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PHYSICAL CHEMISTRY OF SOLUTIONS

The Brilliant Blue FCF Ion-Molecular Forms in Solutions According to the Spectrophotometry Data[1]

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Abstract—The brilliant blue FCF acid–base properties in aqueous solutions have been studied and its ionization constants have been defined by tristimulus colorimetry and spectrophotometry methods. The scheme of the acid–base dye equilibrium has been proposed and a diagram of the distribution of its ionic-molecular forms has been built. It has been established that the dominant form of the dye was the electroneutral form, which molar absorptivity ($\varepsilon_{625} = 0.97 \times 10^5$) increases with the increase of the dielectric permittivity of the solvent. It has been shown that the replacement of polar solvents by less polar ones is causing a bathochromic shift of the maximum absorption band of the dye, the value of which is correlated with the value of the Hansen parameter. Tautomerization constants have been defined in a number of solvents and associated with the value of the Dimroth-Reichardt parameter.

Keywords: brilliant blue FCF, acid–base properties, tristimulus colorimetry, spectrophotometry, solvatochromism, tautomerism

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Synthetic dyes are a broad class of food additives, which are used to provide attractive appearances, as well as to compensate for the loss of natural food colors during processing and storage. Nowadays, synthetic dyes are used much more often than natural ones, but depending on the concentration they may be carcinogens, mutagens and allergens [1]. The main methods for identifying and defining food dyes are chromatography, spectrophotometry and capillary electrophoresis [2]. In general, the state of analytical chemistry of food dyes has been summarized in the review [3].

An important aspect of the study of food dyes in solutions is the establishment of their physicochemical, acid–base and other specific characteristics. Krestov has described thermodynamic evaluation of the processes of solvation, aggregation and acid dissociation of organic dyes [4, 5]. In turn, the dissociation constants (pK) provide the basis for predicting the reactionary ability of compounds and are necessary in ADME studies, as they allow explaining such phenomena as adsorption, distribution, metabolism and elimination substances [6]. Tristimulus colorimetry [7–9] is one of the promising methods of exploring protolytic properties of dyes. Unlike spectrometry, for example, this method can be used to define the pK of all the functional groups of isomeric flavonoids quercetin [10] and morin [11] and sulfonated azo dye tartrazin [12]. The theoretical and practical interest is the comparative study of the protolytic properties of organic dyes, both by tristimulus colorimetry and spectrophotometry in situ with chemometric algorithms for processing light absorption spectra. In nonaqueous solvents with different macrophysical properties the spectrophotometric characteristics of the equilibrium dye forms is changed by solvatochromism or tautomerism [13]. Acid–base properties of one of the widely used food dyes brilliant blue FCF (*N*-(4-((4-(ethyl(3-sulfobenzyl)amino)phenyl)-(2-sulfophenyl)-methylene)cyclohexa-2,5-dien-1-ylidene)-*N*-3-sulfobenzyl)ethanaminium) in the literature are not described probably due to its relatively high resistance to protolysis and proximity to both the spectral characteristics of equilibrium forms and pK values of some functional groups. Relevant information and chemical-analytical characteristics of the brilliant blue FCF in aqueous and organic media are necessary to develop effective methodologies for its extraction or sorption and subsequent determination in foodstuffs and pharmaceuticals.

The purpose of this work is to study the acid–base and spectrophotometric properties of the food dye the brilliant blue FCF in water and nonaqueous solutions

[1] The article was translated by the authors.

by methods of tristimulus colorimetry and spectrophotometry in situ with chemometric algorithms.

EXPERIMENTAL

The E133 brilliant blue FCF food dye was obtained from Rocha DyeChem and purified by further recrystallization from methanol. The stock solution of the dye with a concentration of 1×10^{-3} mol dm^{-3} was prepared by dissolving a precisely weighed portion of the reagent in distilled water, and dissolving in methanol for studying solvatochromism. We used aprotic (chloroform, ethyl acetate, butyl acetate, THF, dioxane-1,4, acetonitrile, methylbenzene, DMSO, DMFA), amphiprotic (methanol, propanol-2, butanol-2, 3-methylbutanol-1) and protophilic (triethylamine) organic solvents, which were further purified according to the recommendations of [13]. The chemicals used in this work were of analytically pure grade.

Electronic absorption spectra were recorded using Specord S 600 (Analytik Jena AG, Germany) and SF-56 (LOMO-Spektr) spectrophotometers in the wavelength range 380–780 nm in 1 cm quartz cells thermostated at 25°C or with an optical probe with an optical path length of 10 mm (Hellma Analytics). The pH of the solutions was controlled with an ESL-63-07 glass electrode coupled with an EVL-1M3 silver-silver chloride reference electrode on an I-160 potentiometer.

In the study of solvatochromism and tautomerism, 0.04 cm^3 of 1×10^{-3} mol/dm^3 methanol dye solution and 5 cm^3 of the organic solvent was added to the graduated test tubes, mixed and the absorption spectra were recorded in quartz cells (l = 1 cm) on a SF-56 spectrophotometer. Tautomerism was studied by the Bershtein equilibrium shift method [14] in some mutually miscible solvents.

The pK determination technique by the tristimulus colorimetry method was described in detail by our previous investigation [10, 11]. The *SCD* (Specific Color Discrimination) values were determined by the equation:

$$SCD = \frac{\Delta S}{\Delta \mathrm{pH}},$$

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

The pK values of the functional groups of brilliant blue FCF were determined by spectrophotometric method using an optical probe. The optical probe use is possible to record the light absorption spectra of solutions in situ without sampling and transferring the sample to the cell, which makes it possible to increase the array of spectrophotometric data and helps to use chemometric algorithms for their processing. This provides a detailed description of the change in the concentration of equilibrium forms of the dye both in time and with a change in the pH of the chemical system. The obtained spectrophotometric data was processed using the SpectroCalc-H5A program. The pK calculation algorithm is based on iteration methods and multiple linear regression analysis by the least squares method [15]. The pK values of brilliant blue FCF functional groups were predicted theoretically based on the structures of its ion-molecular forms with the optimized geometries using software packages Marvin 5.9.1. and ACDLabs Professional 6.0. Geometry of the equilibrium forms of dye was optimized using the MM+ molecular mechanics method implemented in HyperChem Pro software.

RESULTS AND DISCUSSION

The study of the impact of medium acidity in the range from 10 M H_2SO_4 to pH 14 on the light absorption of brilliant blue FCF aqueous solutions showed the presence of absorption bands with different intensity at the above pH range within the whole investigated wavelength interval. Figure 1 illustrates the absorption spectra at the pH values corresponding to the maximum light absorption of the equilibrium acid–base forms of the dye. As seen, in the 10 M H_2SO_4 medium (curve *1*) one broad low-intensity absorption band with a maximum at 430 nm is observed in the dye spectrum related to its protonated form. Additional medium-intensity band with a maximum at 625 nm is revealed in the spectrum at pH 2 (curve *2*), whereas the short-wave band undergoes hypsochromic shift of 20 nm and is significantly reduced in intensity. Further pH increase (curve *3*) leads to an

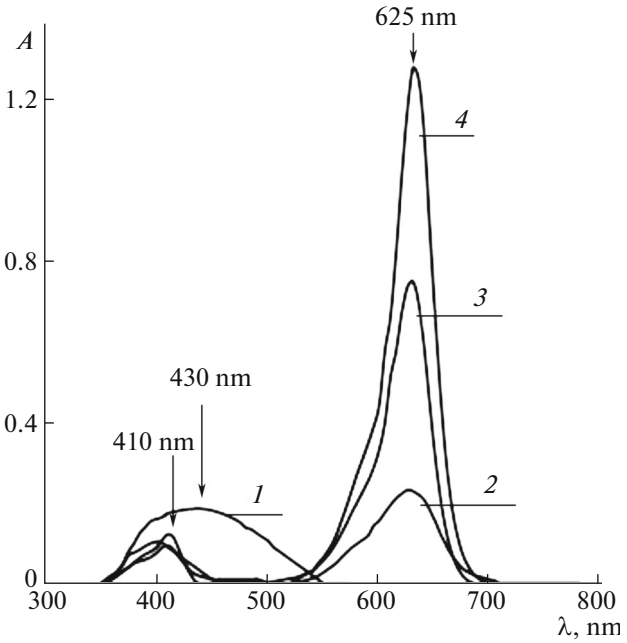


Fig. 1. Absorption spectra of brilliant blue FCF aqueous solution at different acidities: (*1*) 10 M H_2SO_4, (*2*) pH 2, (*3*) pH 6, (*4*) pH 10, $C = 2 \times 10^{-5}$ M.

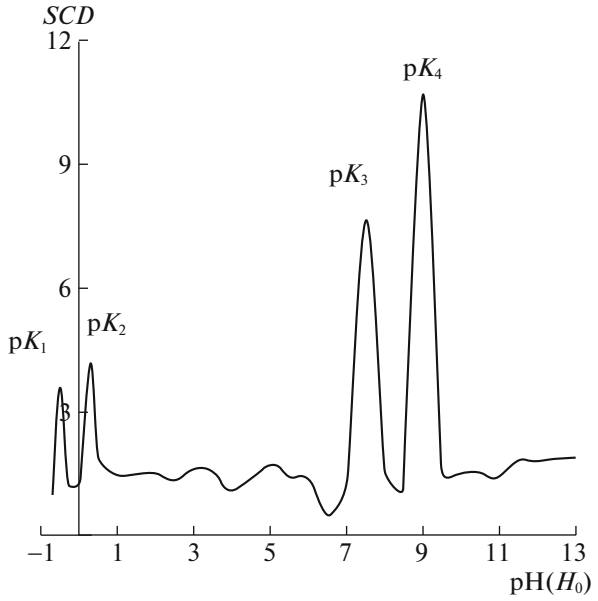
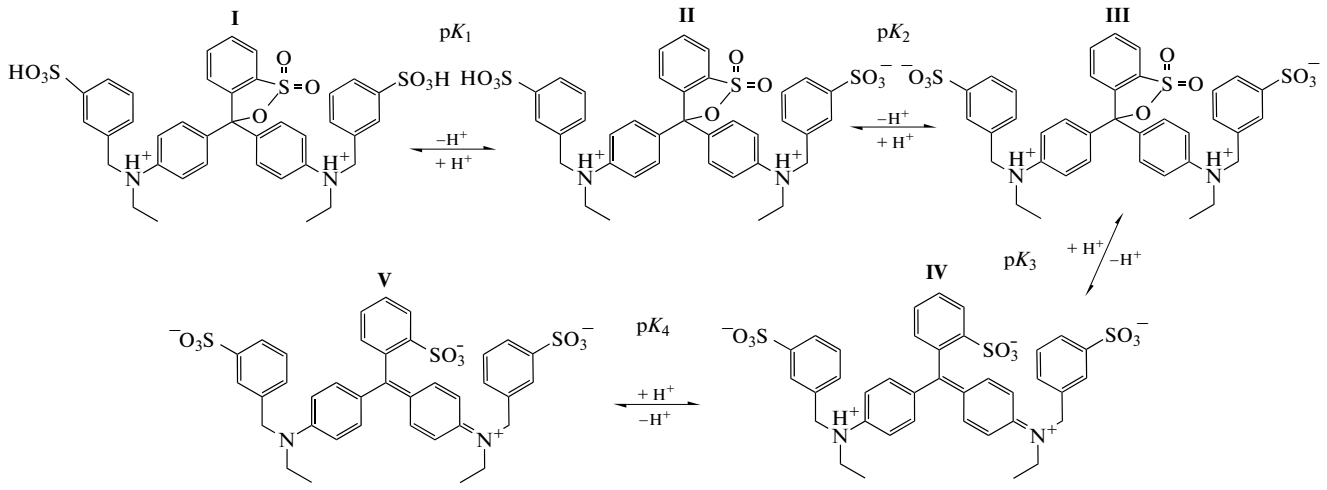


Fig. 2. Change in the specific color discrimination of brilliant blue FCF aqueous solution depending on the medium acidity.

increase in the intensity of both the long-wavelength and short-wavelength light absorption bands. It is noteworthy that the intensity of long-wave band reaches its maximum and then remains constant in the alkaline medium (pH 10, curve *4*). The band at 410 nm does not disappear, probably, indicating the existence of dynamic prototropic equilibrium in the dye solution.

To determine the pK values (Table 1), the light absorption spectra recorded in situ were processed with the SpectroCalc-H_5A program, and those obtained in cuvettes by the tristimulus colorimetry method. The relationship between *SCD* value and pH of the medium is represented in Fig. 2. Herein, four peaks corresponding to a number of sequential acid–base transformations of the dye are observed. The pH values corresponding to the peak of each peak are numerically equal to the pK values of its functional groups (Table 1). Based on the structure of the brilliant blue FCF the appearance of five maxima on the $SCD = f(\mathrm{pH})$ curve should be expected, three of which correspond to the dissociation of sulfo groups and the other two to protolytic equilibria involving nitrogen atoms. Probably, one of the sulfo groups takes part in the formation of the pseudolactone cycle and, consequently, does not dissociate, however may participate in the intramolecular proton transfer.

As shown in Table 1, the ionization constants determined by the tristimulus colorimetry method and spectrophotometry with an optical probe satisfactorily correlate with each other. It should be noted that the dissociation of sulfo groups was not detected by spectrophotometry method since this process isn't accompanied by redistribution of electron density in the molecule and, hence, does not reveal as any effects in the light absorption spectra. In turn, colorimetry allows to record "subtle" changes in the color characteristics of equilibrium ionic-molecular forms that makes it possible to determine pK values of all dye functional groups involving in protolytic equilibria, as well as to assume the existence of prototropic equilibria [7–10]. Generally, based on experimental data obtained by tristimulus colorimetry and spectrophotometry methods as well as their theoretical interpretation, the following probable scheme of acid–base equilibria in aqueous solutions of brilliant blue FCF in a wide range of pH may be proposed:



According to the presented scheme, the dye exists in the protonated lactone form of **I** in a strongly acid medium. Further dissociation of sulfo groups of benzyl fragments takes place leading to **II** and **III** formation, respectively. In a neutral and slightly alkaline medium the deprotonation of one ammonium nitrogen atom takes place followed by tautomerization with the rupture of the lactone cycle and the formation of

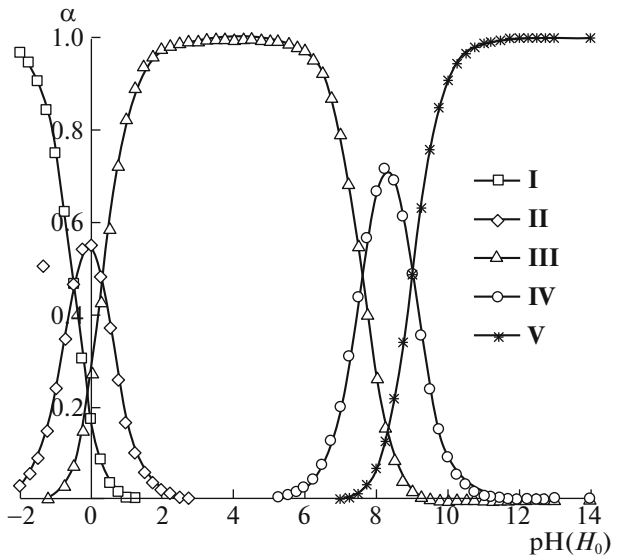
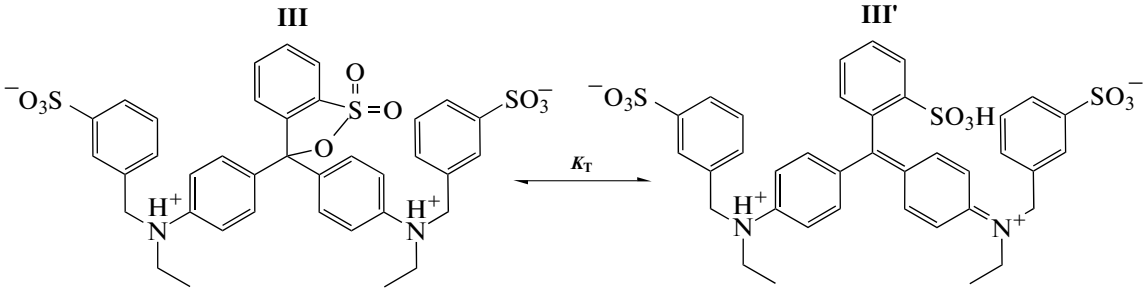


Fig. 3. Chart of the ionic forms distribution of brilliant blue FCF at different acidities in aqueous solutions.

IV. The pH increase leads to deprotonation of the second ammonium atom and the appearance of a new form **V** in the dye solution.

The acid–base forms of the dye in aqueous solution are in dynamic equilibrium depending on pH values and may be represented in the form of appropriate distribution diagram (Fig. 3). The narrow pH ranges for the existence of **I** and **II**, **IV** and **V** forms (Fig. 3) as well as the proximity of the corresponding spectral characteristics explains the ineffectiveness of classical instrumental methods of investigation as evidenced by the absence of literature information about the state of diamond blue FCF in solutions. The spectrophotometric characteristics of the electrically neutral form **III**, which dominates in a wide range of pH (0–8) in water and organic solvents, are summarized in Table 2.

In order to interpret the obtained results and to evaluate the influence of solvent nature, various parameters characterizing their macrophysical properties taken from [13] were used. Analysis of the obtained results allows to conclude that the molar extinction coefficients of the dominant acid–base form **III** in organic solvents almost linearly ($R^2 = 0.87$) increase with growth of their permittivity $\log\varepsilon_\lambda = 0.0047\varepsilon_0 + 4.794$ that is related to its solvation features. It is also noteworthy that positions of the absorption band maximum satisfactorily correlate ($R^2 = 0.83$) with the value of Hansen parameter (δH) $\lambda_{max} = -1.2944\delta H + 649.37$ related to the intermolecular interaction density of brilliant blue FCF with the solvent in the case of hydrogen bonding [16]. When changing the solvent with a high δH value to those with lower or when the polar solvent is replaced by a less polar one, bathochromic shift of the absorption band maximum of the brilliant blue FCF is observed corresponding to $n \rightarrow \pi^*$ electronic transition. An additional confirmation is the disappearance of the long-wavelength band of the dye in a strongly acidic medium (10 M H_2SO_4, Fig. 1, curve *1*) due to complete protonation of the dye. Moreover, along with solvatochromic effects tautomerism of the investigated dye may be detected. Based on a series of experimental data and their interpretation, taking into account the main theoretical positions on the structure of triphenylmethane dyes, the following scheme of tautomeric equilibrium can be proposed:



The corresponding values of the tautomerization constants ($K_T = [III']/[III] = \chi_{III'}/\chi_{III}$, where χ is the mole fraction of the corresponding tautomer) calculated using the Bershtein method [14] are presented in Table 3.

As seen in Table 3, an increase of Dimroth–Reichardt parameter ($E_T(30)$) [13] leads to a linear ($R^2 = 0.93$) increase in K_T indicating the existence of specific "solute–solvent" interactions. It is likely that the specific solvation of tertiary nitrogen atoms in the dye molecule by amphiprotic solvents reduces the stability of the tautomeric form **III** and, the content of tautomer **III'** increases with the growth polarity of the medium.

CONCLUSIONS

Thus, the acid–base properties of brilliant blue FCF in aqueous solutions were studied by tristimulus colorimetry and spectrophotometry methods; ioniza-

Table 1. The ionization constants of brilliant blue FCF in aqueous solutions ($n = 3$, $P = 0.95$)

Method	pK_1	pK_2	pK_3	pK_4
Spectrophotometry in situ	–	–	7.7 ± 0.2	8.9 ± 0.1
Tristimulus colorimetry	–0.5 ± 0.1	0.3 ± 0.1	7.6 ± 0.1	9.0 ± 0.2

To assign the pK value to the corresponding functional group of the brilliant blue FCF, see the ionization scheme of the dye.

Table 2. Spectrophotometric characteristics of the dominant acid–base form of brilliant blue FCF in different nature solvents

Solvent	λ_{max}, nm	$\varepsilon_\lambda \times 10^{-5}$	Solvent	λ_{max}, nm	$\varepsilon_\lambda \times 10^{-4}$
Water	625	0.97	Dioxane-1,4	637	0.65
Methanol	626	1.09	Propanone-2	626	0.81
Propanol-2	626	1.27	Acetonitrile	624	1.46
Butanol-1	626	1.53	Triethylamine	657	0.55
3-Methyl-1-butanol	625	1.08	Chloroform	632	0.74
Ethyl acetate	637	0.47	Dimethyl sulfoxide	632	1.41
Butyl acetate	639	0.47	Dimethylformamide	625	1.59
Tetrahydrofuran	638	0.71	Methylbenzene	648	0.25

tion constants of the corresponding functional groups were also defined. The possibilities of tristimulus colorimetry and spectrophotometry methods in situ in the study of protolytic equilibria in solutions are shown. Probable scheme of acid–base equilibria in aqueous solutions of the dye has been proposed; a diagram of the distribution of its ion-molecular forms in a wide pH range was drawn. It has been established that in a wide range of pH the electrically neutral form of the dye dominates, its molar absorptivity ($\varepsilon_{625} = 0.97 \times 10^5$) is increased with the growth of dielectric permittivity of the solvent. Replacing the polar solvents to the less polar was shown to lead to bathochromic shift of the absorption band maximum, and its position correlates with the value of Hansen parameter. It has been found that besides solvatochromic effects, tautomerism can also be detected by replacing the solvents. Tautomerization constants have been determined in some solvents, the values of which correlate with the Dimroth–Reichardt parameter value indicating the contribution of specific interactions to tautomeric equilibrium.

Table 3. Constants of tautomeric equilibrium in some solvents ($n = 3$, $P = 0.95$)

Solvent	K_T	$E_T(30)$
Water	2.73 ± 0.15	63.1
Propanol-2	0.61 ± 0.11	48.6
Dimethyl sulfoxide	0.62 ± 0.10	45.1
Chloroform	0.14 ± 0.09	39.1
Dioxane-1,4	0.10 ± 0.03	36

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