their absorption bands, λ_{max}) in a wide concentration range in the region of the chosen physiological pH values. As seen from Fig. 2, in all the cases the absorption bands undergo bathochromic shift, with pronounced inflections observed

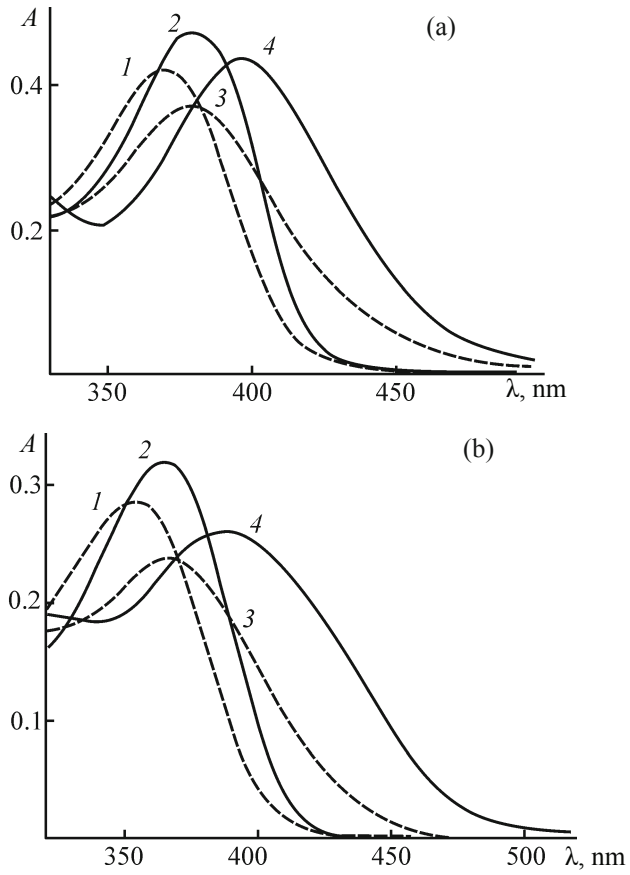


Fig. 1. Absorption spectra of (a) quercetin and (b) rutin solutions at pH (*1*, *2*) 3.0 and (*3*, *4*) 7.4 (*2*, *4*) in the presence of Decamethoxin and (*1*, *3*) without it. $c_{DCM} = 8.0 \times 10^{-3}$ M. (*A*) Optical density of solutions and (λ) wavelength; the same for Fig. 5.

in curves *1–4* at the Decamethoxin concentration $c_{DCM} \sim 4 \times 10^{-3}$ M, which corresponds to CMC of Decamethoxin under these experimental conditions. After the CMC is reached, the spectral characteristics of the flavonoids in the micellar solutions undergo no further changes. Such variation of the spectral characteristics of quercetin and rutin, namely, the bathochromic shift of the absorption bands, suggests polarization of their molecules in Decamethoxin solutions. To confirm this assumption, we studied the variation of acid–base properties of the flavonoids in Decamethoxin solutions.

Quercetin and rutin are weak polybasic acids. Taking into account their first dissociation constants found previously {$pK_{a1(Q)} = 7.28$, $pK_{a1(Rt)} = 7.21$ [7]}, we can conclude that these flavonoids exist in the molecular form at pH < 5 and as mixture of molecular and deprotonated forms at pH > 5. The order of dissociation of separate OH groups in flavonoid molecules has not been precisely determined, but it is believed that the hydroxy groups in positions 3 and 7 of quercetin are the most acidic [10, 11]. Because the pH dependences of the spectral characteristics of quercetin and rutin solutions are similar (Figs. 1, 2) and the rutin molecule does not contain OH group at 3-position, in this case, apparently, the 7-OH group undergoes dissociation first. The specific features of protolytic reactions in Decamethoxin solutions were studied in a wide range of its concentrations and under similar conditions for both flavonoids with the aim to reliably compare their acid–base properties. For quantitative evaluation of the effect exerted by Decamethoxin on the acid–base properties of flavonoids, namely, on their first dissociation constants pK^a_{a1}, we used the calculation method based on spectrophotometric data [12]:

$$pK^a_{a1} = pH_{H_2O} + \log \{[ROH]/[RO^-]\}$$

$$= pH_{H_2O} + \log [(A_{RO^-} - A)/(A - A_{ROH})],$$

where A_{ROH} и A_{RO^-} are the absorbance values of aqueous solutions containing the flavonoid in the molecular (ROH) or deprotonated (RO^-) form, the pH value refers to the solution being analyzed, and A is the absorbance of the flavonoid solution being analyzed. Generally, pK^a_a characterizes essentially the two-phase equilibrium, because pH_{H_2O} refers to the continuous aqueous phase and the [ROH]/[RO^-] ratio refers to the micellar phase. Therefore, pK^a_a is commonly termed the apparent dissociation constant of the reagent [12, 13].

Figure 3 shows the dependence of the found dissociation constants of quercetin and rutin on the Decamethoxin concentration. As can be seen, in the presence of the cationic surfactant the acid properties of flavonoids are enhanced. The shape of the experimental curves for the two flavonoids is essentially different.

For hydrophobic quercetin (Fig. 3, curve *1*) in the region of the formation of associates with Decamethoxin ($c_{DCM} << CMC$), pK^a_{a1} sharply decreases, reaching a minimum at $c_{DCM} \sim 1 \times 10^{-4}$ M: $pK^a_{a1(min)} = 6.2$. Further increase in the Decamethoxin concentration to $c_{DCM} \sim CMC$ leads to the formation of premicellar quercetin associates. In the process, pK^a_{a1} of quercetin increases, reaching

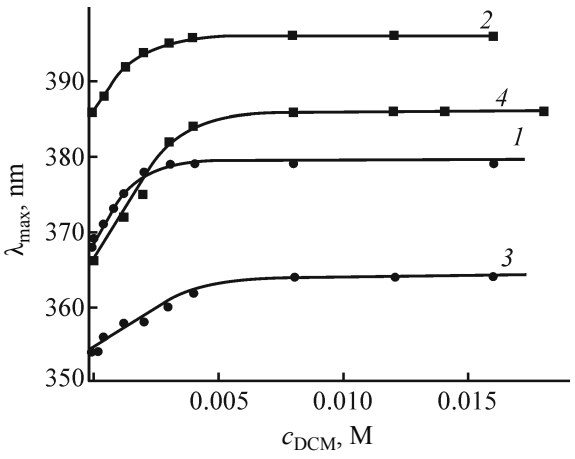


Fig. 2. Plots of λ_{max} in the spectra of (*1*, *2*) quercetin and (*3*, *4*) rutin at different pH values vs. Decamethoxin concentration c_{DCM}. pH: (*1*, *3*) 3.0 and (*2*, *4*) 7.4.

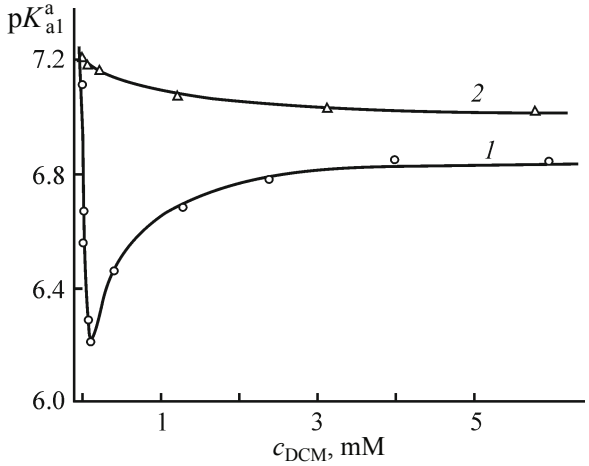


Fig. 3. Plots of the first apparent dissociation constant pK^a_{a1} of (*1*) quercetin and (*2*) rutin vs. Decamethoxin concentration c_{DCM}.

a constant value at $c_{DCM} \geq$ CMC, but remains lower than the initial pK_{a1} value (at $c_{DCM} = 0$). For hydrophilic rutin (Fig. 3, curve *2*), the dissociation constant pK^a_{a1} gradually decreases (without extrema) with an increase in the Decamethoxin concentration and also reaches a constant value in the micellar solution.

A decrease in pK^a_{a1} of quercetin and rutin at low DCM concentrations ($c_{DCM} <<$ CMC) may be due to a change in the character of interaction of their hydroxy groups with water molecules under the action of hydrophobic interaction with hydrocarbon radicals of the cationic surfactant, enveloping the nonpolar core of the flavonoid. Hydrophobization of the ionic associate strongly affects the acid–base properties of the organic reagent incorporated into it.

The lyophilic properties of a substance are commonly characterized by the distribution ratio in the water–*n*-octanol system ($\log P$) [14]. The $\log P$ values given in the literature [15] for quercetin ($\log P = 1.480$) and rutin ($\log P = -2.020$) indicate that quercetin is a moderately hydrophobic compound ($1 < \log P < 3$), whereas rutin is hydrophilic ($\log P < 1$), which accounts for different shapes of the dependences shown in Fig. 3. The increase in the acidity of flavonoids, observed in micellar solutions of Decamethoxin, may be due to the fact that their molecules are arranged in the surface layer of the micelle, with the ionizable groups (as in the case of other indicator dyes [12, 16]) localized in the Stern region, in which the hydroxide ions are concentrated near the positively charged surface of the cationic surfactant micelle. Micellar solutions of Decamethoxin exert weaker effect on the acid–base properties of rutin, because rutinose is oriented toward the aqueous phase, and the energy of bonding of the polar groups of this bulky hydrophilic substituent with water prevents penetration of the rutin molecule into the micelle core to a greater extent, compared to quercetin.

To study the solubility of the flavonoids, we prepared a series of $(0–2) \times 10^{-2}$ M aqueous Decamethoxin solutions, added an excess of dry quercetin or rutin, agitated on a shaker to attain the equilibrium (24 h), centrifuged for 10 min at 2000 rpm, and measured the absorption spectra and pH values of the solutions. The temperature in all the experiments was constant, 293 K.

The experiments showed that, with an increase in the Decamethoxin concentration to $c_{DCM} \sim 4 \times 10^{-3}$ M, the dissolution of quercetin and rutin is accompanied by a decrease in pH of the solution from 7 ($c_{DCM} = 0$) to 5. This fact should be taken into account in spectrophotometric determination of the concentration of flavonoids in the solution, because it was found that their absorption spectra depend both on the surfactant concentration and on pH (Figs. 1, 2). To calculate the solubility of quercetin and rutin in aqueous solution (S_{aq}) and in Decamethoxin solutions (S) from the light absorbance, we used the corresponding molar extinction coefficients determined preliminarily for each Decamethoxin concentration taking into account the specific pH value of the equilibrium solution.

The solubility of quercetin and rutin in Decamethoxin solutions relative to aqueous solution ($S/S_{aq} - 1$) is plotted in Fig. 4 vs. surfactant concentration. Both curves have sharp inflection at $c_{DCM} = 4 \times 10^{-3}$ M, which corresponds to CMC of this surfactant and is well consistent with the data obtained in studying the spectroscopic and protolytic properties of the flavonoid–Decamethoxin system (Figs. 2, 3).

It should be noted that specifically CMC is the principal quantity for substance solubilization. Figure 4 also shows that, with an increase in the Decamethoxin concentration to 0.016 M (curve *1*), the solubility of quercetin increases by an order of magnitude compared to aqueous solution. For rutin, the relative increase in the solubility is considerably less pronounced (Fig. 4, curve *2*), which is probably due to the presence of a bulky hydrophilic substituent, rutinose, in the rutin molecule.

The stability of drugs is an important characteristic. Previous experimental studies showed that the spectral

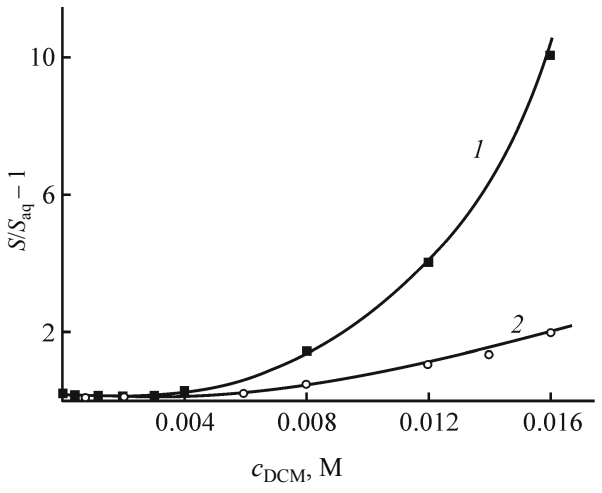
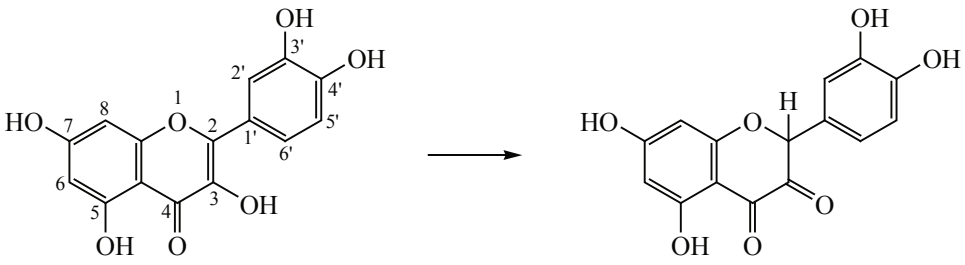


Fig. 4. Solubility of (*1*) quercetin and (*2*) rutin in Decamethoxin solutions relative to aqueous solution, $S/S_{aq} - 1$, as a function of the Decamethoxin concentration c_{DCM} ($T = 293$ K).

Scheme.



Enol form Keto form

characteristics of quercetin in the presence of polyvinylpyrrolidone, human serum albumin, and Miramistin change with time owing to tautomeric transformation of the quercetin molecule [6, 7] in accordance with the scheme.

A spectrophotometric study showed that the absorption spectrum of a neutral quercetin solution in the presence of Decamethoxin changes with time (Fig. 5) similarly: The intensity of the band with λ_{max1} = 402 nm decreases, and a new band at λ_{max2} = 345 nm appears simultaneously.

It should be noted that the λ_{max2} band does not appear with time in the spectrum of a straight quercetin solution under the same conditions. Therefore, it can be assumed that this band corresponds to the formation of the keto form of quercetin as a result of its tautomeric transformation in Decamethoxin solutions.

To confirm the assumption that quercetin undergoes keto–enol tautomeric transition in Decamethoxin solutions, we performed similar experiments with rutin, which is a quercetin derivative with the labile proton of the C_3–OH substituted by the rutinose residue, making the keto–enol tautomerism described by the above scheme impossible. We found that the spectra of rutin in Decamethoxin-containing solutions, as well as in the other organized media studied, do not change with time. Thus, the interaction with Decamethoxin favors keto–enol tautomeric transformation of the quercetin molecule, and the observed hypsochromic shift of the absorption maximum of the quercetin keto tautomer formed upon interaction with Decamethoxin is due to the break of π-conjugation between the γ-pyrone and phenol groups. It should be noted that, in more acidic solutions (pH < 5), in which quercetin exists in the solution in the molecular form, its spectra do not change with time.

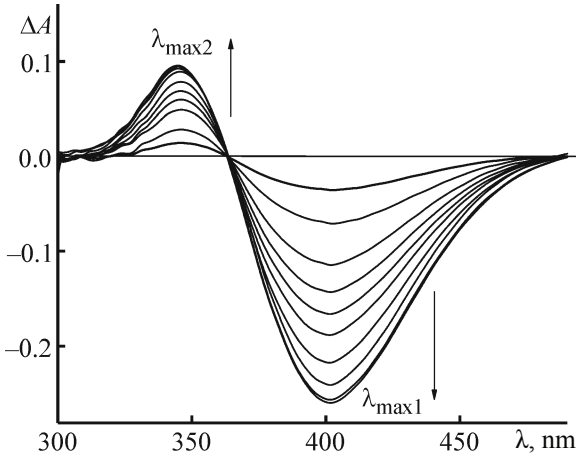


Fig. 5. Evolution (Δ*A*) of the quercetin spectra in the presence of Decamethoxin, monitored over the course of 300 min. Decamethoxin concentration $c_{DCM} = 8 \times 10^{-3}$ M, pH 7.4, l = 1 cm, interval between two successive measurements 30 min.

CONCLUSIONS

(1) Comprehensive physicochemical studies of the interaction of Decamethoxin, a versatile antiseptic belonging to the group of cationic surfactants, with natural bioactive flavonoids in solutions revealed regular trends in variation of the spectral and protolytic properties and of the solubility of flavonoids depending on their hydrophobicity and surfactant concentration in solution. The observed trends change essentially when the critical micelle concentration of the surfactant is exceeded.

(2) In Decamethoxin solutions with the concentration below critical micelle concentration, the formation of associated and mixed micelles leads to a bathochromic shift in the absorption spectra of the flavonoids and to enhancement of their acid properties. In micellar solutions, after reaching the critical micelle concentration, the only effect is the increase in the solubility of quercetin and rutin by factors of 10 and 2, respectively, whereas the

spectral characteristics and protolytic properties remain virtually unchanged.

(3) In neutral and weakly alkaline Decamethoxin solutions, the enol form of quercetin undergoes tautomeric transition to the keto form.

(4) The revealed relationships should be taken into account in the development, standardization, and use of drugs containing flavonoids and Decamethoxin.

REFERENCES

1. Andersen, O.M., *Flavonoids: Chemistry, Biochemistry, and Applications*, New York: CRC, 2006.
2. Mashkovskii, M.D., *Lekarstvennye sredstva* (Drugs), Moscow: Novaya Volna, 2010.
3. *Tekhnologiya i standartizatsiya lekarstv* (Technology and Standardization of Drugs), Georgievskii, V.P. and Konev, F.A., Eds., Kharkov: RIREG, 1996.
4. *Bioflavonoidy kak organoprotektory: kvertsitin, korvitin, kvertin* (Bioflavonoids as Organoprotectors: Quercetin, Korvitin, Kvertin), Moibenko, A.A., Ed., Kiev: Naukova Dumka, 2012.
5. Lipkovs'ka, N.O., Barvinchenko, V.M., and Pogorelii, V.K., *Farm. Zh*., 2000, no. 6, pp. 66–69.
6. Barvinchenko, V.M., Lipkovska, N.O., Fedyanina, T.V., and Pogorelyi, V.K., in *Nanomaterials and Supramolecular Structures: Physics, Chemistry, and Applications*, Springer, 2009, pp. 303−316.
7. Barvinchenko, V.N., Lipkovskaya, N.A., and Fedyanina, T.V., in *Nanomaterialy i nanokompozity v meditsine, biologii i ekologii* (Nanomaterials and Nanocomposites in Medicine, Biology, and Ecology), Kiev: Naukova Dumka, 2011, pp. 346–381.
8. Corrigan, O.I. and Healy, A.M., in *Encyclopedia of Pharmaceutical Technology*, Dekker, 2002, pp. 2639–2653.
9. Bernshtein, I.Ya. and Kaminskii, Yu.L., *Spektrofotometricheskii analiz v organicheskoi khimii* (Spectrophotometric Analysis in Organic Chemistry), Leningrad: Khimiya, 1986.
10. Agrawal, P.M. and Schneider, H.J., *Tetrahedron Lett*., 1983, vol. 24, no. 2, pp. 177–180.
11. Kitson, T.M., Kitson, K.E., and Moore, S.A., *Chem. Biol. Interact*., 2001, vols. 130–132, pp. 57–69.
12. Mchedlov-Petrosyan, N.O., *Differentsirovanie sily organicheskikh kislot v istinnykh i organizovannykh rastvorakh* (Differentiation of the Strength of Organic Acids in True and Organized Solutions), Kharkov: Khar'k. Nats. Univ. im. V.N. Karazina, 2004.
13. Savvin, S.B., Shtykov, S.N., and Mikhailova, A.V., *Russ. Chem. Rev*., 2006, vol. 75, no. 4, pз. 341–349.
14. *Micellization, Solubilization, and Microemulsions*, Mittal, K.L., Ed., New York: Plenum, 1977.
15. *Chemical Information Resources from the National Library of Medicine*. http://sis.nlm.nih.gov/chemical.html.
16. *Fizikokhimiya nanostrukturirovannykh materialov* (Physical Chemistry of Nanostructured Materials), Klimov, B.N. and Shtykov, S.N., Saratov: Novyi Veter, 2009.