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THE ACID-BASE EQUILIBRIUM OF CATIONIC DYES IN THE AQUEOUS SOLUTIONS OF POLY (SODIUM 4-STYRENESULFONATE)

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The acid-base properties have been used to describe the state of indicator dyes in polyelectrolyte microenvironments. The purpose of this study was to determine the absorption spectra and the apparent ionization constant of dyes, K_a^a, in the solutions of poly (sodium 4-styrenesulfonate) (NaPSS) and reveal the regularities in pK_a^a shifts as compared with the values in water, pK_a^w. The well-known dyes neutral red (NR), acridine orange (AO), and methyl yellow (MY) have been used as indicators. The influence of the polyelectrolyte : dye concentration ratio, [P]/[D], on absorption spectra is discussed in terms of metachromasy. The pK_a^a values were determined at [P]/[D] ratio of 1 and 60, the ionic strength varied from 0.01 to 0.05 M. It was found that the absorption spectra of the protonated forms of NR and AO strongly depend on the [P]/[D] ratio due to dye-dye interaction, whereas the absorption spectra of MY virtually don't alter with the change of [P]/[D] ratio. The pK_a^a shifts for all examined dyes were positive mainly due to electrostatic interaction. The optimal conditions for the pK_a^a shifts determination also are considered.

Key words: polyelectrolyte, poly (sodium 4-styrenesulfonate), metachromasy, neutral red, methyl yellow, acridine orange, apparent ionization constant.

Introduction

The interactions of cationic dyes with anionic polyelectrolytes in aqueous solutions have been extensively studied [1-4]. Particularly, the metachromatic effects, the stoichiometry, and the energy of dye-polyelectrolyte interaction have been ascertained for many acid-base indicator dyes. But these data are insufficient for full description of the state of dyes in the polyelectrolyte macromolecules microenvironment. For example, the "protein error" at the pH determination of protein-containing solutions is well-known. This error results from the adsorption of the indicator on the oppositely charged protein and reflects the "surface" pH value rather than the bulk one [5]. Besides, the polyelectrolyte microenvironment may be considered as a non-aqueous medium. I.e., the protonation degree of the indicator changes in polyelectrolyte solution as compared with that in pure water due to the difference between the "surface" and bulk pH values and modification of the indicator properties in non-aqueous microenvironment. Thus, the state of acid-base equilibria described by the so-called "apparent" ionization constants is also inherent characteristic of the indicator dyes.

In the present study, we investigated the acid-base equilibria of the cationic dyes, neutral red (NR), acridine orange (AO), and methyl yellow (MY). Both NR and AO are metachromatic dyes commonly used as probes for determination of the internal pH in many biological objects such as intact tissues, vesicles, chloroplasts [6-8]. On the contrary, the MY hasn't revealed metachromatic behavior. Earlier, the attempts have been made to estimate the state acid-base equilibria of NR [9,10], AO [9], and rhodamine B [4] in poly (sodium 4-styrenesulfonate) (NaPSS) solutions and NR in DNA [11] and to find the electrical surface potential of the polyelectrolyte macromolecules by studying the acid-base behavior of NR [12]. However, these investigations have been generally aimed to different biological objects and haven't been enough classified. For example, the acid-base behavior of cationic dyes in anionic polyelectrolyte solution should be compared with that in the micellar solution of anionic surfactants. It is reasonable since polyelectrolyte macromolecules in solution resemble the surfactant micelles to some degree. Thus, the aim of our investigation is to determine of the apparent ionization constants of dyes mentioned above in NaPSS solutions and to reveal regularity in polyelectrolyte behavior depending on concentration of background electrolyte as well as the concentrations of polyelectrolyte and dyes. In terms of the character of obtained apparent ionization constants, we have to establish the general conditions for the determination of apparent ionization constants in polyelectrolyte solutions of the less examined indicator dyes.

Experimental section

Materials. NaPSS was purchased from Sigma-Aldrich as a powder. The molecular weight (M_w) of NaPSS is, according to manufacturer's specifications, around 70×10^3 g mol^{-1}. The polyelectrolyte was used as received. The stock solutions of NaPSS were prepared with concentration either 0.02 or 0.05 M by placing of the sample in pure water without stirring during one week. Hereafter, the concentrations of NaPSS are expressed in monomer mol dm^{-3} (monomol dm^{-3}). Hydrochloric, acetic, and phosphoric acids, borax, sodium carbonate and sodium chloride used for preparation of working solutions were of analytical grade. The aqueous solution of NaOH was prepared from the saturated stock solution using CO_2-free water and kept protected from the atmosphere. The solutions of NR, MY and AO were prepared from the commercial solids without further purification. NR was diluted in pure water and then filtrated. The NR concentration in the working solutions was 3×10^{-5} M. The precise concentration of NR solution was determined through absorption spectrum in water, using the molar absorptivity value of NR acidic form as $\varepsilon = 17.2 \times 10^3$ mol^{-1} dm^3 cm^{-1} at 530 nm [13]. The initial solutions of MY and AO were prepared at pH 2.0 adjusted by HCl solution [14]. Besides, the ethanol-water solution of MY was prepared. The AO concentration was about 1×10^{-5} M, the precise concentration of AO solution was determined through absorbance value in water at 470 nm with $\varepsilon = 43 \times 10^3$ mol^{-1} dm^3 cm^{-1} that corresponds to the isosbestic point of monomer – dimer equilibrium [15]. The MY concentration was about 2×10^{-5} M, the molar absorptivity was found from absorption spectrum MY in ethanol-water solution. The solvatochromic betaine dye 4-(2,4,6-triphenylpyridinium-1-yl)-2,6-diphenyl phenolate was put to our disposal by Professor C. Reichardt (Philipps-Universität Marburg, Germany). It was dissolved in 96%-ethanol.

The pH values of the solutions have been adjusted using HCl for pH < 4, or buffer solutions: acetate (pH 3.7 – 5.4), phosphate (pH 5.8 – 8.0), borate (pH 8.0 – 10.0), and carbonate (sodium carbonate and hydrochloric acid, pH 8.8 – 10.0). The pH values around 11 – 12 have been adjusted by diluted sodium hydroxide. The total ionic strength of the bulk (aqueous) phase has been maintained by appropriate NaCl additions.

Methods. The ionization of cationic acid in solution can be described by the below equation:

$$HR^+ \rightleftharpoons R + H^+,\ K_a \tag{1}$$

Therefore, the apparent ionization constant is expressed as:

$$pK_a^a = pH_w + \log \frac{[HR^+]_t}{[R]_t}. \tag{2}$$

The acid-base couple HR^+/R is assumed to be (partly) located within the polyelectrolyte microenvironment and equilibrated with the pH value of the continuous (aqueous) phase, pH_w. Such apparent ionization constant was input by analogy with micellar systems [16]. The subscript *t* (total) denotes that the concentration is expressed in moles per dm^3 of the whole solution. If it deals with the acid-base indicator, the equilibrium concentrations ratio $[HR^+]_t/[R]_t$ can be determined via spectrophotometry:

$$pK_a^a = pH_w + \log \frac{A_R - A}{A - A_{HR}}. \tag{3}$$

The pH_w value may be maintained by buffer solutions and determined using a glass electrode in a cell with liquid junction.

In fact, the absolute value of pK_a^a shift expressed as $\Delta pK_a^a = pK_a^a - pK_a^w$ equals the "protein error" discussed by Danielli [5]. It can be represented in the following way:

$$pH_x = pK_a^w - \log \frac{[HR^+]_t}{[R]_t}, \tag{4}$$

$$pH_x - pH_w = \Delta pH = pK_a^w - pK_a^a, \tag{5}$$

$$\Delta pK_a^a = -\Delta pH, \tag{6}$$

where pH_x is pH that measured via the absorption spectra of the indicator and using the pK_a^w value in the calculations, ΔpH is "protein error". It should be noted that pH_x is not pH of the particles surface in

general case. It is true only if the influence of polyelectrolyte as non-aqueous media, expressed by the activity coefficients of transfer, is negligibly small as opposed to the electrostatic effects of the macromolecules, as shown by the equation (7):

$$pH_x - pH_w = -\log \frac{{}^{w}\gamma_R^{non\text{-}aq}}{{}^{w}\gamma_{HR}^{non\text{-}aq}} + \frac{\psi \cdot F}{2.303 \cdot R \cdot T} \quad (7)$$

and eq. (8) [17]:

$$pH_{surface} - pH_w = \log {}^{w}\gamma_{H^+}^{non\text{-}aq} + \frac{\psi \cdot F}{2.303 \cdot R \cdot T} \quad (8)$$

${}^{w}\gamma_i^{non-aq}$ are the activity coefficients of transfer from water to non-aqueous medium for the *i*-th particle, ψ – the electrical surface potential of the polyelectrolyte macromolecules.

Absorption spectra were measured with Hitachi U-2000 spectrophotometer against pure water as blanks, at 25°C. The pH determinations were performed by using R 37-01 potentiometer and pH-121 pH-meter (Russia) with an ESL-43-07 glass electrode (Gomel, Belarus) in a cell with liquid junction (3.0 M KCl). An Ag|AgCl electrode was used as a reference electrode. The glass electrode was calibrated with standard buffer solutions: pH 9.18, 6.86, 4.01, and 1.68 at 25°C. In addition, the NaPSS particle size (*d*) distribution and zeta-potentials (ζ) were determined via dynamic light scattering (DLS) by N. N. Kamneva using Zetasizer Nano ZS Malvern Instruments apparatus, in the laboratory of the National University of Food Technologies, Kiev, Ukraine.

Results and discussion

Size of NaPSS particles. The particle size distributions by intensity, volume, and particle number of NaPSS determined via DLS are represented in Figure 1. According to these measurements, the average size (*d*) of particles is of the order of 9.3 ± 0.5 nm (by number) for the salt-free NaPSS solution of concentration 1.8×10^{-3} M and 13.2 ± 0.8 nm (by number) for NaPSS solution of the same concentration at $I = 0.05$ M. In this case, the average diameter is the diameter of the sphere which diffuses at the same speed as the particle being measured. The large intensity peaks at 300 nm are related to large light scattering of small amount of the polyelectrolyte molecule aggregates.

As to the particles shape, the DLS method per se doesn't give any information about asymmetry of NaPSS particles. However, as it is generally known, polyelectrolyte molecules possess significant aspect ratio parameter. The numerical simulations as well as DLS and viscosity measurements of two NaPSS samples, with average molecular weight $M_w = 15.8 \times 10^3$ and 70×10^3 g mol^{-1}, were carried out by Adamczyk et al. [18,19]. For NaPSS with average molecular weight $M_w = 15.8 \times 10^3$ g mol^{-1}, aspect ratio parameter varies between 11.8 (for $I = 2 \times 10^{-3}$ M) and 4.5 (for $I = 0.15$ M) using experimental data of intrinsic viscosity, the corresponding values of the equivalent length of the molecule vary between 16.3 to 8.5 nm, whereas theoretical predictions give 12.5 – 8.5 nm. For the same polyelectrolyte, the hydrodynamic radius determined by DLS was 3.1 nm (for $I = 5 \times 10^{-3}$ M) and 4.0 nm (for $I = 0.15$ M) [18]. According to numerical simulations, the hydrodynamic radius is 2.5 nm for a spheroid, 2.6 nm for a cylinder, 3.1 nm for a semi-circle (torus), and 4.8 nm in the case of a circle (torus). Thus, deformation of macromolecules is expected to increase its hydrodynamic radius, i.e., decrease the diffusion coefficient. So, in terms of simulation the shape of NaPSS molecules is semi-circle, i.e. molecules aren't bent significantly [18]. For NaPSS with average molecular weight $M_w = 70 \times 10^3$ g mol^{-1} in 4.9×10^{-4} monomol dm^{-3} polyelectrolyte solution, the hydrodynamic radius is 11.7 nm for $I = 1 \times 10^{-3}$ M and 13.8 nm for $I = 0.15$ M, and in 2.5×10^{-3} monomol dm^{-3} NaPSS solutions it equals 6.9 nm for $I = 1 \times 10^{-3}$ M [19]. According to the numerical simulations, the hydrodynamic radius is 10.7 nm for a cylinder, 12.3 nm for a semi-circle, and 16.3 nm in case of a circle. [19]. Thus, the polyelectrolyte molecules are rods at low ionic strength and bent to the semi-circle when the ionic strength increases.

So, in spite of lower values of hydrodynamic radius obtained in our investigation, NaPSS molecules are expected to be enough rigid and to have a semi-circle form. The value of the ζ-potential was estimated as –21.8±0.5 mV in the salt-free NaPSS solution and –15.4±3.0 mV with 0.05 M NaCl (Smoluchowski equation was used in processing the data). The decrease in ζ-potential is consistent with surface charge screening by sodium chloride.

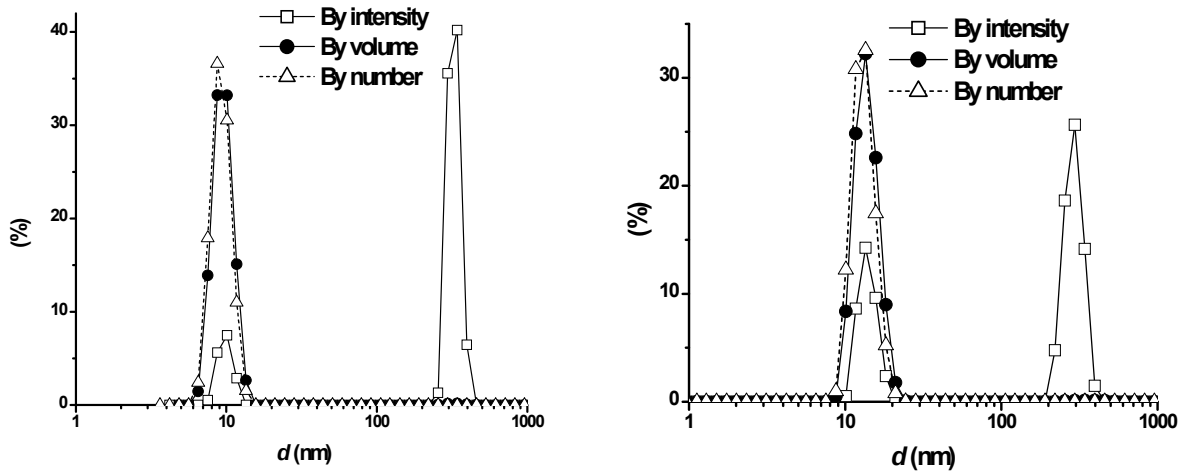


Figure 1. The size distribution of NaPSS in 1.8×10^{-3} M aqueous solution without (left) and with 0.05 M NaCl (right).

The interaction between NaPSS and dye molecules. We have examined the influence of the concentration of the polyelectrolyte on the visible spectra of AO, NR and MY according to technique reported elsewhere [1-3,20-24]. It allows determining the stoichiometry of the dye-polyelectrolyte complexes and choosing appropriate conditions for ascertainment of the apparent ionization constants.

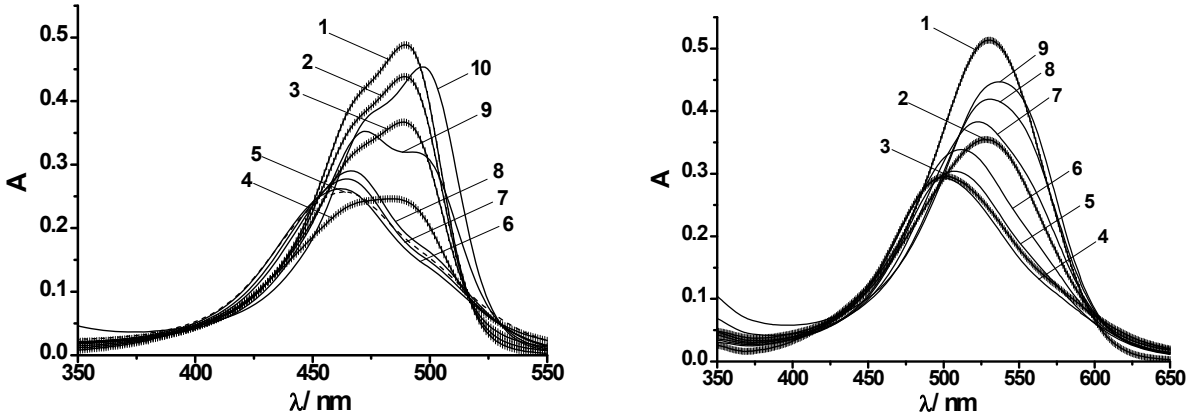


Figure 2. The absorption spectra of acridine orange in the presence of NaPSS at various P/D ratios at pH 7.0; c (AO) = 9.5×10^{-6} M; 1 – P/D = 0 (λ_{max} = 490 nm), 2 – P/D = 0.20, 3 – P/D = 0.50, 4 – P/D = 1.0, 5 – P/D = 1.5, 6 – P/D = 2.0, 7 – P/D = 5.0, 8 – P/D = 10, 9 – P/D = 100, 10 – P/D = 500. (left)

The absorption spectra of neutral red in the presence of NaPSS at various P/D ratios at pH 2.0; c (NR) = 2.98×10^{-5} M; 1 – P/D = 0 (λ_{max} = 530 nm), 2 – P/D = 0.34, 3 – P/D = 1.0, 4 – P/D = 1.7, 5 – P/D = 9.4, 6 – P/D = 17, 7 – P/D = 67, 8 – P/D = 170, 9 – P/D = 290 (right); the curves 3 and 4 (right) correspond to the γ-band, curve 9 corresponds to the monomer band, α_2.

In the presence of NaPSS the absorption maxima of the protonated charged species of AO and NR were revealed to be blue-shifted, and their molar absorptivities decrease in comparison with the corresponding values in aqueous solutions. The NaPSS induces the metachromasy of AO, with the appearance of the dimer band, β, at [P]/[D] ratio equals ca. 1, and monomer band, α_2, which appears at a large excess of polyelectrolyte. The metachromasy consists in the appearance of several colors in solution of a single dye. Thus, the increase in the [P]/[D] value from 0 to 1 has resulted in the change of the monomer band of free AO, α_1 (Figure 2, left, curve 1), to the β dimer band (Figure 2, left, curves 5-6). The subsequent increase in the [P]/[D] value to about 10^2 has led to the appearance of the monomer band of AO fixed in the complex with NaPSS, α_2 (Figure 2, left, curve 10). The α and β bands of AO have been described in the literature [9,25]. The so-called γ-band, which corresponds to the higher

aggregates of the dye [25], was not observed under the conditions of our experiment. The polyelectrolyte is known to stack the dye molecules when the difference between the concentrations of the NaPSS (in monomol dm^{-3}) and the dye is small [25]. However, the excess of the polyelectrolyte binding sites tends to unstack dye molecules. The occurrence of the γ and α_2 bands in the NR spectra involved with the increase in the polyelectrolyte concentration should be also considered as a kind of metachromatic effect. The similar changes in the absorption spectra are observed for the anionic dye methyl orange in the presence of the strong cationic polyelectrolyte [26].

The stoichiometry of the dye-polyelectrolyte metachromatic complex was determined using the absorbance values at the wavelengths corresponding to the largest absorbance changes [2], i.e., at 490 nm for AO and 530 nm for NR. The number of polyelectrolyte sites per one bound dye molecule was obtained from the ε vs. [P]/[D] dependence (Figure 3), by extrapolation of the two linear pieces of the curve. For both dyes the stoichiometry of complex was found to be about 1:1 that is consistent with the appearance β or γ bands at the given [P]/[D] ratio. Such a stoichiometry has confirmed relative rigidity and prolateness of macromolecules [2] as it was concluded from DLS experiment.

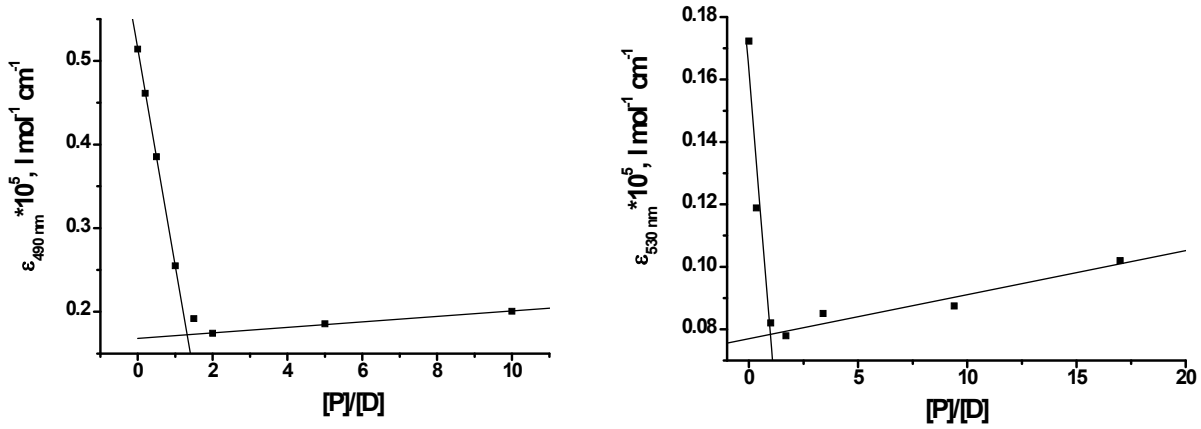


Figure 3. Stoichiometry for AO – NaPSS complex (left) and NR – NaPSS complex (right). The molar absorptivity values of dye-NaPSS solutions vs. [P]/[D].

It should be noted that the monomer band, α_2, undergoes a small bathochromic shift as compared with the monomer band of the free dyes, α_1. Similar bathochromic shifts are observed for the non-metachromatic cationic indicator dyes, for instance, MY, as represented in Figure 4 (left).

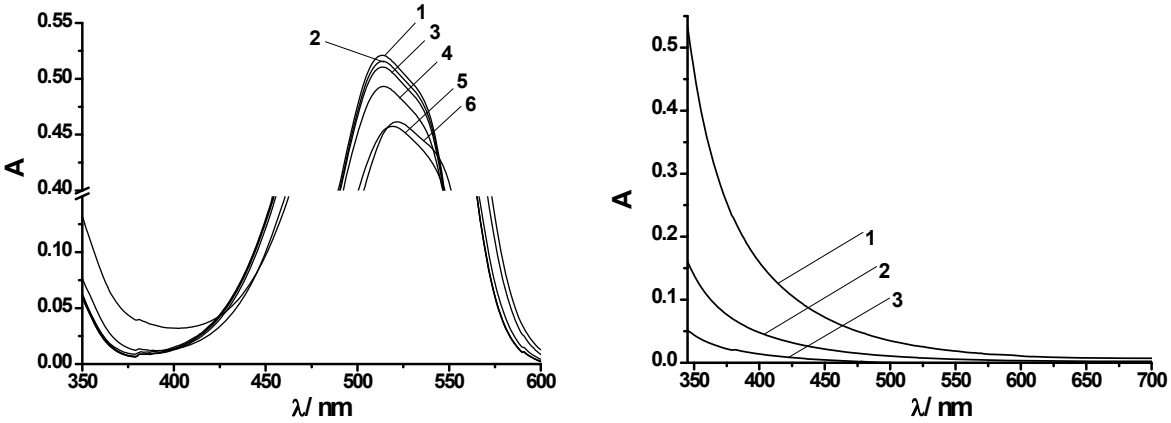


Figure 4. The absorption spectra of methyl yellow in the presence of NaPSS at various P/D ratios at pH 1.3; c (MY) = 1.78×10^{-5} M; λ_{max}([NaPSS]/[MY] = 0) = 514 nm; 1 – P/D = 0, 2 – P/D = 0.1, 3 – P/D = 1.0, 4 – P/D = 10, 5 – P/D = 100, 6 – P/D = 490. (left)

The absorption spectra of NaPSS at various polyelectrolyte concentrations: 1 – c (NaPSS) = 0.05 M, 2 – 0.015 M, 3 – 0.005 M, measured against water. (right)

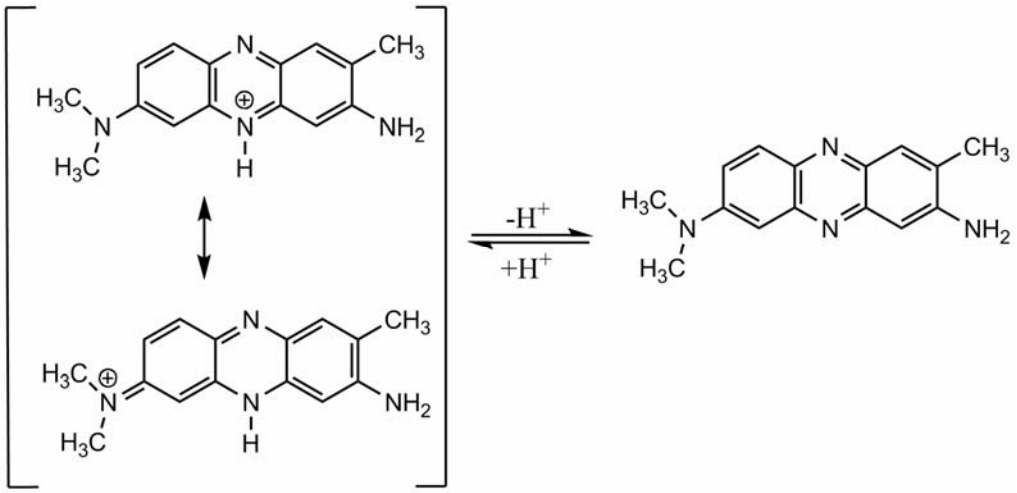
In terms of ascertained regularities, the determination of apparent ionization constant of these indicator dyes demands NaPSS concentration that one can observe the α_2 band. In this case, the conditions of determination are similar to those in surfactant micellar solution, when one micelle should fix only one dye molecule, i.e., the interaction between dye molecules should be excluded [17,27,28]. Such conditions were used by Neumann [12] for polyelectrolyte surface potential determination, the ratio between anionic polymer sites and dye molecules was kept at ca. 1000. However, the high NaPSS concentration may result in difficulties at solution preparation or pH measurements. As well, low [P]/[D] values correspond to the dilute regime of polyelectrolyte solutions whereas [P]/[D] > 100 meets the semi-dilute regime. Besides, when the concentration of NaPSS arises, the light scattering by macromolecules will become considerable. Therefore, the absorbance value of the dyes becomes commensurate with that of NaPSS, that decreases the accuracy of the results despite measuring of the working solution spectra against the blanks containing NaPSS (Figure 4, right). On the other hand, the pK_a^a value of NR is observed earlier [10] to increase with polyelectrolyte concentration up to a constant value when [P]/[D] ratio is about 2. Therefore, let us choose two [P]/[D] ratios as 1 and 60. Thereby we can preliminary estimate the influence of NaPSS on dye molecule state.

The problem of the completeness of binding of the dyes by the macromolecules should be also discussed. The protonated species of AO, NR and MY should be bound by NaPSS due to electrostatic interaction at least. In particular, the (partial) binding of the dyes was confirmed by observed alteration of the absorption spectrum of the solvatochromic betaine dye 4-(2,4,6-triphenylpyridinium-1-yl)-2,6-diphenyl phenolate. We revealed that microscopic polarities, $E_T(30)$, of the macroion are 59.9 kcal/mol (λ_{max} = 477 nm) and 58.3 kcal/mol (λ_{max} = 490 nm) at 0.002 and 0.01 M NaPSS, respectively, which are slightly higher than in the micelles of sodium dodecyl sulfate (SDS). Such a result is consistent with that obtained by Neumann et al. [12]. As was reported by Baumgartner et al. [10], the absorbance maximum of the NR basic species is not altered by NaPSS at [P]/[D] from 0 to 10. It may mean that the NR neutral molecule hasn't been bound by NaPSS that is in line with the absence of electrostatic interaction between dye and NaPSS molecules. However, in the first place the lack of spectra alterations has denoted the absence of the metachromatic effects for basic species. Meanwhile, the conclusion about the unbinding of the basic species may be found false. Nevertheless, the determined apparent ionization constant is a total characteristic which includes metachromatic, electrostatic, hydrophobic and other effects, so its determination is of importance.

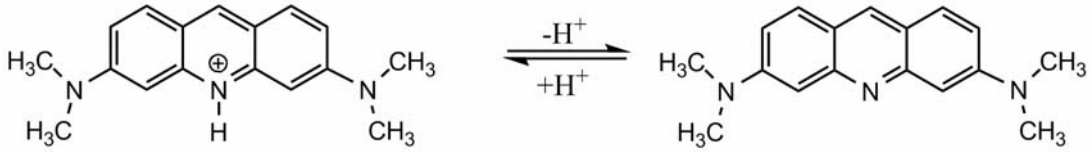
It should be noted that the association of NR with the anionic colloidal electrolyte in solution below the critical micelle concentration occurs in spite of the absence of surfactant aggregates. Furthermore, the shift of the acid-base equilibrium state owing to the NR – surfactant associate formation has been used for the quantitative determination of alkylbenzene sulfonates [29]. The binding of NR by amphiphilic surfactants gives addition evidence of the dye molecules fixation on the polyelectrolyte macroion.

The influence of NaPSS microenvironment on protolytic properties of the indicator dyes. Scheme 1 represents the acidic dissociation of NR. However, it should be noted that, according to some authors [9,30], this acid-base transition may occur in two steps. First, the uncharged NR is converted into the protonated form of the dye with no resonating charge with pK = 7.35. Next, with pH decrease the resonance structure shown in Scheme 1 appears. The pK of the second transition is 5.89. Thus, the pH-dependent spectra of NR in pure water haven't exhibited clear isosbestic point [30]. This, however, seems to be unlikely because of independence of the state of tautomeric equilibrium of the dye on the pH value. Besides, the molar absorptivity decreases when the dye concentration rises and the slight blue shift of the maximum of absorbance is observed. These phenomena have been attributed to the formation of dimers or higher aggregates of dye (dye-induced metachromasy). The pK_a^w values, calculated earlier by Dell'Antone et al. [9] from the mid-point of the plots of absorbance of the alkaline band at 440 nm against pH, are 6.0 at 0.004 M NR and 6.7 at 2×10^{-5} M NR, i.e., the aggregation of dye molecules has caused the slight decrease of the pK_a^w values. On the other hand, Drummond et al. [27] have attributed the "aberrant" pH-titration behavior of NR in pure water to pH-dependent self-aggregation of NR without accounting of overlapping equilibria in terms of well-defined pK values and clear isosbestic points in the 1,4-dioxane-water mixtures and micellar solutions. The pK_a^w value was estimated as 6.5 by indirect method [27]. Nikol'skiy et al. [31] have also ascer-

tained by means of the oxidation potential method that the protonated species of NR is associated as the dimer and tetramer. The method of oxidation potential leads to $pK_a^w = 6.5 \pm 0.1$ for 1×10^{-5} M NR and 6.7 ± 0.1 for 2×10^{-4} M NR [31]. These values are in a good agreement with those obtained by spectrophotometric method. It appears that exactly the association of NR has caused problems with pK_a^w determination. The AO dissociation behavior is represented in Scheme 2.



Scheme 1. The ionization of neutral red in aqueous solutions.



Scheme 2. The ionization of acridine orange in aqueous solutions.

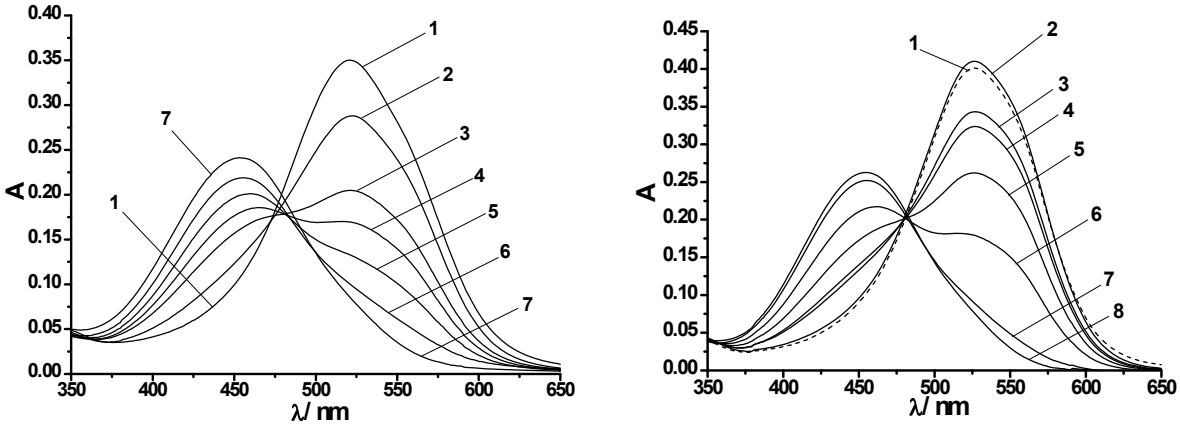


Figure 5. The absorption spectra of neutral red at [NaPSS]/[NR] = 60 and $I = 0.05$ M; c (NR) = 2.98×10^{-5} M; HR^+: λ_{max} = 521.5 nm, pH 2.0; R: λ_{max} = 454 nm , pH 12.0; 1 – pH 2.0, 2 – pH 7.40, 3 – pH 8.04, 4 – pH 8.25, 5 – pH 8.53, 6 – pH 8.98, 7 – pH 12.0; 2 – phosphate buffer, 3-6 – carbonate buffer. (left)

The absorption spectra of neutral red at [NaPSS]/[NR] = 60 and $I = 0.01$ M, with 1.6 M EtOH; c (NR) = 3.04×10^{-5} M; HR^+: λ_{max} = 526 nm, pH 5.4; R: λ_{max} = 455 nm , pH 12.0; 1 – pH 2.0, 2 – pH 5.4, 3 – pH 7.52, 4 – pH 7.64, 5 – pH 7.84, 6 – pH 8.24, 7 – pH 9.0, 8 – pH 12.0; 3, 4 – phosphate buffer, 5, 6 – borate buffer, 7 – carbonate buffer. (right)

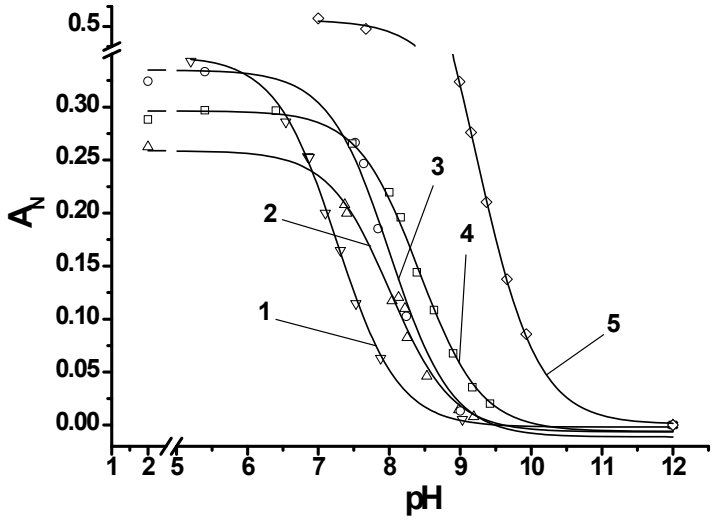


Figure 6. The dependences of normalized absorbance [$A_N(\lambda) = A(\lambda) - A_{pH\ 12}(\lambda)$] of neutral red (1 cm optical path cell) vs. pH; 1 – I = 0.05 M, 0.02 M SDS, 0.8 M BuOH, λ = 540 nm; 2 – I = 0.05 M, P/D = 60, λ = 520 nm; 3 – I = 0.01 M, P/D = 60, 1.6 M EtOH, λ = 526 nm; 4 – I = 0.01 M, P/D = 60, λ = 523 nm; 5 – I = 0.05 M, 0.02 M SDS, λ = 540 nm.

We have determined pK_a^a values of NR under different conditions, namely at I = 0.05 M and I = 0.01 M as well as at I = 0.01 M in the presence of 1.6 M ethanol. As is known, the conformations of polyelectrolyte macromolecules depend on the ionic strength. There are extended conformations in salt-free solution (rigid rods) and coil conformations in 4 M NaCl solution [32]. So, at the ionic strength of 0.01–0.05 M the macromolecules have intermediate conformations, which more resemble rods. In all cases, the [P]/[D] = 60 value was maintained.

In Figure 5, the acid-base transitions in two systems are shown. The clear isosbestic point hasn't been observed in aqueous solutions of NR. Nevertheless, it should be noted that in the presence of 1.6 M (or 0.096 v/v) ethanol, the isosbestic point is much clearer. In addition, the isosbestic point follows clockwise (Figure 5), i.e., its shift is ordered. Presumably, such a behavior of the isosbestic point relates to specific interactions of the dye molecules with the polyelectrolyte chain. In Figure 6, the normalized absorbance values at different pH and conditions ("titration" curves) are summarized. The absorbance values correspond to the wavelength of maximum absorbance of the NR cationic species. "Titration" curves for surfactant-based systems are given for comparison.

The pK_a^a values calculated utilizing three wavelengths within the spectral range near 520 nm by equation (3) are gathered in Table 1. At I = 0.01 and 0.05 M, pK_a^a = 8.39±0.05 and 7.96±0.08 respectively, whereas at I = 0.01 M in the presence of ethanol pK_a^a = 8.01±0.18. However, if the calculations are implemented at 440 nm, following Dell'Antone et al. [9], the corresponding pK_a^a values are 8.48±0.07, 8.17±0.04, and 7.98±0.16.

The pK_a^a values of AO have been determined at I = 0.05 M, while the [P]/[D] value equals 60 or 500. The AO exhibits high molar absorptivity, so even [P]/[D] = 500 allows to determine the absorption spectra against water as blank. At [P]/[D] = 60 and 500 pK_a^a = 11.48±0.11 and 11.66±0.12, calculated utilizing three wavelengths within the spectral range near 470 and 497 nm, respectively. Thus, these pK_a^a values practically equal. The pK_a^w value of AO is 10.4 [27], than ΔpK_a^a = +1.08 and +1.26.

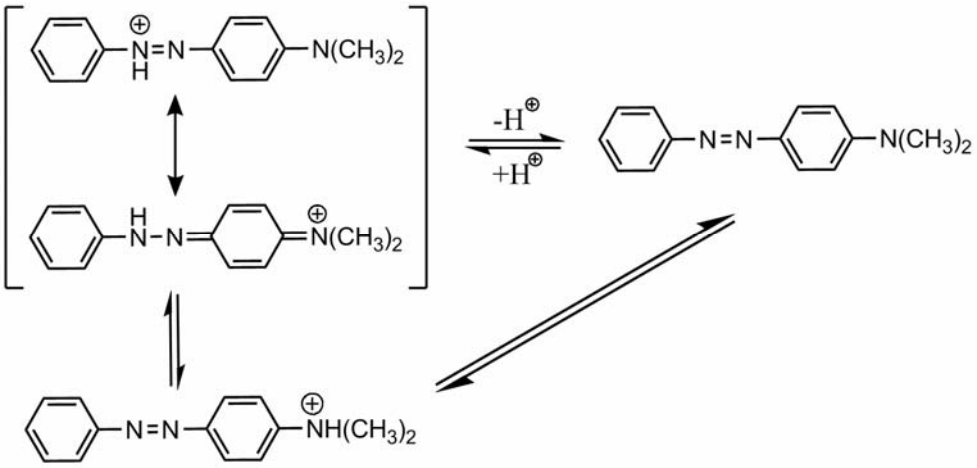
In whole, the obtained values are conformed to those in micellar pseudophase. For example, ΔpK_a^a of NR at I = 0.05 M in 0.02 M SDS solution equals +2.71 (our data, to be published soon), ΔpK_a^a of AO in 0.025 M SDS solution equals +2.00 [27].

Table 1. The indices of the apparent ionization constants of the dyes in NaPSS-based aqueous systems.

Dye	pK_a^w	*I*, M	[NaPSS]/[D] = 1		[NaPSS]/[D] = 60	
			pK_a^a	ΔpK_a^a	pK_a^a	ΔpK_a^a
Neutral red	6.5[a]	0.05	–		7.96±0.08	+1.5
		0.01	–		8.39±0.05 8.01±0.18[b]	+1.9 +1.5
Methyl yellow	3.01±0.01[c]	0.05	3.25±0.04	+0.24	3.63±0.05 3.58±0.06[b]	+0.62 +0.57
		0.01	3.15±0.03	+0.14	3.97±0.06	+0.96

[a] from ref. [27]
[b] 1.6 M EtOH
[c] from ref. [14]

Scheme 3 represents the protolytic equilibrium of MY. It includes the acid-base and tautomeric equilibria as given by Tawarah [14] and Drummond [28]. In water, the absorbance of the basic species at pH 9.0 was found to decrease with time (color changes from intense yellow to pale yellow) and to reach a constant value after ca. 45 min on the addition of NaOH solution [14]. Similar situation was observed in the present work particularly at low ionic strength. These effects are supposed to be caused by aggregation of the basic species. The lowering of pH results in the appearance of the mono-protonated form as the azonium–ammonium tautomeric mixture in definite HCl concentration range (in water, this corresponds to the range of 0.032 to 0.46 M HCl [14]). In water, the azonium tautomer exhibits the absorbance maximum at 518 nm, whereas that of the ammonium tautomer is at 316 nm [14].



Scheme 3. The ionization of methyl yellow in aqueous solutions.

We have determined the pK_a^a values of MY at $I = 0.05$ and $I = 0.01$ M, while the [P]/[D] ratio equaled 1 or 60, and also at $I = 0.05$ M and [P]/[D] = 60 in the presence of 1.6 M ethanol. The absorption spectra of MY at $I = 0.05$ M are represented in Figure 7. The MY is highly soluble in ethanol–water mixture that results in higher absorbance values in case if the working solutions are prepared by diluting the initial alcoholic solution (Figure 7, left). The basic species in ethanol–water mixture is fairly steady, the isosbestic point is reasonably clear. Nevertheless, the absorbance of MY in aqueous polyelectrolyte solutions (Figure 7, right) undergoes sharp fall on NaOH additions. When the working solutions are prepared by acetate buffer, the first added component of the buffer mixture has to be acetic acid and the second one is sodium hydroxide. As well the first added component of phosphate buffer used for basic form has to be phosphate acid and the second one is sodium hydroxide. In this case, the spectra of species mixture are stable in time but absorbance of the MY base decreases as time passes. Such a method of solution preparation has allowed to determine the MY spectra at $I = 0.05$. The isosbestic point follows clockwise as in the case of NR. The partial decoloration of the solutions

of the basic form analogous to behavior of MY in pure water is attributed to MY aggregate formation [14]. Otherwise, it may be caused either by adsorption on the glass of the flasks or by precipitation.

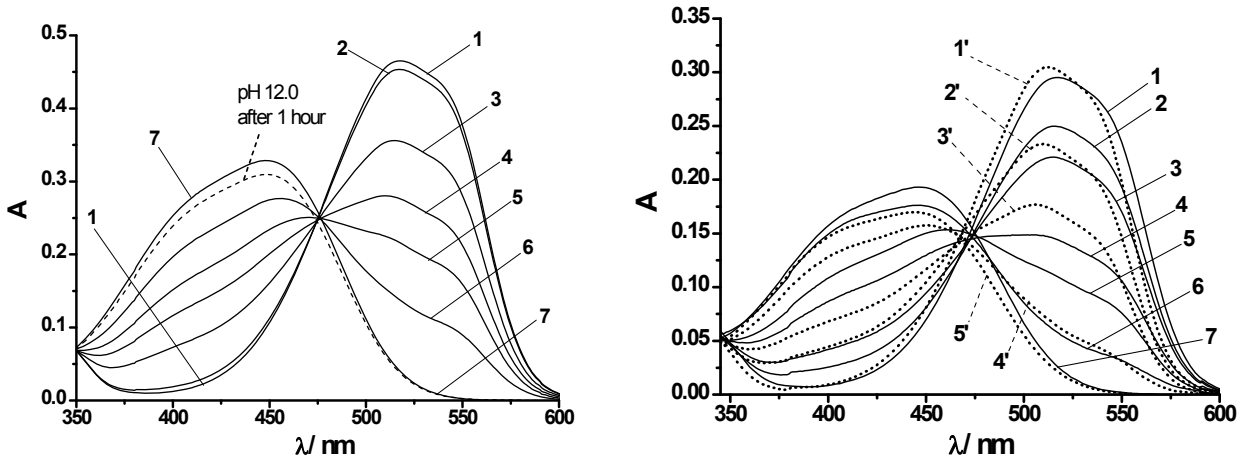


Figure 7. The absorption spectra of methyl yellow at [NaPSS]/[NR] = 60 and I = 0.05 M with 1.6 M EtOH; c (MY) = 1.78×10^{-5} M; HR^+: λ_{max} = 517 nm, pH 1.3; R: λ_{max} = 448.5 nm, pH 12.0. R species solution is unstable, the next day the precipitate appeared in the solution; 1 – pH 1.3, 2 – pH 2.0, 3 – pH 3.13, 4 – pH 3.48, 5 – pH 3.78, 6 – pH 4.07, 7 – pH 12.0. (left)

The absorption spectra of methyl yellow at [NaPSS]/[MY] = 60 (solid) and [NaPSS]/[MY] = 1 (dot), at I = 0.05 M; the initial solution of MY was obtained at pH 2.0; c (MY) = 1.17×10^{-5} M; at [NaPSS]/[MY] = 60 HR^+: λ_{max} = 517 nm, pH 1.3, R: λ_{max} = 446 nm, pH 7.8; 1 – pH 1.3, 2 – pH 2.86, 3 – pH 3.13, 4 – pH 3.80, 5 – pH 3.98, 6 – pH 4.55, 7 – pH ~ 7.8; at [NaPSS]/[MY] = 1 HR: λ_{max} = 512.5 nm, pH 1.3, R: λ_{max} = 444.5 nm, pH 7.8; 1′ – pH 1.3, 2′ – pH 2.82, 3′ – pH 3.18, 4′ – pH 3.98, 5′ – pH ~7.8. The first added component of acetate buffer is acetic acid and the second is sodium hydroxide. (right)

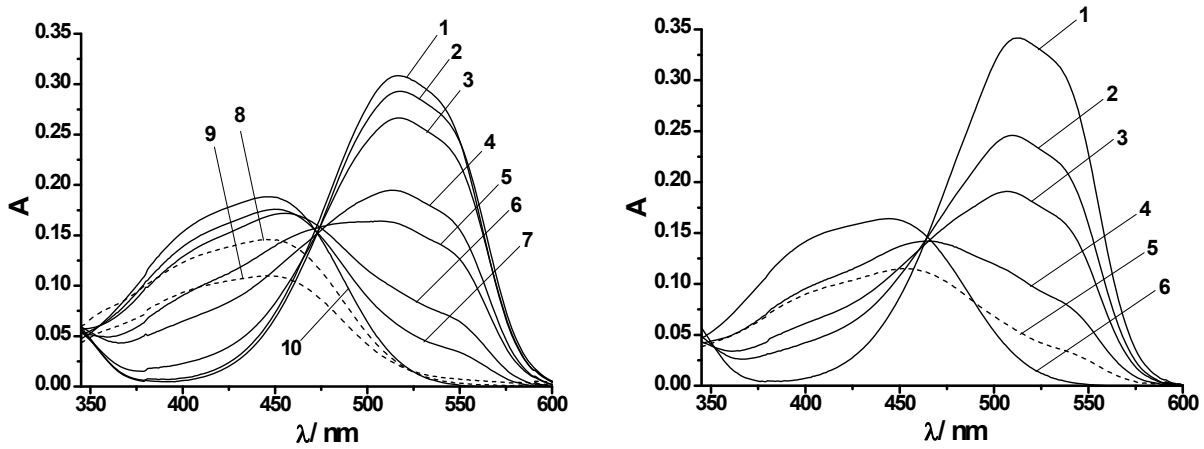


Figure 8. The absorption spectra of methyl yellow at [NaPSS]/[NR] = 60 and I = 0.01 M; initial solution of MY was obtained at pH 2.0; c (MY) = 1.13×10^{-5} M; HR^+: λ_{max} = 516 nm, pH 1.3; R: λ_{max} = 446 nm, pH 7.8 (I = 0.05 M); 1 – pH 1.3, 2 – pH 2.0, 3 – pH 3.15, 4 – pH 3.80, 5 – pH 4.04, 6 – pH 4.49, 7 – pH 4.80, 8 – pH 6.0, 9 – pH 7.8 (I = 0.01 M), 10 – pH 7.8 (I = 0.05 M). (left)

The absorption spectra of methyl yellow at [NaPSS]/[NR] = 1 and I = 0.01 M; initial solution of MY was obtained at pH 2.0; c (MY) = 1.13×10^{-5} M; HR^+: λ_{max} = 512.5 nm, pH 1.3; R: λ_{max} = 444.5 nm, pH 7.8 (I = 0.05 M); 1 – pH 1.3, 2 – pH 2.81, 3 – pH 3.12, 4 – pH 3.63, 5 – pH 4.0, 6 – pH 7.8 (I = 0.05 M). (right) At I = 0.01 M it is impossible to obtain spectra of R species due to practically instantly decrease in absorbance values.

Figure 8 shows the absorption spectra of MY at $I = 0.01$ M. It was found that the lowering of ionic strength results in instant partial decoloration of solutions, which contained the indicator base only (pH 7.8, pH 6.0), regardless of the preparation method. As well if $I = 0.01$ M and [P]/[D] = 1, the solution discolors even at pH 4.0, in spite of that it contains the mixture of species. It hasn't been clearly identified yet, whether the polyelectrolyte leads to the absorbance decrease since this effect is observed in water as well [14].

The monoprotonated species of MY in polyelectrolyte solution, as well as in water, is the the equilibrium mixture of azonium and ammonium tautomers, but the tautomer ratio diverges from that in water. In the NaPSS solution, the ratio of absorbance of the protonated species at 516 nm to that of the neutral molecules at 445 nm is smaller as compared with the corresponding ratio in water, reported by Tawarah and Abu-Shamleh [14]. Hence, in the presence of the polyelectrolyte the fraction of the azonium tautomer has decreased.

In Figure 9, the dependences of normalized absorbance of MY vs. pH values are shown, which correspond to the pK_a^a values given in Table 1. At [P]/[D] = 60, the ΔpK_a^a values are +0.62 and +0.96 for $I = 0.05$ M and $I = 0.01$ M, respectively. Thus, polyelectrolyte effects on the acid-base equilibrium of MY are similar to those for NR, but they are less in absolute magnitude. At [P]/[D] = 1 obtained polyelectrolyte effects on pK_a^a are not so expressed as at [P]/[D] = 60. The micellar effects on acid-base equilibrium of MY are smaller than those of NR as well. At $I = 0.05$ M ΔpK_a^a for MY in 0.02 M SDS solution equals +1.56 [17]. Hence, the behavior of NR, AO, and MY in the presence of NaPSS qualitatively coincides with their behavior in SDS micellar solution, but the shift of the acid-base equilibria state is less expressed in NaPSS solution than in micellar medium.

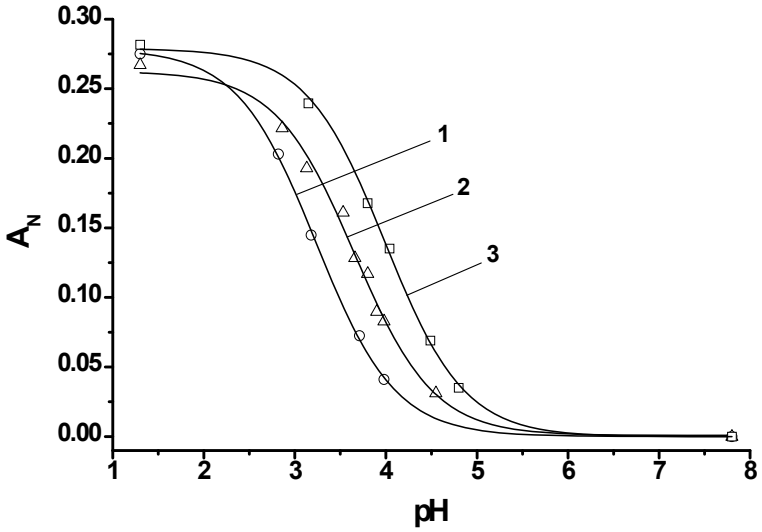


Figure 9. The dependences of normalized absorbance [$A_N(\lambda) = A(\lambda) - A_{pH\ 12}(\lambda)$] of methyl yellow (1 cm optical path cell) vs. pH; 1 – I = 0.05 M, P/D = 1, λ = 512 nm; 2 – I = 0.05 M, P/D = 60, λ = 516 nm; 3 – I = 0.01 M, P/D = 60, λ = 516 nm.

Conclusion

The protonated species of cationic dyes NR, AO, and MY are fixed by NaPSS polyelectrolyte macromolecules. The absorption spectra of the protonated NR and AO strongly depend on the [P]/[D] ratio due to dye–dye interactions, whereas the absorption spectra of the protonated MY undergo (practically) no changes with variation of [P]/[D] ratio. The absorption bands of the protonated forms of NR and AO at [NaPSS]/[Dye] = 60 exhibit the hypsochromic shift as opposed to bathochromic one for SDS micelles, whereas the band MY undergoes slight long-wavelength shift. The band shifts for NR and AO should be attributed to the metachromatic effect. The pK_a^a values for all the above mentioned indicators exceed the corresponding pK_a^w in water, but the differences are less than those observed in the SDS micellar solutions. The decrease in ionic strength from 0.05 to 0.01 M results in the rise of the

pK_a^a value on about 0.3 unit. The zeta-potential value becomes less negative on NaCl adding due to the surface charge screening. In terms of observed pK_a^a alteration and band shifts we suggest that the acid-base equilibria of all dyes must be examined at [P] >> [D].

The obtained pK_a^a values characterize the total polyelectrolyte influence on the acid-base equilibria of indicator dyes. The pK_a^a shifts are mainly of electrostatic nature owing to electrostatic interaction between the cationic species of dyes and polyelectrolyte anionic sites and simultaneous increasing H^+ concentration in the vicinity of macroanions. However, electrostatic effects aren't the only interactions which shift the pK_a^a values. For example, when cationic species of NR or MY are adsorbed at the NaPSS polyanion, they are situated in partially non-aqueous media, that is represented by activity coefficient of transfer in equations (7) and (8). Besides, the expected incomplete binding of deprotonated indicator species and metachromatic effect obtained for NR and AO lead to more complicated reason for the pK_a^a shifts in polyelectrolyte solutions than in micellar ones.

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References

1. R. Nandini; B. Vishalakshi, Spectrochim. Acta Mol. Biomol. Spectrosc. (2010), Vol. 75, P. 14.
2. J. S. Tan; R. L. Schneider, J. Phys. Chem. (1975), Vol. 79, P. 1380.
3. M. Shirai; T. Nagatsuka; M. Tanaka, Die Makromol. Chemie (1977), Vol. 178, P. 37.
4. I. Moreno-Villoslada; M. Jofré; V. Miranda; R. González; T. Sotelo; S. Hess; B. L. Rivas, J. Phys. Chem. B (2006), Vol. 110, P. 11809.
5. J. F. Danielli, Biochem. J. (1941), Vol. 35, P. 470.
6. S. Manente; S. D. Pieri; A. Iero; C. Rigo; M. Bragadin, Anal. Biochem. (2008), Vol. 383, P. 316.
7. J. C. LaManna, Metab. Brain Dis. (1987), Vol. 2, P. 167.
8. W. Ausländer; W. Junge, FEBS Lett. (1975), Vol. 59, P. 310.
9. P. Dell' Antone; R. Colonna; G. F. Azzone, Eur. J. Biochem. (1972), Vol. 24, P. 566.
10. E. Baumgartner; R. Fernandez-Prini; D. Turyn, J. Chem. Soc. Faraday Trans. 1 (1974), Vol. 70, P. 1518.
11. F. G. Walz; B. Terenna; D. Rolince, Biopolymers (1975), Vol. 14, P. 825.
12. M. G. Neumann; I. A. Pastre; A. M. Chinelatto; O. A. El Seoud, Colloid Polym. Sci. (1996), Vol. 274, P. 475.
13. S. P. Moulik; B. K. Paul; D. C. Mukherjee, J. Colloid Interface Sci. (1993), Vol. 161, P. 72.
14. K. M. Tawarah; H. M. Abu-Shamleh, Dyes and Pigments (1991), Vol. 16, P. 241.
15. M. E. Lamm; D. M. Neville, J. Phys. Chem. (1965), Vol. 69, P. 3872.
16. N. N. Kamneva; A. Y. Kharchenko; O. S. Bykova; A. V. Sundenko; N. O. Mchedlov-Petrossyan, Jour. Moll. Liq. (2014), Vol. 199, P. 376.
17. N. O. Mchedlov-Petrossyan, Pure Appl. Chem. (2008), Vol. 80, P. 1459.
18. Z. Adamczyk; B. Jachimska; T. Jasiński; P. Warszyński; M. Wasilewska, Colloids Surf., A (2009), Vol. 343, P. 96.
19. Z. Adamczyk; M. Zembala; P. Warszyński; B. Jachimska, Langmuir (2004), Vol. 20, P. 10517.

20. O. Ortona; V. Vitagliano; R. Sartorio; L. Costantino, J. Phys. Chem. (1984), Vol. 88, P. 3244.
21. C. Peyratout; E. Donath; L. Daehne, J. Photochem. Photobiol. A Chem. (2001), Vol. 142, P. 51.
22. R. Nath; S. Dasgupta; S. Ghosh; A. Mitra; A. Panda, J. Disp. Sci. Technol. (2010), Vol. 31, P. 1447.
23. A. B. Fradj; R. Lafi; S. B. Hamouda; L. Gzara; A. H. Hamzaoui; A. Hafiane, Spectrochim. Acta Mol. Biomol. Spectrosc. (2014), Vol. 131, P. 169.
24. A. B. Fradj; S. B. Hamouda; H. Ouni; R. Lafi; L. Gzara; A. Hafiane, Sep. Purif. Technol. (2014), Vol. 133, P. 76.
25. D. F. Bradley; M. K. Wolf, Proc. Natl. Acad. Sci. USA (1959), Vol. 45, P. 944.
26. F. Quadrifoglio; V. Crescenzi, J. Colloid Interface Sci. (1971), Vol. 35, P. 447.
27. C. J. Drummond; F. Grieser; T. W. Healy, J. Chem. Soc. Faraday Trans. 1 (1989), Vol. 85, P. 551.
28. C. J. Drummond; F. Grieser; T. W. Healy, J. Chem. Soc. Faraday Trans. 1 (1989), Vol. 85, P. 561.
29. N. O. Mchedlov-Petrossyan; S. A. Shapovalov; P. A. Perov; E. I. Markova; A. P. Rudoy; USSR № 1575107, G01 № 21/78, Bull. № 24, (1990).
30. P. Bartels, Z. phys. Chemie (1956), Vol. 9, P. 95.
31. B. P. Nikol'skiy; V. V. Pal'chevskiy; A. A. Pendin; K. M. Yakubov Oksredmetriya; Himiya: Leningrad, 1975.
32. G. M. Pavlov; A. S. Gubarev; I. I. Gavrilova; E. F. Panarin, Polym. Sci. Ser. A (2011), Vol. 53, P. 1003.

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А. Ю. Харченко, Е. Г. Москаева. Кислотно-основные равновесия катионных красителей в водных растворах поли (натрий 4-стиролсульфоната).

Кислотно-основные свойства описывают состояние индикаторных красителей в полиэлектролитном микроокружении. Цель данного исследования состояла в определении кажущейся константы ионизации красителей, K_a^a, в растворах поли (натрий 4-стиролсульфоната) (NaPSS) и обнаружении закономерностей в сдвигах $\mathrm{p}K_a^a$ по сравнению со значениями в воде, $\mathrm{p}K_a^w$. Хорошо известные красители, нейтральный красный (NR), акридиновый оранжевый (AO) и метиловый желтый (MY), были использованы в качестве индикаторов. Влияние концентраций полиэлектролита и красителя, [P]/[D], на спектры поглощения рассмотрено с учетом явления метахромазии. Значения $\mathrm{p}K_a^a$ определены для соотношений [P]/[D] 1 и 60, ионная сила варьировалась от 0.01 до 0.05 М. Установлено, что спектры поглощения протонированных форм NR и AO сильно зависят от соотношения [P]/[D] в связи с взаимодействием молекул красителя между собой. Спектры поглощения MY практически не изменяются с изменением соотношения [P]/[D]. Сдвиги $\mathrm{p}K_a^a$ для всех исследованных красителей положительные, в основном за счет электростатического взаимодействия. Также рассматриваются оптимальные условия для определения сдвигов $\mathrm{p}K_a^a$.

Ключевые слова: полиэлектролит, поли (натрий 4-стиролсульфонат), метахромазия, нейтральный красный, акридиновый оранжевый, метиловый желтый, кажущаяся константа ионизации.

А. Ю. Харченко, О. Г. Москаєва. Кислотно-основні рівноваги катіонних барвників у водних розчинах полі (натрій 4-стиролсульфонату).

Кислотно-основні властивості характеризують стан індикаторних барвників у поліелектролітному мікрооточенні. Мета даного дослідження полягала у визначенні уявної константи іонізації барвників, K_a^a, у розчинах полі (натрій 4-стиролсульфонату) (NaPSS) і виявленні закономірностей у зсувах $\mathrm{p}K_a^a$ у порівнянні зі значеннями у воді, $\mathrm{p}K_a^w$. Добре відомі барвники, нейтральний червоний (NR), акридиновий оранжевий (AO) і метиловий жовтий (MY), були використані як індикатори. На основі теорії метахромазії розглянуто вплив концентрацій поліелектроліту та барвника, [P]/[D], на спектри поглинання. Значення $\mathrm{p}K_a^a$ визначені

для співвідношень [P]/[D] 1 і 60, іонна сила варіювалася від 0.01 до 0.05 М. Було встановлено, що спектри поглинання протонованих форм NR та АО сильно залежать від співвідношення [P]/[D] у зв'язку з взаємодією молекул барвника між собою. Спектри поглинання MY практично не змінюються зі зміною [P]/[D]. Зсуви $\mathrm{p}K_a^a$ для всіх досліджених барвників були позитивними, в основному за рахунок електростатичної взаємодії. Також розглядаються оптимальні умови для визначення зсувів $\mathrm{p}K_a^a$.

Ключові слова: поліелектроліт, полі (натрій 4-стиролсульфонат), метахромазія, нейтральний червоний, акридиновий оранжевий, метиловий жовтий, уявна константа іонізації.

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