Spectrophotometric Determination of the Acid Dissociation Constants of Plasmocorinth B

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Plasmocorinth B (2-(1-hydroxy-4-chloro-2-phenylazo)-1, 8-dihydroxynaphthalene-3, 6-disulfonic acid disodium salt) was proposed as a colorimetric reagent for calcium in serum by Yanagisawa,[1] and it was studied then by Brush;[2] more recently this reagent has been utilized as an indicator of the chelometric titration[3] of calcium in the presence of magnesium.

This paper will report on a more detailed study of the absorption spectra and the acid dissociation constants of this reagent by the spectrophotometric method.

Experimental

Reagents.—A reagent obtained from the Sumitomo Dye Co. (SPB*) and the Dôjin Pharmaceutical Co. (DPB**), with the trade name Plasmocorinth B, was used for the studies without any further purification. It was dried at 60°C in a vacuum, cooled in a phosphorus pentoxide desiccator, and weighed. A solution (3×10^{-4} mol./l.) was prepared and stored in the dark.

Buffer Solution and Sodium Perchlorate Solution.—The method of preparation has been described earlier.[4]

Procedure and Apparatus.—The measurements of the absorption spectra at various pH values were made as follows: Five milliliters of a 3×10^{-4} mol./l. reagent solution was placed in a 50 ml. volumetric flask, and an adequate volume of 1 M perchloric acid, 1 M sodium hydroxide or a buffer solution (acetic acid-sodium acetate, ammonium acetate-ammonia or ammonia-ammonium chloride) was added in order to adjust the pH value. 0.1 ml. of a 0.01 M EDTA solution was added to mask trace amouts of calcium or magnesium contained in sodium hydroxide or ammonia, because the definite isosbestic points can not be obtained in the absence of EDTA, especially at a higher pH. Then, a 1 M sodium perchlorate solution was added to maintain the ionic strength at 0.1, and the volume was made up to 50 ml. with water. The absorbance was then measured with a Shimadzu spectrophotometer QR-50 type, and a Hitachi automatic recording spectrophotometer EPS-2 type, with 1 cm. cells, using water as a reference, while the pH value was measured with a Tôa-Denpa glass electrode pH meter, Model HM-8A.

All the measurements were made at 25°C.

Results and Discussion

Spectral Properties of the Reagent. — The absorption spectra of the reagent solution (3×10^{-5} M) are shown in Fig. 1 over various pH values.

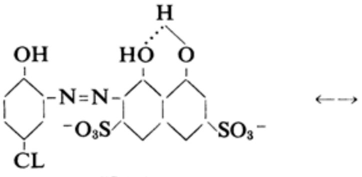
Below pH 4, the reagent gives an absorption spectrum with one absorption maximum at 530 mμ; when the pH of the solution is increased to 8.8, the maximum shifts to 610 mμ. Between pH 1 and 8.8, four isosbestic points exist, at 367, 413, 456 and 573 mμ.

Above pH 9.3, an absorption maximum appears at 576 mμ, and between pH 9.3 and 13, the two isosbestic points are at 467 and 612 mμ.

Between pH 8.8 and 9.3, there are no isosbestic points, so that the curves pass neither the point at 573 mμ nor that at 612 mμ.

Since the reagent faded gradually at a higher pH, the effect of *l*-ascorbic acid and hydroxylamine on the absorbance at the maxium (576 mμ) was studied at pH 13.0. The effect of neither reagent is good, but in the presence of 1.5×10^{-6} M *l*-ascorbic acid the absorbance is constant for ten minutes.

The Acid Dissociation Constant of the Reagent.—Judging from the presence of the isosbestic point, the structures H_3R^{2-} and H_2R^{3-} between pH 1 and 8.8 and the structures H_2R^{3-} and HR^{4-} between pH 9.3 and 13.0 are expected:



H_3R^{2-}

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[1] F. Yanagisawa, *J. Biochem.* (*Japan*), **42**, 3 (1955).

[2] J. S. Brush, *Anal. Chem.*, **33**, 789 (1961).

[3] G. S. Kovacs and K. E. Tarnoky, *Anal. Chim. Acta*, **21**, 297 (1959).

* Anal. Found: N, 5.40 Calcd. for $C_{16}H_9O_9N_2S_2Na_2$-Cl: N, 5.51%.

** Anal. Found: N, 4.91

[4] H. Miyata, This Bulletin, **36**, 382, 386 (1963).

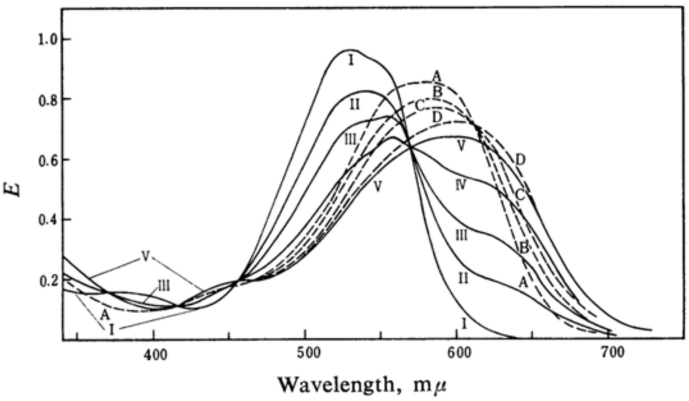
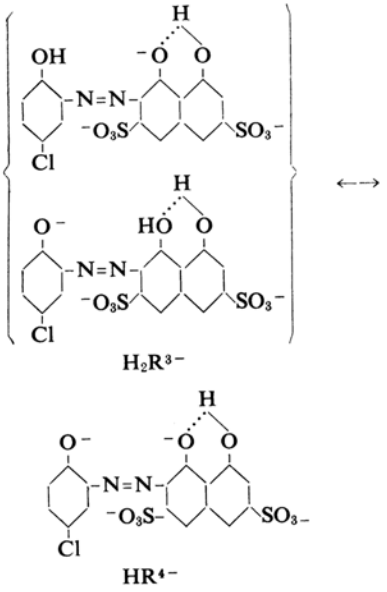
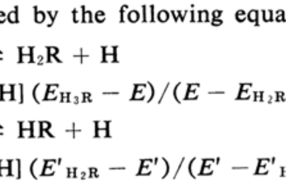


Fig. 1. Absorption spectra of Plasmocorinth B (SPB).
I, pH ~3.86; II, pH 6.64; III, pH 7.25; IV, pH 7.73; V, pH 8.47.
A, pH 13.01; B, pH 10.93; C, pH 10.52; D, pH 9.33



H_2R^{3-}



HR^{4-}

The dissociation constants, K_1 and K_2, can be calculated by the following equations:

$$H_3R \rightleftharpoons H_2R + H \tag{1}$$

$$K_1 = [H]\,(E_{H_3R} - E)/(E - E_{H_2R}) \tag{2}$$

$$H_2R \rightleftharpoons HR + H \tag{3}$$

$$K_2 = [H]\,(E'_{H_2R} - E')/(E' - E'_{HR}) \tag{4}$$

where E_{H_3R}*, E_{H_2R} and E represent, respectively, the absorbances of H_3R, H_2R and the mixture of H_3R and H_2R at 600 mμ, and E'_{H_2R}, E'_{HR}** and E' represent, respectively, the absorbances of H_2R, HR and their mixture at 640 mμ.

E_{H_3R}* and E'_{HR}** are equal to the absorbances at pH 1~4 and pH 12.4~13 respectively; E_{H_2R} and E'_{H_2R} are, however, not obtainable directly from the experimental absorbances, because the difference between pK_1 and pK_2 is small and the species H_2R always exists togethere with H_3R or HR, even under the most favorable conditions. Thus, at pH 8.8~9.3 the absorption curves have no isosbestic points, although H_2R is predominant.

Consequently, E_{H_2R} and E'_{H_2R} must be calculated by the following equations reported by Yoshioka et al.:[5)]

$$E_{H_2R} = (a_1E_2 - a_2E_1)/(a_1 - a_2)$$
$$a_1 = [H_1]\,(E_{H_2R} - E_1)$$
$$a_2 = [H_2]\,(E_{H_3R} - E_2)$$
$$E'_{H_2R} = (b_1E'_1 - b_2E'_2)/(b_1 - b_2)$$
$$b_1 = [H'_1]/(E'_1 - E'_{HR})$$
$$b_2 = [H'_2]/(E'_2 - E'_{HR})$$

In the equations, E_1 (or E'_1) and E_2 (or E'_2) represent the absorbances at the hydrogen ion concentrations $[H_1]$ (or $[H'_1]$) and $[H_2]$ (or $[H'_2]$) respectively.

On the basis of these calculations, the acid dissociation constants of the reagent have been estimated and compared with the values of Brush:[2)]

		pK_1	pK_2	pK_3
Brush[2)]		8.0	10.5	11.9
The author	SPB	7.1_5	10.4_8	—
	DPB	7.1_6	10.4_8	—

The value of pK_3 could not be found from the absoption spectra of highly alkaline solutions; however, judging from the pK_2 values of chromotropic acid (15.6)[6)] and

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* E_{H_3R}=0.090 for SPB; 0.075 for DPB.

** E'_{HR}=0.250; 0.223.

[5)] M. Yoshioka et al., *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **83**, 229 (1962).

[6)] J. Heller and G. Schwarzenbach, *Helv. Chim. Acta*, **34**, 1876 (1951).

phenylazo chromotropic acid (14.64)[7], (pK_2 corresponding to the pK_3 of this reagent) and from the absorption spectra of some azo compounds coupled with chromotropic acid,[4,8] Brush's pK_3 value (11.9) seems to be doubtful.

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[7] J. Bjerrum et al., "Stability Constants, Part I: Organic Ligands," The Chemical Soc., London (1957), p. 95.

[8] Unpublished work of H. Miyata.