Spectrophotometric Analysis of the Relationship between Dissociation and Coloration, and of the Structural Formulas of Phenolphthalein in Aqueous Solution

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The absorption spectra of phenolphthalein (H_2PP) in phosphate buffers of pH 5 to 13 were analyzed in order to determine the features of the dissociation and coloration of H_2PP . The spectra demonstrated that HPP^- , while keeping a γ -lactone ring, was colorless, and that only PP^{2-} was red, where $pK_1=9.05$, and $pK_2=9.50$; further, the reaction of hydroxide ion with PP^{2-} to produce colorless $PP(OH)^{3-}$ was rather slow, where $pK_3=12$. On the contrary, the color of the aqueous solution of phenolsulfonphthalein ($H_2^+PS^-$), having a zwitter ion structure and no lactone ring, was orange-red, which dissociated to yellow HPS^- and finally to PS^{2-} , having a similar red color and absorption spectrum to PP^{2-} . These results clearly demonstrate the relationship between the color and the prolonged conjugated system.

Keywords Phenolphthalein, phenolsulfonphthalein, absorption spectrum, structural formula and color, dissociation exponent

Phenolphthalein (H_2PP) and phenolsulfonphthalein (H_2PS) are the key compounds of various kinds of phthaleins and sulfonphthaleins, respectively. The structural formula and chemical name of H_2PS had been postulated as a γ -sultone in almost all books; until recently, Yamaguchi *et al.* denied the γ -sultone and proposed the molecular structure of the zwitter ionic form of H_2PS ($H_2^+PS^-$) using X-ray crystallography.¹

On the contrary, H_2PP having a γ -lactone ring is colorless, and its aqueous solution gives only one kind of red color in the alkaline region. The molecular structure of the species of red color had not been determined. Although Ozeki *et al.* estimated PP^{2-} as the red species, they reported that the value of pK_1 (10.1) was larger than that of pK_2 (9.3).² These values are unreasonable since among the successive acid-dissociation constants, K_1 should always be larger than K_2 .

In this paper, the absorption spectra of H_2PP in phosphate buffers of various pH were analyzed in order to determine the features of the dissociation and coloration of H_2PP while taking the spectra of its monoglucuronide and diphosphate, and the spectra of $H_2^+PS^-$ as references.

Experimental

Materials

Phenolsulfonphthalein was kindly supplied by Daiichi Pharmaceutical Co., Ltd. Phenolphthalein and phenolphthalein monoglucuronide (Wako Pure Chemical

Industries Ltd.), phenolphthalein diphosphate tetrasodium salt hydrate (Aldrich Chem. Co., Ltd.) and other chemicals were of analytical reagent grade.

Buffers

Phosphate buffers of 0.2 M were prepared with sodium dihydrogenphosphate, hydrochloric acid, sodium hydroxide and redistilled water.

Preparation of sample solutions

After 3.21 mg of H_2PP was dissolved in 1 ml of ethanol and diluted to 10 ml with the buffer of pH 10, the resulting solution (1 mM) was diluted 100 times with the buffers to obtain 10^{-5} M sample solutions of H_2PP .

A 1 mM solution of $H_2^+PS^-$ in the buffer of pH 8 was diluted 200 times with the buffers to obtain 5×10^{-6} M sample solutions of $H_2^+PS^-$. Similar methods were used to prepare 10^{-5} M sample solutions of phenolphthalein diphosphate and phenolphthalein monoglucuronide.

Instruments and measurements

A Hitachi U-3210 spectrophotometer with a scan speed of 120 nm/min and a band path of 2 nm, and a pair of matching cells of 1 cm light pass were used to measure the absorption spectra at $25 \pm 2^\circ C$, where the buffers were used as references. A Toa-HM-20E glass electrode pH meter was used at $25 \pm 2^\circ C$ and calibrated with standard solutions of pH 1.68, 4.01, 6.86 and 10.02. A Macintosh Quadra 840AV Personal Computer of Apple Co., Ltd. was used to analyze the absorption spectra.

Fig. 1 Absorption spectra of phenolphthalein. The final concentration is 10^{-5} M. The rough pH values of solutions are given on the spectra.

Fig. 2 Graphic estimation of the molar fraction of PP^{2-} , assuming its absorbance to be 0.35 (○), 0.375 (●) and 0.40 (×). —: the theoretical curve for a monobasic acid with $pK=9.6$.

Results and Discussion

The absorption spectra of the H_2PP solutions in Fig. 1 were measured as soon as possible in the alkaline region, since the spectra were found to change with the speed, depending on the concentration of the hydroxide ion (as described later).

In the visible region, since the absorption spectra of H_2PP had only one maximum point at 552.8 nm, the molar fractions of the red species were calculated from the absorbances at 552.8 nm by trial and error (Fig. 2). The values obtained by assuming the absorbance of the species to be 0.375 indicated a pK of approximately 9.6. However, the values at pH below 9 were lower than theoretical ones for a monobasic acid (Fig. 2). These results indicated the presence of a colorless species (HPP^-) between H_2PP and the red

Fig. 3 Preliminary estimation of pK_1 of H_2PP by drawing with the absorbance at 255.2 nm.

Fig. 4 Molar fractions of H_2PP , HPP^- , PP^{2-} and $PP(OH)_3^-$. Theoretical curves assuming $pK_1=9.05$ and $pK_2=9.50$ (—), and additionally $pK_3=12$ (---), where the acid dissociation constant of HA is defined as $K=a_{H^+}[A^-]/[HA]$. The observed absorbances at 552.8 nm under the conditions given in Fig. 1 (●), and after standing for equilibration (▲).

species (PP^{2-}).

From the spectra in the ultraviolet region, 255.2 nm was selected, at which point the absorbance maintained a constant value at pH above 10, and decreased with a lowered pH. A similar sigmoid curve was obtained, where a pK value of approximately 9 was indicated (Fig. 3).

Consequently, as shown in Fig. 4, the molar fractions (—) of H_2PP , HPP^- and PP^{2-} were calculated using the assumed pK_1 and pK_2 values and the trial-and-error method, to coincide with the observed values of PP^{2-} (●) from the following equations for a dibasic acid:³

$$[H_2PP] + [HPP^-] + [PP^{2-}] = \Sigma P,$$

$$a_{H^+}^2 + a_{H^+} \times 10^{-pK_1} + 10^{-pK_1-pK_2} = \Sigma,$$

$$[H_2PP]/\Sigma P = a_{H^+}^2/\Sigma,$$

$$[HPP^-]/\Sigma P = a_{H^+} \times 10^{-pK_1}/\Sigma,$$

$$[PP^{2-}]/\Sigma P = 10^{-pK_1-pK_2}/\Sigma.$$

The absorbance at 552.8 nm equals $\epsilon_{PP^{2-}}[PP^{2-}]$.

Fig. 5 Absorption spectra of phenolphthalein monoglucuronide. The final concentration is 10^{-5} M. In this case, water was used as the reference.

Fig. 6 Absorption spectra of phenolsulfonphthalein. The final concentration is 5×10^{-6} M.

As the result, the values of pK_1 and pK_2 were estimated to be 9.05 and 9.50, respectively.

Phenolphthalein diphosphate, in which both of the hydroxy groups were substituted, showed a similar absorption spectrum to that of H_2PP in the 6 to 11 pH range where the γ -lactone ring was proved to be stable.

As shown in Fig. 5, phenolphthalein monoglucuronide, in which one of the hydroxy groups was substituted,

showed a similar spectrum to that of H_2PP at pH below 7, while another spectrum, perhaps corresponding to HPP^- , appeared at a pH above 11. These spectra, having an isosbestic point, demonstrated the existence of two kinds of molecular species in the range of pH 6 to 12 with a pK value of about 9. The spectral change which occurred in the ultraviolet region in an alkaline solution indicated the dissociation of the hydroxy group together with the maintenance of the lactone ring.

Fig. 7 Structural formulas and colors of the existent molecular species in aqueous solutions of H_2PP and H_2+PS^- .

The lactone form of colorless HPP⁻ was further supported by a comparison of the spectra of H₂PP (Fig. 1) with those of orange red H₂⁺PS⁻, which was a strong acid possessing no lactone ring¹, and dissociated a phenolic proton to yellow HPS⁻ at pH 4 to 5, as shown in Fig. 6 (also in Fig. 7); pK₁=1.2 was calculated from the spectra of pH 0, 1 and 4 using a similar trial-and-error method to that in Fig. 4, and pK₂=7.70 was obtained from the absorbances at 559.0 nm.

The reaction of hydroxide ion with PP²⁻ to produce PP(OH)³⁻ was rather slow, and caused a disturbance of the spectra shown in Fig. 1. From the rates of the decrease in the absorbances at 552.8 nm with solutions of H₂PP at a pH above 11, the rate constant of the OH⁻ addition reaction was estimated to be about 30 M⁻¹ h⁻¹ at 25°C. By a measurement after a sufficient standing time, pK₃ of H₂PP was determined to be 12 using the equations for a tribasic acid³ (Fig. 4, ---) together with the spectrum of PP(OH)³⁻, having an absorption maximum at 244 nm.

From the data obtained above, the structural formulas of existent molecular species in aqueous solutions of H₂PP and H₂⁺PS⁻ were estimated (Fig. 7).

Figures 1, 6 and 7 clearly demonstrate the differences between H₂PP and H₂⁺PS⁻. Both PP²⁻ and PS²⁻, having analogous structural formulas, display a red color with similar absorption spectra. There remains a possibility of partially maintaining the lactone ring in PP²⁻, as in the parenthesis of Fig. 7, since the molar extinction coefficient of PP²⁻ is much lower than that of PS²⁻. On the contrary, H₂PP and HPP⁻, which maintain the γ -lactone ring, are colorless because of isolated benzene-rings, while H₂⁺PS⁻ and HPS⁻, which maintain a prolonged conjugate system, are colored.

Further comparisons with their O-bridged analogous, fluorescein (H₂FL) and sulfonefluorescein (H₂⁺SF⁻) are interesting.³

Table 1 The pK values of the molecular species of H₂PP, H₂⁺PS⁻, H₂FL and H₂⁺SF^{-a}

Charge	Neutral form			
	H ₂ PP	H ₂ FL	H ₂ ⁺ PS ⁻	H ₂ ⁺ SF ⁻
1+		2.24 ^b		
0	9.05	4.20	1.2	3.10 ^b
1-	9.50	6.39	7.70	6.25
2-	12			

a. The acid dissociation constant of HA is defined as

$$K = a_{\text{H}^+}[A^-]/[HA]$$

The zwitter ionic structures of H₂⁺PS⁻ and H₂⁺SF⁻ were estimated by Yamaguchi¹ *et al.* and Kanazawa⁴ *et al.*, respectively.

b. The values are taken from ref. 3.

As shown in Table 1, the stability of the lactone ring in H₂PP is greatly lowered due to the fixed plane structure in H₂FL, which thus has different pK values from those of H₂PP. Further, the bridging oxygen in H₂⁺SF⁻ will suppress the dissociation of =OH⁺ with a resonance effect, and accelerate that of -OH with an inductive effect to cause the larger pK₁ and smaller pK₂ values of H₂⁺SF⁻ than those of H₂⁺PS⁻.

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(Received May 28, 1996)

(Accepted September 18, 1996)