===== **ARTICLES** =====

Redox and Acid–Base Properties of 2,6-Dichlorophenolindophenol Immobilized on a Polymethacrylate Matrix

N. A. Gavrilenko, A. V. Sukhanov, and O. V. Mokhova

Tomsk State University, pr. Lenina 36, Tomsk, 634050 Russia

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Abstract—The effect of the immobilization of 2,6-dichlorophenolindophenol (**DCIP**) on a polymethacrylate matrix on its redox and acid–base properties was studied. The ionization constant and formal redox potentials of the immobilized DCIP were determined by solid-phase spectrophotometry. It was shown that the acidity constant and the formal potentials of DCIP decreased upon the immobilization in the polymethacrylate matrix. The results of studying the interaction of ascorbic acid with immobilized DCIP are presented.

The use of reagents immobilized on solid supports is a trend in analytical chemistry. The advantages of using immobilized reagents are that the consumption of the reagent is reduced and toxic solvents are not used. Immobilized reagents can be used to determine elements in the solid phase by spectroscopy, as ready-to-use analytical forms in visual test methods, and as recognizing devices in sensor methods of analysis.

DCIP is a well studied analytical reagent for the volumetric and photometric determination of ascorbic acid and other reductants [1, 2]. The effect of the noncovalent immobilization of DCIP on amberlite XAD-4 on its properties was studied [3, 4] to develop an optical sensor for ascorbic acid [5]. The properties of DCIP immobilized on reversed-phase silica gels and ion-exchangers were investigated [6]. Based on the results, indicator tubes and indicator powders were proposed for the solid-phase spectrophotometric and test determinations of iron(II).

In the present work, a transparent polymethacrylate matrix is proposed for immobilizing DCIP. Previous studies showed that the ability of reagents to be immobilized without loss in transparency of the matrix could be combined with their ability to enter the analytical reaction [7, 8]. This allowed the direct measurements of the absorbance of the matrix with the immobilized reagent by solid-phase spectrophotometry. The results of the investigation of acid–base and redox properties of DCIP immobilized in a polymethacrylate matrix are given for the further development of an optical element for the determination of ascorbic acid.

EXPERIMENTAL

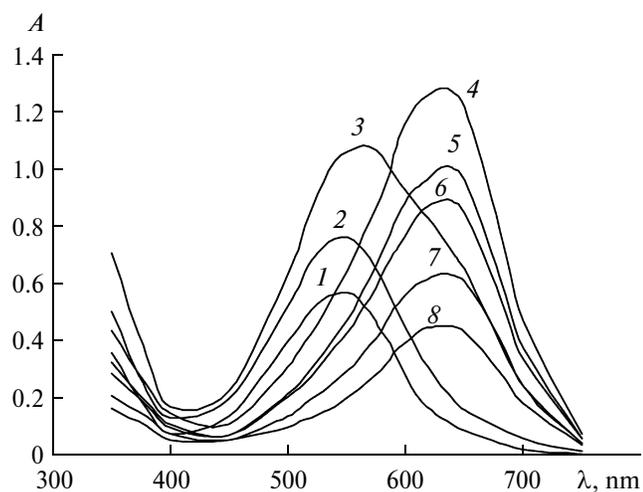The polymethacrylate matrix was prepared in the form of a transparent plate, 0.60 ± 0.04 mm in thickness, by radical block polymerization according to the procedure described in [9]. Smaller plates, 6×8 mm in size and 0.05 g in weight, were cut from the initial plate. A sodium salt of 2,6-dichlorophenolindophenol was used. A 3.5×10^{-4} M solution of the reagent was prepared by dissolving a weighed portion in twice-distilled water; the solution was filtered and its titer was determined by titration with Mohr's salt solution. Initial solutions of potassium hexacyanoferrate(II) and potassium hexacyanoferrate(III), sodium hydroxide, and sodium chloride were prepared by dissolving their accurately weighed portions in twice-distilled water. The required pH level of the solutions was maintained using a universal buffer solution. All reagents were of cp or analytical grade.

2,6-Dichlorophenolindophenol was immobilized on the polymethacrylate matrix by adsorption from an aqueous solution under batch conditions. For this purpose, 10-mL portions of the reagent solutions with different pH were mixed with the matrix plates for 5 min.

The acid–base properties of the immobilized DCIP were studied using solid-phase spectrophotometry. To determine the ionization constant of the oxidized form of the immobilized indicator, the polymethacrylate matrix plates with DCIP was shaken with 50 mL of a buffer solution with a certain pH and constant ionic strength of 0.1 M. When the equilibrium was set, the pH value of the solution was measured and the absorption spectra of the polymer matrix with the immobilized indicator were recorded. The ionization constant (pK_a) was calculated by the equation

$$pK_a = pH + \log[\overline{HR}]/[\overline{R^-}]$$
$$= pH + ((A_{mix} - A_{HR})/(A_{R^-} - A_{mix})),$$

where A_{HR} and A_{R^-} were the absorbance of the matrix plates under conditions when the reagent was entirely

Fig. 1. Absorption spectra of DCIP on the polymethacrylate matrix after adsorption from solutions with pH (*1*) 2.0, (*2*) 2.5, (*3*) 3.2, (*4*) 5.0, (*5*) 6.7, (*6*) 7.3, (*7*) 8.0; 3.5×10^{-4} M DCIP, $t = 5$ min.

in the molecular (HR) or ionic (R^-) form, respectively, and A_{mix} was the absorbance of the matrix plate when the reagent occurred in both forms. The wavelength region was used for measurements, where the largest difference between the values of A_{HR}, A_{R^-}, and A_{mix} was observed.

The redox properties of DCIP in solution and in the immobilized state were studied using procedures described in [3, 6]. The redox equilibrium of the indicator in the solution was studied by observing the changes in the absorbance of the indicator with respect to changes in the redox potential of a mediator brought into contact with the indicator. For this purpose, 5 mL of a 3.5×10^{-4} M solution of DCIP was added to solutions with different ratios of potassium hexacyanoferrate(III) to potassium hexacyanoferrate(II), constant ionic strength of 1 M, volume of 25 mL, and a certain pH value. Then, the potential of the indicator electrode and the absorbance of the reagent solution at the absorption maximum were measured for each pH value. The redox equilibrium of the immobilized DCIP was studied by recording the change in the absorption of the polymethacrylate plate with the immobilized indicator in accordance to the varying redox potential of the solution brought into contact with the plate. For this purpose, the polymethacrylate plate with the immobilized indicator was put into solutions with different ratios of potassium hexacyanoferrate(III) to potassium hexacyanoferrate(II), constant ionic strength of 1 M, volume of 30 mL, and a certain pH value; the mixture was stirred until the redox equilibrium was set. Then, the potential of the indicator electrode in the solution and the absorbance of the polymethacrylate plate with the immobilized DCIP in the oxidized state were mea-

sured. The formal redox potential was found by the equation $E^{0'}$ (mV) $= E - \dfrac{58}{2} \log [c_{ox}/(c - c_{ox})]$, where c and c_{ox} were the initial and residual concentration of the oxidized form of DCIP in the solution or in the polymethacrylate plate, respectively, and E was the potential of the indicator electrode in the solution, mV. The value $\log [c_{ox}/(c - c_{ox})]$ was calculated as log $\log [A_i/(A - A_i)]$, where A was the absorbance of the initial DCIP solution or the polymethacrylate matrix with the immobilized DCIP and A_i was the absorbance of the DCIP solution or the polymethacrylate matrix with the immobilized DCIP after its contact with the solution of hexacyanoferrate(II)−hexacyanoferrate(III).

The absorbance and absorption spectra of the reagent and the polymethacrylate matrix were measured on Evolution 600 and Spekol 21 spectrophotometers against an unmodified polymethacrylate matrix. An I-160 laboratory potentiometer with a glass electrode and a silver−silver chloride electrode was used for potentiometric measurements. Redox potentials were measured with a platinum electrode and a silver−silver chloride electrode.

RESULTS AND DISCUSSION

2,6-Dichlorophenolindophenol is a well studied redox indicator [10]. The reduced form of DCIP is colorless, while the oxidized form, which acts as a redox indicator, is reddish pink in acidic solutions (molecular form, HR, $\lambda_{max} \approx 510$ nm) and dark blue in alkaline solutions (ionic form, R^-, $\lambda_{max} \approx 605$ nm). The dark blue color is more intense than the red color; the indicator in the molecular form is unstable and precipitates in an acidic medium.

The absorption spectra of the polymethacrylate matrix after the immobilization of DCIP from aqueous solution at different pH values are presented in Fig. 1. It is seen that, after adsorption from solutions with pH 2−3.5, the reagent occurs in the matrix preferably in the molecular form with the absorption maximum at 530 nm, while in solutions with pH 4−8, it is presented in the ionic form with the absorption maximum at 630 nm. The absorption maxima of the molecular and ionic forms of DCIP are shifted to the longer-wave region with respect to the aqueous solutions (Table 1). According to the literature data [11], the long-wave shifts of the absorption bands occur because of nonspecific interactions. In further experiments, an indicator solution in a phosphate buffer solution (pH 6.7) was used to immobilize DCIP, because the indicator was stable under these conditions [10].

In the study of the acid−base properties of the immobilized reagent, the absorption spectra of the polymethacrylate matrix with the immobilized DCIP

were obtained after its contact with solutions with different pH values (Fig. 2). The ionization constants of the immobilized indicator and indicator in the solution, determined from these spectra, are presented in Table 1. The ionization constant for the immobilized DCIP is lower than that for the reagent in aqueous solutions. It should be noted that in the investigation of the acid–base properties, the reagent was not washed out from the polymer matrix at any acidity of the solution, which indicated that the reagent is well retained. Moreover, the molecular form of DCIP becomes more stable after immobilization on the matrix.

The formal redox potentials of DCIP in solution and in the immobilized state were determined as functions of the pH of the solution value (Table 2). In the present work, as in [3], the formal potential of DCIP in the solution was measured at pH 4−6, while in the immobilized state, at pH 2−6. The indicator in the molecular form is unstable at pH < 4. However, as was mentioned above, the stability of the indicator on the polymethacrylate matrix increased, which allowed the measurement of the formal potential of the immobilized DCIP at pH < 4. Therefore, the immobilization of DCIP on the polymethacrylate matrix ensures the determination of reductants in more acidic media because of increasing stability of the molecular form of the reagent on the polymethacrylate matrix. At pH > 6, the indicator is not reduced completely, because the potential in the solution cannot be decreased enough since hexacyanoferrate(II) solution with a higher concentration cannot be prepared.

It is seen from the data presented in Table 2 that the oxidative activity of the immobilized DCIP decreases with respect to its activity in the solution because of

Table 1. Values of λ_{max} and pK_a of DCIP (I) on the polymethacrylate matrix and (II) in aqueous solution

| Medium	λ_{max}, nm		pK_a
	Molecular form	Ionic form	
I	530	630	6.12 ± 0.17*
II	510	605	5.7 [12]

Note: * $n = 3$, $P = 0.95$.

Table 2. Formal redox potentials ($E^{0'}$, mV) of DCIP (I) in aqueous solution and (II) on the polymethacrylate matrix

| pH	I	II
2.0	–	451 ± 21
3.0	–	420 ± 14
4.0	466 ± 4	385 ± 11
5.0	394 ± 6	356 ± 10
6.0	332 ± 10	330 ± 13

diffusion and steric hindrances. The formal potentials of both the immobilized DCIP and dissolved DCIP coincide at pH 6. With a decrease in pH, the difference between the formal potential of DCIP in the immobilized state and in the solution increases (Fig. 3). Such character of the redox potential of DCIP in the immobilization indicates that, at lower pH values, the reduced form of DCIP is bound to the polymethacrylate matrix less effectively than the oxidized form and that this difference decreases with increasing pH, because the indicator transforms into the ionic form (R^-) [12].
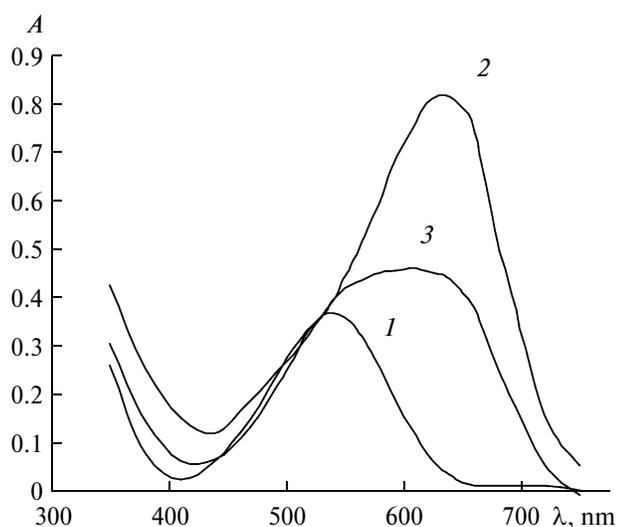
Fig. 2. Absorption spectra of the polymethacrylate matrix containing (*1*) HR form, (*2*) R^- form, and (*3*) both forms of DCIP
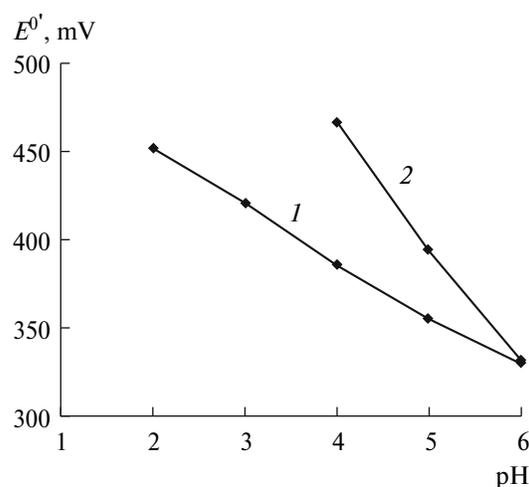
Fig. 3. pH-Dependence of the formal potentials of DCIP (*1*) immobilized on the polymethacrylate matrix and (*2*) in the solution.
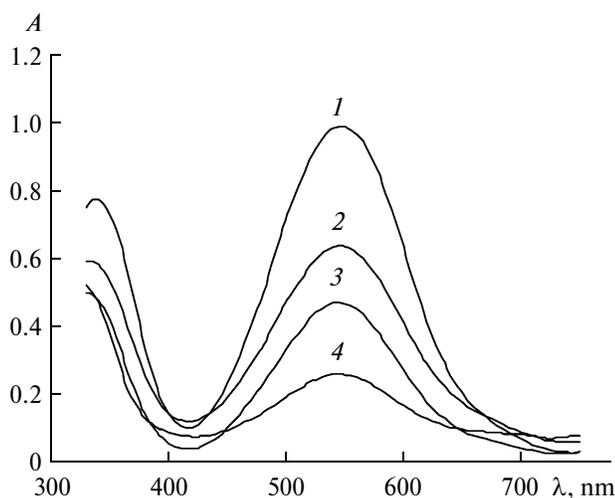
Fig. 4. Absorption spectra of the polymethacrylate matrix with immobilized DCIP after its contact with the solution of ascorbic acid, c_{Asc}, mg/L: (*1*) 0, (*2*) 50, (*3*) 70, and (*4*) 100.

Fig. 5. Dependence of the absorption spectra of the poly-methacrylate matrix with immobilized DCIP on the concentration of ascorbic acid in solution, $V = 50$ mL, $t = 15$ min, pH 3.
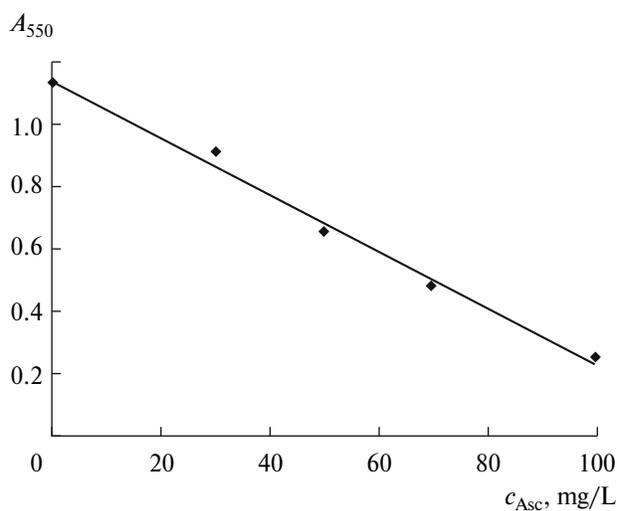
We studied the interaction of the immobilized DCIP with ascorbic acid for assessing the possibility of using the material obtained for determining ascorbic acid. The color intensity of the polymethacrylate matrix with immobilized DCIP decreases after its contact with the ascorbic acid solution. The discoloration is accompanied with the decrease in absorbance proportional to the concentration of ascorbic acid in the solution (Fig. 4). The study of the effect of the pH of the ascorbic acid solution showed that the decrease in absorbance was the largest at pH 3–4. The dependence of the decrease in the absorbance at 550 nm on the concentration of ascorbic acid at pH 3 and a contact time of 15 min are presented in Fig. 5. The calibration curve is described by the equation $A_{550} = 1.140 - 0.009c_{Asc}$ ($r = 0.996$), where c_{Asc} is the concentration of ascorbic acid, mg/L.

The results of determining ascorbic acid in a rosehip extract by the proposed method are presented, compared to the results obtained by the volumetric procedure recommended in [13] for determining ascorbic acids in extracts (Table 3). The rosehip extract was prepared by the procedure described in [13, p. 239].

Thus, the investigations performed in this work showed that the polymethacrylate matrix with immobilized DCIP can be used as an optical element for determining reductants, in particular, ascorbic acid.

Table 3. Determination of ascorbic acid in the rosehip extract ($n = 3$, $P = 0.95$)

Method	Found, mg/100 g	RSD, %
Proposed	1250 ± 170	5
Volumetric [13]	1250 ± 90	3

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