SPECTROPHOTOMETRY OF LITMUS AND RELATED INDICATORS

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Litmus is one of the oldest and most widely used acid-base indicators. The color change takes place over a rather wide pH range in the neighborhood of pH 7. Litmus is prepared by digesting certain lichens, particularly *Roccella tinctoria* and *Lecanora tartarea,* with sodium carbonate and ammonia in the presence of air. If the sodium carbonate is omitted the product is orcein. Litmus can be separated into three indicator components by selective solvent extraction (Elman *et al.,* 1928). The ether-soluble component is erythrolein, the alcohol-soluble component is erythrolitmin, and the alcohol-insoluble material is azolitmin.

Materials similar to those obtained from lichens can be obtained from orcinol or resorcinol (Thorpe, 1948). The material obtained from orcinol on treatment with sodium carbonate and ammonia has been called synthetic litmus, and in the absence of sodium carbonate the product is called orcein. The orceins obtained from orcinol and that from lichens are very similar and usually no distinction is made between the two. Musso (1956) has shown that both are complex mixtures and very similar in nature.

Commercially available litmus is a mixture of colorless plant debris, inorganic salts, and indicator components. Azolitmin and orcein are also commercially available.

In spite of its wide use, it appears that no systematic study of the spectral and indicator properties of litmus, its components, and related compounds has been reported. Spectrophotometric studies to date have been fragmentary and primarily related to use as dyestuffs (Desbleds, 1928) or histological stains (Elman *et al.,* 1928; Ringer, 1950). The present study was undertaken to provide some data on these materials and provide information for structure determination work.

Experimental

Apparatus. Spectral curves were measured with a Cary Model 11 recording spectrophotometer, using 2-cm. quartz cells. Absorbance measurements at fixed wave lengths were made with a Beckman Model B spectrophotometer using 1-cm. Pyrex cells. A Beckman Zeromatic pH meter was used for all pH measurements.

Reagents. All reagents were of the highest quality commercially available. Of the purchased indicators used, litmus and bromthymol blue were from Fisher Scientific Co., orcein was from Eastman Kodak, and commercial azolitmin was from Matheson, Coleman and Bell. All were used without further purification. Other indicators were prepared by methods subsequently described.

Buffer Solutions. Buffer solutions of constant ionic strength were prepared according to the method of Elving, Markowitz, and Rosenthal

(1956) from citric acid, disodium hydrogen phosphate and potassium chloride. The solutions were prepared in 0.5 *M* ionic strength and diluted with an equal volume of water to give the 0.25 *M* ionic strength buffer solutions used in all spectrophotometric measurements. A 0.0833 *M* solution of disodium hydrogen phosphate, ionic strength 0.25, pH 9 was used for measurements at high pH. In one series of experiments, Clark and Lubs phosphate buffers, ionic strength about 0.1 *M*, were used.

Litmus Components. Commercial litmus was extracted with small portions of hot water until the extract came away colorless. The combined extracts were filtered and evaporated to dryness. The residue was triturated with 3 *M* hydrochloric acid. The material that remained insoluble was removed by filtration, washed with water, and dried. This material, which contained most of the indicating matter of litmus and amounted to about 10% of the original litmus, was placed in a Soxhlet extractor and extracted with ethyl ether, and then with 95% ethanol. Evaporation of the ether gave a red gum, erythrolein; evaporation of the ethanol gave a dark red solid, erythrolitmin. The ether- and ethanol-insoluble residue, azolitmin, was an almost black, hard, crystalline material. The composition of a typical sample of crude litmus is given in Table I.

Stock solutions of azolitmin and erythrolitmin were prepared by dissolving 0.25 g. samples in 5 ml. of 0.1 *M* sodium hydroxide and diluting to 100 ml. Stock solution of whole litmus was prepared by refluxing

TABLE I.

Composition of a typical litmus sample.

Component	%
Plant debris	56.8
Inorganic salts	33.2
Erytholein	0.7
Erythrolitmin	4.5
Azolitmin	4.8

10.00 g. of whole litmus with 500 ml. of water for ten hours filtering and diluting the filtrate to 1.000 l. The pH of the stock solution was 9.8.

Synthetic Indicators. A mixture of 0.05 mole of orcinol or resorcinol, 10 ml. of concentrated ammonia, 11 g. of sodium carbonate, and 70 ml. of water was heated on a steam bath in an open flask for twenty-four hours. The solution was cooled and diluted to give 1 l. of stock solution. The solution obtained from the reaction with orcinol was too concentrated for use in the procedure subsequently described. An intermediate dilution of 5 ml. of the reaction solution to 100 ml. with water gave a suitable stock solution. The material derived from orcinol is hereafter designated as orcolitmin, and that from resorcinol is designated resorcolitmin.

Commercial Indicators. Stock solutions of orcein and bromthymol blue were prepared by dissolving 0.050 g. samples in 5 ml. of 0.1 *M* sodium hydroxide and diluting to 100 ml. Commercial azolitmin stock solution was prepared by dissolving a 0.500 g. sample in 10 ml. of 0.1 *M* sodium hydroxide and diluting to 100 ml.

Procedure. Solutions for spectral measurements were prepared by diluting 1- to 5-ml. aliquots of the appropriate stock solution to 100 ml. with buffer solution of the desired

pH. One solution of high pH, one of low pH, and three with pH's near the pK_I, all with the same concentration of indicator, were prepared for each series of measurements. Immediately after preparation the exact pH of each solution was determined and the absorbance at 580 mμ was measured on a Beckman Model B spectrophotometer, and the spectral response curves for the high and low pH solution in the range 400 to 700 mμ were recorded with a Cary Model 11 spectrophotometer. The indicators used were only slightly soluble in acidic solution, and precipitates formed in some acid solutions on standing.

Beer's law studies were made using solutions of high and low pH only, with varying concentrations of indicators.

The same procedure was used in ionic strength experiments except that the calculated amount of additional potassium chloride to give the desired ionic strength was added to the indicator aliquot before dilution with buffer solution.

Solutions of erythrolein were prepared directly from the solid indicator in Clark and Lubs phosphate buffers which were about 0.1 *M* ionic strength.

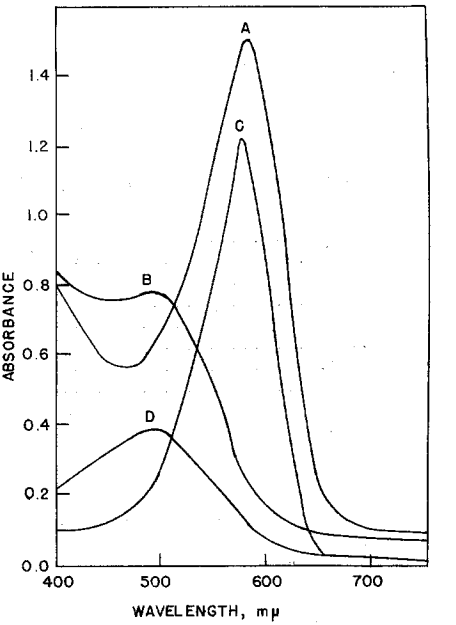

Fig. 1.—Absorption spectra of litmus and orcolitmin.
A=litmus, 5.0 x 10^{-1} g./1., pH 9;
B=litmus, 5.0 x 10^{-1} g./1., pH 3;
C=orcolitmin, 6.2 x 10^{-3} g./1., pH 9;
D=orcolitmin, 6.2 x 10^{-3} g./1., pH 3.

Results and Discussion

It has been generally assumed that each of the three indicator components shown in Table I represented a single chemical compound. Information available to 1948 has been summarized in Thorpe's *Dictionary of Applied Chemistry* (1948). Musso (1956) has shown orcein to be a complex mixture, and this suggests that each of the litmus components is also a mixture. Observations in this laboratory confirm that each component is a mixture, a conclusion based on variable elemental composition, paper chromatography, and spectrophotometric evidence. Only the latter will be discussed in this paper.

Spectral response curves for whole litmus and for orcolitmin, the indicator derived from orcinol, are presented in Figure 1. The curves for all the litmus components and commercial azolitmin are similar to those for whole litmus, and those for orcein and resorcolitmin, the material

TABLE II.—Spectral data.

	pH 3		pH 9	
	λ max mμ	A_s 1000 cm.² g⁻¹	λ max mμ	A_s 1000 cm.² g⁻¹
Whole Litmus	490	0.8	583	1.54
Erythrolein	486	0.8	574	1.3
Erythrolitmin	495	5.6	584	10.2
Azolitmin	498	6.0	587	9.1
Commercial Azolitmin	502	1.9	596	3.8
Orcein	497	37.0	580	138.0
Orcolitmin	497	28.6	580	97.5
Resorcolitmin	490	10.9	590	16.5

prepared from resorcinol, are similar to that of the synthetic litmus. Absorption maxima and absorbancy indices, at high and low pH, for all indicators studied, are given in Table II. There were small variations in spectra of litmus components isolated in one separation when compared with those obtained in other separations. The minimum observed in the 425-475 mμ range for solutions of low pH was not always distinct, leaving a shoulder rather than a peak in the 475-500 mμ range. The most remarkable aspect of the spectra was that while the absorbancy indices varied greatly from one indicator to another, there were only minor differences in the shape of the spectral curves.

Indicator constants were determined in citric acid—disodium hydrogen phosphate buffer with potassium chloride added to maintain a constant ionic strength of 0.25 *M*. The buffers were prepared according to the directions of Elving, Markowitz, and Rosenthal (1956), using one-half the specified concentrations. Additive absorbances were assumed. Deviations from Beer's law for whole litmus and orcolitmin were measured and found to be smaller than experimental error for both the acid and the salt form of both indicators. Assuming ideal behavior the indicator constants were calculated by the equation

$$pK_I = pH_{buf} + \log \frac{A_{Buf} - A_{Alk}}{A_{Acid} - A_{Buf}}$$

where pH_{buf} is the pH of the particular buffered indicator solution, A_{Buf} is the absorbance of that solution, A_{Acid} is the absorbance of a strongly acidic indicator solution, and A_{Alk} is the absorbance of a strongly alkaline indicator solution. The total concentration of indicator was the same for all solutions of a given series, although the ratios of acid form to salt form were different. The results of these experiments are given in Table III. The values for bromthymol blue were measured to give a check on the procedure, and may be compared with the values of 7.10 at 0.1 *M* ionic

strength and 6.9 at 0.5 *M* ionic strength given by Kolthoff and Rosenblum (1937).

Ideally the value obtained for the pK_I of a given indicator should be independent of the pH of the buffer solution in which the measurement is made. However, this is found to be true only for bromthymol blue. Bromythymol blue is a pure compound, but all the other indicators were known or expected to be mixtures, and the spectrophotometric behavior is consistent with this conclusion. The change in the fraction of indicator molecules ionized with change in pH is most rapid when the pH is at the pK_I. Hence, for a mixture of two indicators, the change will be more rapid for the indicator with a pK_I closer to the pH of the solution being examined. Thus, the apparent pK_I of the mixture will be dominated by the indicator whose pK_I is closest to the pH of a given solution. The degree of dominance is dependent not only on pK_I and pH factors but also on the relative absorbances of the species involved. In the present instance, since the pure indicator compounds have not been isolated, no quantitative calculations are possible. The magnitude of this effect can be estimated by calculations for a hypothetical mixture. Assume an equimolar mixture of two indicators each having identical absorbances at a given wave length, in strongly acidic solution, the absorbances in acid and alkaline solution being different. Assume a pK_I of 7.00 for one indicator and a pK_I of 6.00 for the other. If one then makes the usual assumptions of ideal behavior for each component, the apparent pK_I value will be 6.62 in a solution of pH 7, 6.50 in a solution of pH 6.50, and 6.38 in a solution of pH 6.00. A pK_I value of 6.50 would have been found for this mixture if the commonly used graphical procedure was employed in which absorbance is plotted vs. pH for a series of solutions, and pK_I value is taken as the pH at which the absorbance is half-way between that for the strongly acidic and strongly basic solutions.

The effect of ionic strength on pK_I values was determined for litmus and orcolitmin. Relatively high ionic strengths were selected in order to minimize the contribution of the

TABLE III.—Indicator constants in 0.25*M* ionic strength buffer.

Indicator	Solution pH	Calculated pK_I
Whole Litmus	6.22	6.24
	6.60	6.39
	6.98	6.51
Erythrolein[a]	6.3	6.4
	6.8	6.6
	7.2	6.8
Erythrolitmin	6.28	6.48
	6.64	6.58
	7.03	6.69
Azolitmin	6.29	6.72
	6.66	6.76
	7.08	6.93
Commercial Azolitmin	6.30	6.35
	6.63	6.43
	7.08	6.51
Orcolitmin	5.95	6.06
	6.32	6.08
	6.84	6.12
Resorcolitmin	6.30	5.86
	6.62	5.99
	7.03	6.24
Orcein	6.39	6.17
	6.65	6.20
	7.03	6.35
Bromthymol Blue	6.28	6.98
	6.60	6.99
	7.00	6.99

[a] pK_I determinations for erythrolein were made in Clark and Lubs phosphate buffers with ionic strength about 0.1*M*.

indicator stock solution to overall ionic strength and because litmus is not frequently used for the determination of pH of very dilute solutions. Solutions of ionic strength 0.25 *M*, 0.50 *M*, 0.75 *M*, 1.00 *M*, 1.25 *M* were used, and were prepared by adding the required potassium chloride to the standard 0.25 *M* buffers.

Again pK_I values were calculated using buffers of varying pH, and as previously described, the pK_I value increased as the pH of the solution increased. The effect of change in ionic strength on pK_I values was comparable in magnitude to the effect of changing buffer pH as shown in Table III. It was not practical to operate at a constant pH because changes in ionic strength affected the pH of the buffer. In Table IV the average pK_I values of three determinations, each at a different pH, are given for solutions with varying ionic strength. The data shown are representative of those obtained from several samples of indicator. In every case examined the pK_I values were significantly lower at 0.50 *M* ionic strength than at 0.25 *M*, but while further increases in ionic strength tended to decrease the pK_I the results were less consistent and the changes were less pronounced.

All of the indicators investigated are prepared by air oxidation, and each indicator is a mixture of compounds. As a result it is difficult to obtain uniform indicator samples in successive preparations. Instrumental errors in pH and optical absorbance measurements limit the accuracy to $\pm$ 0.1 pK_I unit and

TABLE IV.—Effect of ionic strength on pK_I values for litmus and orcolitmin

Ionic Strength	pK_I Values[a]	
M	Litmus	Orcolitmin
0.25	6.38	6.09
0.50	6.31	5.98
0.75	6.20	6.07
1.00	6.18	5.96
1.25	6.25	6.01

[a] pK_I values given are averages for three determinations, each in a buffer solution of different pH.

reproducibility to $\pm$ 0.05 pK_I unit. Values given in Tables II and III represent results of typical experiments rather than averages. Whole litmus and orcolitmin were the only indicators for which several determinations were made, and values from 0.2 unit lower to 0.2 unit higher were observed, while one sample of orcolitmin consistently gave results about 0.4 of a unit higher, the value varying with buffer pH. The separation process apparently affected litmus components, since all of the individual components exhibited higher pK_I values than whole litmus.

These experiments confirm on a quantitative basis, what is generally known in a qualitative way. Neither litmus nor any of the related indicators are suitable for quantitative work or for colorimetric determination of pH because of the variation of pK_I with pH, the large salt effect, and the uncertainty of composition of a given sample of indicator. The orcolitmin has narrower absorption bands than litmus and the visual color changes are consequently clearer. Orcolitmin also has a much higher absorbancy index and is as easily prepared as litmus solution. On this basis it is recommended as a substitute for litmus. There is no appar-

ent reason why it could not be used for the preparation of indicator paper that would be superior to litmus paper.

Summary

The visible spectra and indicator constants for litmus, three of its components, two related synthetic indicators, and two related commercial products have been measured. All have apparent pK_I values between 6 and 7, but evidence is presented to show that all are mixtures of indicating substances. The effect of ionic strength on the behavior of litmus and the synthetic indicator prepared from orcinol has been determined and found to be large.

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