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ACID–BASE PROPERTIES OF AZO DYES IN SOLUTION STUDIED USING SPECTROPHOTOMETRY AND COLORIMETRY

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Colorimetry and spectrophotometry with chemometric data processing were used to study the acid–base properties of azo dyes in aqueous solution. The capabilities of both methods were compared. Ionization constants of all the functional groups of the azo compounds studied could be determined relative to the change in the specific color difference depending on the acidity of the medium. The colorimetric functions of ion-molecular forms of azo compounds used as an analytical signal allow us to obtain complete information on the acid–base equilibrium in a wide acidity range.

Keywords: *azo dyes, colorimetry, spectrophotometry, ionization constants, acid–base properties.*

Introduction. Advances in chemical analysis have contributed to the development of the theory of ionic equilibria and the complexation of metal ions with organic ligands [1]. Complex formation in solution has been studied rather thoroughly. A considerable number of procedures have been proposed for the determination of the stoichiometry of complexes and their stability constants such as the Ostromyslenskii–Job, Bent–French, Starik–Barbanel, and the Edmonds–Birnbaum methods as well as for establishing the type and charge of the coordinating metal ions and ligand (Nazarenko method) [2]. In turn, the study of acid–base equilibria is an important part of the investigation of the physicochemical and metal-binding properties of organic ligands, which are often polyfunctional compounds, in establishing reaction mechanisms, in optimizing the conditions for analytical chemical determination, and planning experiments involving the use of extraction and ion exchange [3]. The numerical characterization of the acid–base properties of a compound is the proton transfer equilibrium constant pK_a [4], which is one of the physicochemical properties determining the major pharmacological and pharmacodynamic parameters of molecules in ADME (absorption, distribution, metabolism, and excretion) studies [4, 5]. We should note that the pK_a term determines the direction and extent of protolytic processes and the position of acid–base equilibria and, thus, the type, charge, and concentration ratio of ion-molecular forms of molecules.

These considerations account for the present interest in the development and improvement of methods for the study of acid–base processes, establishment of the ratio of concentrations of equilibrium forms of a compound, and calculation of the corresponding pK_a values. Considerable work is underway to develop experimental methods for the determination of pK_a values in addition to theoretical methods based on the the QSPR method using quantitative structure–property relationships. Modern methods for studying acid–base properties of compounds have some disadvantages including special requirements for purity as well as light, heat, and chemical stability of the compound over the entire range of medium acidities. The possibilities of the classical methods for pK_a determination such as spectrophotometry, potentiometry, and conductometry and recently proposed methods such as capillary electrophoresis and various types of high-efficiency liquid chromatography have been discussed by some authors [4–6]. We note that physicochemical methods for studying acid–base equilibria in solution are constantly being improved and modernized. To a large extent, this improvement is related to spectrophotometry in the UV and visible regions due to the accessibility of suitable equipment, simplicity of obtaining the corresponding analytical signal, and the use of various mathematical and chemometric algorithms for data treatment [7]. Colorimetry is a promising method for the study of acid–base equilibria. Various authors [8–12] have shown that the use of color saturation functions as well as the specific and total color difference is a convenient and efficient means for determining the pK_a values of functional groups of organic compounds. The possibilities of this method were analyzed in two reviews [13, 14], while Shokrollahi and Zare [15] proposed a simple but insufficiently effective method for determining pK_a values using a tablet scanner and graphics editor.

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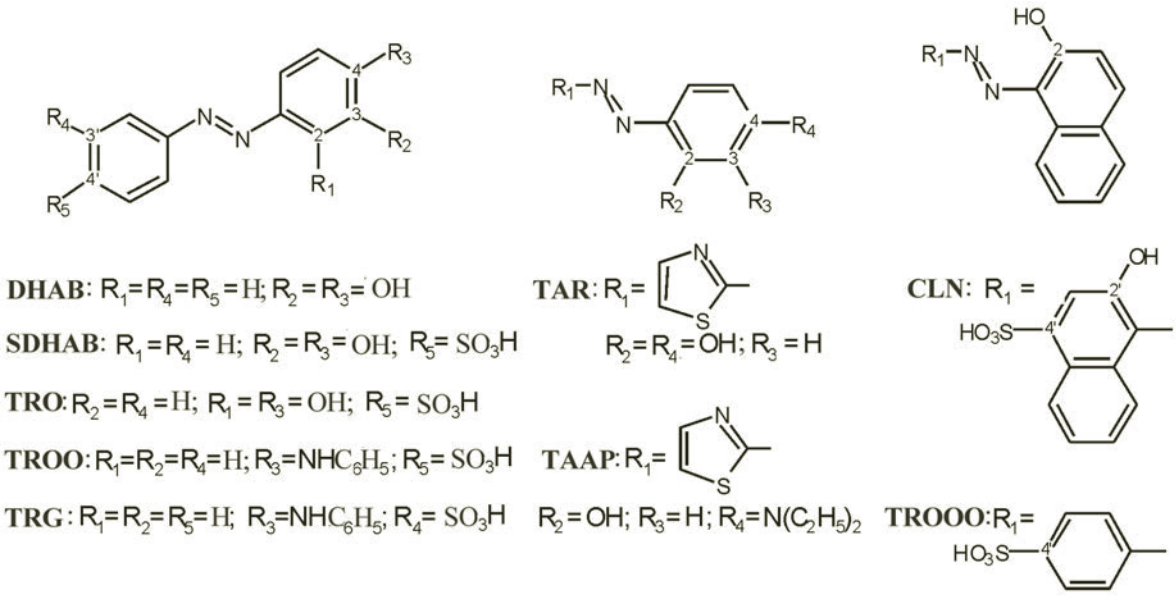
Hence, further study of methods for the determination of the pK_a values of organic compounds using colorimetric functions calculated on the basis of recorded electronic absorption spectra in the visible region holds both practical and theoretical importance. We selected a series of polyfunctional azo dyes as models.

Experimental. Solutions of azo dyes 3,4-dihydroxyazobenzene (DHAB), 4'sulfo3,4dihydroxyazobenzene (SDHAB), calcon (mordant black 17, CLN), 4(thiazolylazo)resorcinol (TAR), 4(2thiazolylazo)5diethyl*m*aminophenol (TAAP), tropeoline O (TRO), tropeoline OO (TROO), tropeoline OOO (TROOO), and tropeoline G (TRG) with concentration $1 \cdot 10^{-3}$ mole/L were prepared by dissolving a weighed sample of the compound in 50% aqueous ethanol:



Acidity was established by using solutions of sulfuric acid and sodium hydroxide. The purity of the reagents was at least analytical-grade.

The electronic absorption spectra were taken on an SF56 spectrophotometer at 380–780 nm in quartz cells maintained at constant 25°C with path length 1 cm. The acidity of the medium was monitored using an ÉSL6307 glass electrode paired with an ÉVL1M3 silver chloride reference electrode in an I160 ionometer calibrated relative to standard buffer solutions.

In order to determine the pK_a values, samples of 0.5–1.0 mL starting dye solution was added to a series of 25mL test tubes, the corresponding medium acidity was established in the range from 5 M H_2SO_4 to pH 13, and brought to the mark. In the case of unclear separation of the maxima and for greater differentiation of the pK_a values, the pH change step was reduced to 0.1. The following colorimetric functions were used: *X*, *Y*, *Z*) color coordinates in the CIEXYZ system; *L*, *A*, *B*) color coordinates in the CIELAB system; color saturation (*S*) and specific color difference (*SCD*). The calculation procedure was described in detail in our previous work [8]. The electronic absorption spectra were treated in the SpectroCalc-H_5A program to obtain the spectrophotometric determination of pK_a [16].

The order of dissociation of the functional groups of the azo compounds studied was predicted theoretically starting with the structures of their ion-molecular forms with geometry optimized by molecular mechanics methods (MM+). Calculation of pK_a values and their assignment to the corresponding functional groups in the Marvin 5.9.1 and ACDLabs Professional 6.0 program packages were carried out using the QSPR method [17].

Results and Discussion. The absorption spectra of the azo compounds DHAB and TAAP are given as examples in Fig. 1. The spectrophotometric study of acid–base equilibria in solutions of azo dyes is a rather complicated problem since protonation–deprotonation of the azo group, as a rule, is not accompanied by a significant change in the absorption spectrum of the dye. The determination of the pK_a values of auxochromic acid groups can be readily achieved when the absorption spectra have an isosbestic point. Thus, one strong absorption band with maximum at 350 nm is seen in the absorption spectrum for DHAB (Fig. 1a) in acid media (pH 1–5), while increasing the pH of the medium leads to a bathochromic shift to 445 nm. In addition, the absorption spectra shown in Fig. 1a have a pronounced isosbestic point at 390 nm. It is much more difficult to study acid–base equilibria in solutions of polyfunctional and heterocyclic azo dyes. In such cases, the overlap of the ionization processes of a series of functional groups and tautomerism do not permit isosbestic points or the assignment of bands to a specific equilibrium ionic or molecular form. The absorption spectra of TAAP may be seen as an example (Fig. 1b).

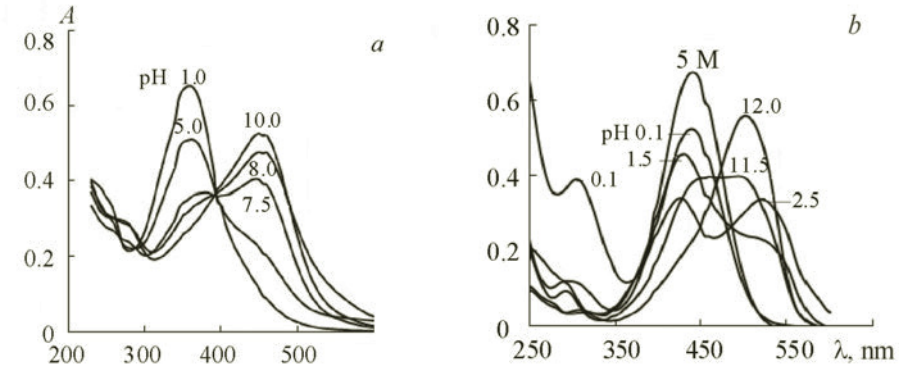


Fig. 1. Electronic absorption spectra of azo dyes DHAB (1) and TAAP (2) at different medium acidities.

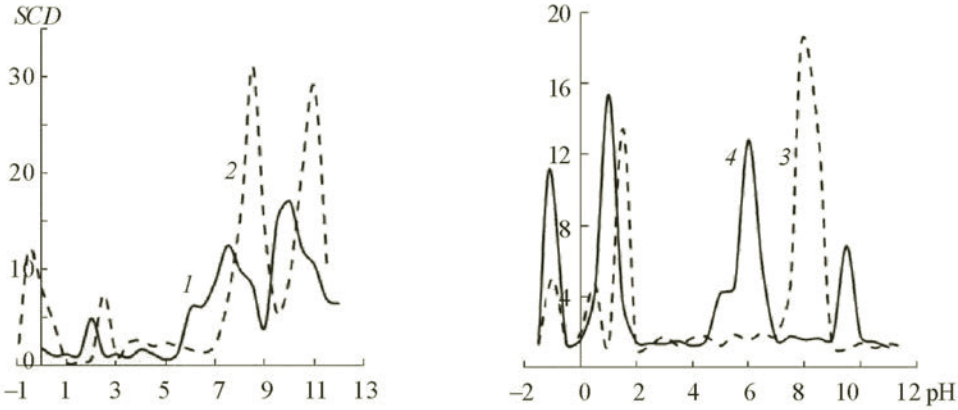


Fig. 2. Effect of pH of the medium on the change in the specific color difference of solutions of DHAB (1), SDHAB (2), TAR (3), and TAAP (4).

The spectrum of TAAP in 5 M H_2SO_4 has one strong band with maximum at 445 nm. However, at pH 0.1, its intensity drops and a second band appears with maximum at 300 nm. Increasing the pH leads to disappearance of the band at 300 nm, decrease in light absorption at 445 nm, and formation of a new band with maximum at 515 nm. An increase in intensity and hypsochromic shift of the band to 495 nm is observed in alkaline media. On the whole, there are no isosbestic points in the absorption spectra of TAAP.

The specific color differences of the azo dye solutions relative to pH of the medium were calculated for the colorimetric pK_a determination (Fig. 2). The curves for *SCD* (specific color difference) as a function of pH for DHAB, SDHAB, TAR, and TAAP have clearly differentiated peaks, whose number corresponds to the number of functional groups of the dyes capable of undergoing protolysis in the acidity range examined. Similar curves were obtained for the other dyes studied. According to previous recommendations [8, 9], the pK_a values were determined as the pH corresponding to the maxima of the *SCD* parameter on the curves for *SCD* vs. pH. We should note that broadened peaks and the appearance of shoulders (Fig. 2) are probably related to tautomeric equilibria, which are most characteristic for azo dyes possessing an OH group in the position *para* to the azo group.

Spectrophotometry with an iterational calculation algorithm and QSPR approaches were used to confirm the correctness of the determination of the azo dye pK_a values (Table 1). The pK_a values in Table 1 obtained by colorimetry and spectrophotometry are in accord, which indicates their correctness. However, the dissociation constants of strongly acidic sulfo groups and, in most cases, ionization constants of azo groups cannot be determined spectrophotometrically. Differentiation of functional groups with similar acid–base characteristics as well as determination of equilibria involving strongly acid sulfo groups and the corresponding pK_a values are possible using the advantages of colorimetric functions as an analytical signal. Thus, the spectrophotometric data of the entire visible range are considered for calculating these functions, which eliminates the problem of an incorrect selection of a photometric analytical wavelength in the case of the superposition of tautomeric

TABLE 1. Ionization Constants of Azo Dyes in Solutions (n = 3, P = 0.95)

Dye	Group	Colorimetry	Spectrophotometry	QSPR
DHAB	–N=N–	2.0 ± 0.1	–	–
	3–OH	7.6 ± 0.2	7.8 ± 0.1	8.1
	4–OH	10.1 ± 0.1	9.9 ± 0.2	–
SDHAB	4′–SO_3H	–0.7 ± 0.1	–	–1.0
	–N=N–	2.5 ± 0.2	–	–
	3–OH	8.5 ± 0.2	8.3 ± 0.2	8.8
	4–OH	11.2 ± 0.1	11.1 ± 0.1	11.7
CLN	–SO_3H	–0.7 ± 0.1	–	–0.6
	4′–N=N–	0.6 ± 0.2	0.5 ± 0.2	–
	o–OH	7.4 ± 0.1	7.5 ± 0.1	7.5
	n–OH	12.2 ± 0.1	12.3 ± 0.1	11.9
TAR	–N=	–1.1 ± 0.1	–	–1.2
	–N=N–	1.0 ± 0.1	0.9 ± 0.1	–
	2–OH	6.0 ± 0.2	5.9 ± 0.1	7.2
	4–OH	9.8 ± 0.1	9.9 ± 0.2	9.2
TAAP	–N=	–1.0 ± 0.1	–0.9 ± 0.2	–1.1
	–N=N–	0.6 ± 0.2	0.5 ± 0.1	–
	4–$N(C_2H_5)_2$	1.5 ± 0.1	1.6 ± 0.1	1.7
	2–OH	7.8 ± 0.2	7.7 ± 0.1	7.5
TRO	4′–SO_3H	–0.8 ± 0.1	–	–0.9
	–N=N–	0.5 ± 0.1	0.7 ± 0.1	–
	2–OH	6.5 ± 0.1	6.6 ± 0.1	–
	4–OH	12.0 ± 0.2	11.8 ± 0.2	12.0
TROO	4′–SO_3H	–0.6 ± 0.1	–	–
	–N=N–	0.8 ± 0.1	0.9 ± 0.1	0.8
	–NH–	2.0 ± 0.1	1.9 ± 0.1	1.3
TROOO	4′–SO_3H	–0.7 ± 0.1	–	–
	–N=N–	2.0 ± 0.1	1.9 ± 0.1	–
	2–OH	8.5 ± 0.1	8.3 ± 0.1	11.8
TRG	3′–SO_3H	–0.8 ± 0.1	–	–1.0
	–N=N–	0.9 ± 0.1	1.0 ± 0.2	–
	–NH–	2.1 ± 0.2	2.2 ± 0.1	1.2

equilibria in dissociation processes. Furthermore, the molar coefficients of colorimetric functions are one or two orders of magnitude greater than molar absorption coefficients [13], which should permit the determination of fine differences in the spectral characteristics of equilibrium acid–base forms. The possibilities of the QSPR method are limited although, in many cases, this method allows determination of pK_a values, especially of sulfo groups, which permits additional confirmation of the correctness of the results obtained by the colorimetric method. We should note that in some cases such as SDHAB, TAR, TROO, TROOO, and TRG, the pK_a values determined by the QSPR method differ markedly. This discrepancy is attributed to the inadequacy of present algorithms, which has been noted repeatedly by Zevatskii [5] and Reijenga [6]. However, results obtained by the QSPR method permit the correct assignment of pK_a values to the corresponding functional groups. The

TABLE 2. Comparison of the Advantages (+) and Disadvantages (–) of Methods for the Determination of pK_a Values

Method	Amount of compound	pK_a range	Accuracy	T, I, ε	Time/cost
Potentiometry	– –	–	+	+	+ +
Conductometry	– –	–	+	–	+ +
Voltamperometry	–	+	+	+	+
HELC	++	–	+	–	–
Capillary electrophoresis	++	+	++	++	+
Calculation/QSPR	++	++	– –	– –	+
Spectrophotometry	+	+	++	++	+
Colorimetry	+	++	++	++	++

pK_a values of functional groups of tropeolines and TAR given in Table 1 are in satisfactory accord with the corresponding handbook data [18, 19].

Comparison of colorimetry with other methods proposed for the determination of pK_a values according to Reijenga et al. [6] holds interest. Table 2 shows that the requirement of using solutions with high concentrations and, thus, rather large amounts of compound is a significant limitation of potentiometric and conductometric methods. These methods permit the determination of pK_a values in a limited range but these values are rather accurate and simply found. Similarly, the volt-amperometric method, which also permits determination of the effect of temperature (T), ionic strength (I), and organic solvents (ε) on the pK_a value. High-efficiency liquid chromatography (HELC) facilitates work with very small amounts of the compound to be tested but is more costly and complicated than the other methods relative to cost and simplicity. We should note that capillary electrophoresis is a rather efficient method for studying the protolytic properties of compounds but has the disadvantage of not permitting determination of pK_a values of functional groups with similar acid–base properties. Disadvantages of calculation methods for the determination of pK_a values include their insufficient precision and the impossibility of evaluating the effect of temperature, ionic strength, and nature of the solvent on these values. Spectrophotometry and colorimetry are not inferior to electrophoresis but are simpler and more available. An advantage of colorimetry is the possibility of the simultaneous determination of pK_a values of all the functional groups of a compound capable of undergoing protolysis, including groups with similar acidity, which additionally permits us to save time in the complete study of acid–base properties of a compound. On the whole, the use of values of the colorimetric functions of ionmolecular forms of dyes as an analytical signal permits us to obtain a complete picture of the existing acid–base equilibria in a broad range of medium acidity.

Conclusions. Comparative analysis of the possibilities of the major physicochemical methods and colorimetry in the study of protolytic equilibria and the determination of ionization constants in solution shows that, in contrast to spectrophotometry, colorimetry permits determination of the ionization constant of sulfo groups of azo dyes, whose dissociation, though slight, affects the magnitude of colorimetric functions. In the colorimetric determination of pK_a values, the superposition of tautomeric equilibria on the ionization processes and the coexistence of several ion-molecular forms capable of undergoing protolysis in narrow pH ranges does not have a significant effect, while the error of an incorrect selection of the wavelength in photometric determination of a solution is minimized.

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