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# Solvatochromic and protolytic properties of the indicators in the solidified gelatin gel medium

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## ABSTRACT

Fixed on the transparent triacetate cellulose substrate of the commercial film, the solidified gelatin gel was used for the immobilization of acid-base and solvatochromic betaine dyes. The results of microscopic studies showed that while immobilizing the reagents, the structure and morphology of the gel don't change. Indicators are distributed uniformly in the gelatin layer of the film. Polarity parameters ($E_T$ (30) and $E_T^N$) of the solidified gelatin gel film medium and gelatin gel on the glass substrate, obtained in vitro have been estimated on account of the shifts of the spectral absorption bands of the immobilized in the film Reichardt's betaine indicators. It was found that at pH far from the isoelectric point (pH 4.5), the polarity of the medium of the solidified gelatin layer of the film is in the intermediate position between the respective values of the acetone and ethanol. This polarity verges towards the polarity of such solvent media as nitromethane, pentan-2-ol, propylene carbonate. The influence of the gelatin matrix on the change, comparing with aqueous solutions, of the index values of the ionization constants of the acid-base indicators correlates in sign with effects, observed for the same indicators in micellar solutions of anionic and cationic surfactants. Films with immobilized dyes on it can be recommended for use in optical pH sensors when visual and spectrophotometric pH controlling.

## 1. Introduction

Modification of sorbents by immobilizing the analytical reagents or fixing of the functional-analytical groups on their surface forms the basis of the production of various solid phase reagents [1].

Optically-transparent modified polymeric materials are considered promising: the materials may be used in photometry, visual colorimetry or in sensory devices as sensing elements. Such materials include, for example: the sol-gel materials based on silicon dioxide [2]; polymethacrylate matrixes [3]; polystyrene films and ion exchangers based on styrene copolymer with divinylbenzene [4,5]; triacetylcellulose [6,7]; ethyltcellulose [8]; gelatin films, in particular, ready layers of solidified gelatin gel of commercial photographic films [9–17]. Such films are flexible, have good mechanical, chemical and thermal stability. Modified photographic films are applied as indicator means [12,13,15–17], the matrix of the solidified gelatin gel is used as a medium for template synthesis of metal-macrocyclic complexes [9–11]. To predict the conditions for chemical reactions behavior in the gelatinous medium, results of complex studies of the properties of the reaction medium itself are needed.

Applied to the transparent polymer substrate, based on cellulose ethers, the solidified gelatin gel has a porous structure and a high degree of hydration. Cross-linked polymer structure ensures effective penetration of chemical compounds in the gelatinous mass, their uniform distribution, steric availability of reagents for the occurrence of chemical reactions with their participation. As a result of high water content in gelatin gel, the reagents' behavior in a film is similar to their behavior in aqueous solutions. Hereat, the solubility of the reagents in the gel can be several tens of times greater than their solubility in water [9,12].

When choosing the optimal conditions of reagents extraction in a gelatin matrix of photofilm it is important to know the pH value at the isoelectric point of the solidified polymer, pI. For this purpose, the viscosimetric studies of solutions, prepared from solidified gelatin gel of the photophilm, were carried out in the paper [16]. The estimated value of pI was 4.5 ± 0.1. At pH $<$ 4.5 the total grid charge of the gelatin matrix is positive, and at pH $>$ 4.5 it is negative.

Immobilization of reactants in a solid matrix may change their

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properties due to microenvironment changes. Protolytic and spectral properties of indicators may change. Thus, the authors [18,19] explained the observed spectral and protolytic effects by advantageous fixation of the neutral form of the indicator on the surface or in mass of uncharged polymer; formation of hydrogen bonds [20]; electrostatic interaction of ionic forms of the indicators with grid-charge gel [20,21] or charged surface of modified silica [22]; changing of the conformation of the dye molecules [23]; aggregation [24] and hydrophobic interactions [17].

On the other hand, the revealed effects can characterize a set of properties of the medium itself with located reagent. An indispensable tool for such studies are dyes, in particular, acid-base and solvatochromic indicators. The study of the introduction of acid-base indicators allows us to define local electric charge of the environment [25,26]. Local polarity of the medium may be estimated by way of using solvatochromic betaine indicators [27–39].

Reichardt's betaine indicators were used as solvatochromic probe when studying the properties of direct and reverse microemulsions [29,39], micellar systems based on cationic [30,31] and anionic surfactants [31]. The studies of the interactions in binary mixtures are informative: nonionic surfactant Triton X-100 and various alcohols with different hydrocarbon chain length [32]; a set of alkyl derivatives of aminoacids with acetonitrile, ethyl acetate or *n*-butanol [33]; methanol with alkylbenzoates and alkylalcohols with methylbenzoate [34]; 1-butyl-3- methylimidazole acetate, solvent of lignocellulose material, with water, methanol or dimethylsulfoxide [35]; 2-hydroxyethylammonium formate with *N,N*-dimethylformamide, dimethylsulfoxide or dimethylamine [36]. To evaluate the polarity of the sorbent surface, Reichardt's dyes were used. Thus, Reichardt's betaine standard indicator was used to evaluate the polarity of the graft octadecyl silica C 18, modified by sodium dodecylsulfate and *n*-pentanol, at different temperatures [37]. The same indicator was used for sensing the surface layer of amine contained ormosils [38].

The purpose of this work was to study the processes of hydrophobic and electrostatic interaction of indicators with a matrix of solidified gelatin gel of the commercial photofilm. For the estimation of the medium polarity, seven Reichardt's betaine dyes of different hydrophobicity and with different geometry of substituents as solvatochromic probes were chosen. To evaluate the effect of the gelatin matrix on the acid-base properties of immobilized indicators, two dyes of the trioxifluorone series − pyrogallol red and bromopyrogallol red, were chosen.

## 2. Experimental

### 2.1. Materials

We used photo film for offset printing of the company AGFA. Samples of the films were colorless and transparent, silver halides had been completely removed beforehand using commercial solutions of Agfa Graphics NV (Belgium). To prepare aqueous solutions of gelatin, photographic gelatin was used. To prepare gelatin aqueous solutions, the reactive of gelatin with qualification "photographic" was used.

The indicators pyrogallol red (PGR) and bromopyrogallol red (BPGR) were of analytical grade (firms "Neva Reactive" and "ACROS Organics"). Reichardt betaine indicators: 2,6-diphenyl-4-(2,4,6-triphenylpyridinium-1-yl) phenolate (I); 4-(2,4,6-tri(4-*tert*-butylphenyl)pyridinium-1-yl)-2,6-di(4-*tert*-butylphenyl) phenolate (II); sodium 4-[4-(4-phenylcarboxylate)-2,6-diphenylpyridinium-1-yl]-2,6-diphenylphenolate (III); 2,6-diphenyl-4-[2,4,6-tri(4-perfluorohexylphenyl)pyridinium-1-yl] phenolate (IV); 2,6-cyclonanomethylen-4-(2,4,6-triphenylpyridinium-1-yl) phenolate (V); 2,6-cyclododecamethylen-4-(2,4,6-triphenylpyridinium-1-yl) phenolate (VI); 2,6-dipyridyl-4- (2,6-dipyridyl-4-phenyl-1-pyridinium) phenolate (VII), were put to our disposal by Professor C. Reichardt (Phillips University of Marburg, Germany).

Initial solutions of indicators were prepared via dissolving the precise amount of appropriate reactives in distilled water (PGR, BPGR, indicators III and VII) or in ethyl alcohol with the volume fraction of 96% (indicators I, II, IV–VI). Indicator solutions were prepared by dissolving the stock solutions by distilled water or ethanol.

The pH values of the solutions were adjusted using HCl for pH $< 3$, acetate buffer for pH 3–6 and NaOH for pH $> 11$. The initial solution of NaOH was prepared from saturated stock solution using $CO_2$-free water and kept protected from the atmosphere. All reagents were of analytical grade.

### 2.2. Apparatus

The absorption spectra of dye solutions and indicator films were measured with a photocolorimeter KFK-3 (Russia) or a spectrophotometer UV-1800 (Shimadzu, Japan) against solvent blanks or colorless film. The films were fixed in the holder of the cuvette section of the apparatus.

The pH determinations of the solutions under study were performed by potentiometry using the compensation scheme with uncertainty ± 0.3 мВ (an Ag|AgCl electrode was used as a reference electrode EVL-1M3, Russia) or using the pH-meter ion meter "Expert–001" (Russia) with a combined electrode, calibrated beforehand with standard buffers.

The study of the structure of the photofilm and distribution of the colored reactants in solidified gelatin gel was performed using an inverted optical microscope Nikon Digital Eclipse Ti-E with 60 times magnification mode and a scanning electron microscope JEOL JSM-7500F ("JEOL", Japan).

### 2.3. Procedure

#### 2.3.1. Preparation of the gelatin gel films on the glass substrate

0.24 g of gelatin was dissolved in 4 ml of distilled water under heating on a water bath. The solution was cooled for 10 min and poured evenly on the 3 × 10 cm glass slide. The resulting samples were dried in air for 4 days and stored at room temperature in the dark.

#### 2.3.2. Immobilization of indicators in gelatin film

Samples of gelatin films with immobilized indicators PGR and BPGR with the size of 2.5 × 3.5 cm and 0.7 × 2.0 cm were prepared according to the procedures, described in the papers [42,43]. The concentration of indicators in the modifying solution, with constant pH value was equal to $(1–3) \times 10^{-4}$ mol $L^{-1}$; the contact time of the films with solutions varied from 30 to 60 min.

Reichardt's indicators were extracted for 1 h into the colorless samples of the films from water-ethanol solutions at the concentration of dye in the range $1 \cdot 10^{-4}$–$1 \cdot 10^{-3}$ mol $L^{-1}$ and at pH value of 12. The volume ratio of ethanol/water was 2/3 for the indicators I, III, IV, V, VII and 3/2 for the indicators II, VI. The obtained colored films were stored at room temperature in the dark. Reichardt's betaine indicators were extracted to the gelatin layer on the glass substrate the same way as the colorless samples of the films.

The dried samples of indicator films saved their color over a year.

#### 2.3.3. Spectrophotometric determination of apparent acidity constants of PGR and BPGR indicators in a two-phase system water/gelatin film

The films with immobilized indicator were immersed in the solution with a given pH for one minute, and were dried in air for 1 h at 18–22 °C. The spectra were measured for colored films against colorless film at the wavelength of maximum absorption of the immobilized indicator; pH values of solutions were controlled via potentiometry.

Heterophase acid-base equilibrium was described in a general form by equation (particles in the gelatin phase were marked by a bar over the formula; the charge of the particles was omitted):

$$\overline{HR} \Leftrightarrow \overline{R} + H^{+}, \quad K_a^{app} \tag{1}$$

To evaluate the apparent constant of acidity of the indicator, which