THE SPECTROPHOTOMETRIC MEASUREMENT OF THE INDICATOR CHARACTERISTICS OF SOME NEW SULPHONPHTHALEINS

By

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To

Professor Malcolm H. Haring

who suggested the problem, and
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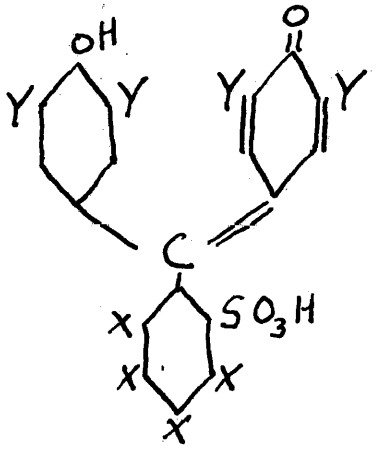
INTRODUCTION

The very valuable properties of the sulphonphthaleins as indicators for the hydrogen ion have been recognized for a long time. Especially since the careful studies of Clark and Lubs[1] supplemented by the work of Cohen[2] this class of organic substances has enjoyed wide usage in determinations of hydrogen-ion concentration. The sulphonphthaleins possess several advantages as indicators over some of the other classes of substances whose color is controlled by the hydrogen-ion concentration of the solution. Among these advantages are brilliance of color, ease of preparation in a pure state, and reliability.

In 1928 Twiss[3] succeeded in synthesizing di- and tetra- halogenated ortho-sulphobenzoic acids and their anhydrides, making possible the preparation of a series of sulphonphthaleins having four halogen atoms in the sulphobenzoic acid part of the molecule. The general formula for the compounds of this series is as follows:



where X is any halogen, and Y may be halogen, methyl, or hydrogen.

In 1928 Harden[4] prepared eleven of the possible members of the series, and studied them qualitatively as indicators and as aids in the study and diagnosis of pathological

conditions.

The present work is a spectrophotometric investigation of the indicator characteristics of seven of the compounds made by Harden. The substances studied were as follows:

Phenoltetrabromosulphonphthalein (Phenol-4Br)
Phenoltetrachlorosulphonphthalein (Phenol-4Cl)
o-Cresoltetrabromosulphonphthalein (o-Cresol-4Br)
o-Cresoltetrachlorosulphonphthalein (o-Cresol-4Cl)
Tetrabromophenoltetrabromosulphonphthalein (4-Br-phenol-4Br)
Tetrabromophenoltetrachlorosulphonphthalein (4-Br-phenol-4Cl)
Dibromo-o-cresoltetrachlorosulphonphthalein (2-Br-o-cresol-4Cl)

Determination of the Salt Errors and Protein Errors of the indicators was not undertaken.

THEORETICAL DISCUSSION

Indicators. Throughout this paper the word "indicator" is used in the somewhat restricted sense of an "acid-base indicator," or, more fully, a hydrogen-ion concentration indicator. In this sense, then, an indicator may be defined as any substance whose color is controlled by the hydrogen-ion concentration of its environment. The mere satisfaction of the requirement of the definition, however, is not by any means a criterion as to whether or not a given substance is a good, or even a statisfactory, indicator. This consideration is emphasized by comparing Clark's[5] general list of indicators, where some 185 substances classed as indicators are given, with a selected list of "good" indicators such as that of Sørensen, who lists 21 substances, or that of Clark and Lubs[1] and Cohen[2] who list 13.

The following characteristics are possessed by a good indicator:

(1) The indicator must be a weak acid or base, so that its own ionization may not disturb the hydrogen-ion concentration which is being measured.

(2) It must have a high tinctorial strength, so that only very small amounts of it need to be mixed with the solution under investigation.

(3) It should show a large change of color over a relatively short range of hydrogen-ion concentration. This of course makes for accuracy in measurement.

(4) There must be a negligible time-lag between

a change in hydrogen-ion concentration and the corresponding change in the color of the indicator. For most indicators this change is practically instantaneous, but in a number of cases an appreciable time lag is observed. The presence of the time lag does not in itself constitute more than a rather serious inconvenience; but it is usually found that different preparations of the same indicator may have very different rates of transformation, in which case the indicator is practically useless for accurate work.

(5) The color condition of the indicator must be dependent upon the hydrogen-ion concentration alone. Indicators whose molecules are very large and tend to form colloidal aggregates usually will vary in color with the size of the aggregates, as well as with hydrogen-ion concentration. Since the size of the aggregates depends at least somewhat on the presence of neutral salts in the solution, such indicators are said to have "salt errors". The presence of proteins, too, may modify the color of the indicator, leading to a so-called"protein error". This effect is particularly important when the indicator is to be used in connection with biological work where proteins often are likely to be present. As a matter of experimental fact, practically all indicators are susceptible to salt and protein errors, but fortunately in most cases the error is small. When an indicator is to be used in the presence of large amounts of salt, or with proteins, it should be carefully studied for its errors so that corrections may be applied.

A quantitative theory of indicator behavior was first proposed by Ostwald. He started with the experimental evidence that indicators behave as weak acids or bases, and applied the laws of equilibrium to them. He postulated that the undissociated, or molecular, form of the indicator possessed one color, and the dissociated, or ionic, form possessed another color. In the case of an indicator acid, the dissociation would be

$$HIn \rightleftharpoons H^+ + In^-$$

and the corresponding dissociation constant

$$K_{diss} = \frac{[H^+][In^-]}{[HIn]} \qquad (1)$$

Now the degree of dissociation, or fraction of dissociation, of the acid is given by the ratio

$$\alpha = \frac{[In^-]}{[HIn] + [In^-]} \qquad (2)$$

where the denominator is equivalent to the total concentration of the indicator. Dividing (1) by (2) and solving for α gives the very important relation

$$\alpha = \frac{K_{diss}}{K_{diss} + [H^+]} \qquad (3)$$

which emphasizes the fact that changes in $[H^+]$ produce corresponding changes in α. Thus since $[H^+]$ in a specific case determines α, $[H^+]$ indicates the <u>degree</u> to which properties associated with the anion or properties associated with the undissociated residue, or both, are present in mass. In terms of the postulate of Ostwald, this means that the degree of dissociation of the acid is synonymous with the degree of <u>color transformation</u> of the indicator, and

that the amount of color transformation is dependent only on the $[H^+]$ and the K_{diss} of the substance.

As the knowledge of the connection between the color of substances and their molecular structure progressed, it became evident that the situation with indicators is not as simple as the postulate of Ostwald indicates. It is now known that certain organic groups, by their presence in the molecule, "produce" color, although the actual physical mechanism of the production of the color is not yet apparent. Lewis[6] with his theory of vibrating electrons and "odd molecules" has made a start, but no complete connection between molecular structure and light absorption has so far been worked out.

Study of the structures of typical indicators shows that the color-producing (or color-changing) reaction must be such as to produce a tautomeric shift of structure from a colorless configuration to one containing a chromophore (color-causing) group; or, in the case of a two-color indicator, a tautomeric shift from one chromophore structure to another. Thus the single equilibrium treated by Ostwald must be expanded to two or more equilibria, at least one of which involves the tautomeric shift between two types of structure. To illustrate the principle in outline, assume that there are two tautomers, HIn and HIn', and that HIn' alone is capable of ionization as an acid. The equilibria are $HIn \rightleftharpoons HIn' \rightleftharpoons H^+ + In^-$. For the first equilibrium,

$$\frac{[HIn']}{[HIn]} = K_1 \qquad (4)$$

and for the second, $\frac{[H^+][In^-]}{[HIn']} = K_2$ (5)

Combination of these equations leads to

$$\frac{[H^+]\ [In^-]}{[HIn]} = \frac{K_1}{K_2} = K' \qquad (6)$$

Suppose now that In^- furnishes one color and either HIn or HIn' another color. Since equation (6) has the same form as equation (1) it is obvious that the color change will depend on the $[H^+]$ in exactly the manner already described, the only difference being that K_{diss} is replaced by K', and that α, while still the "degree of color transformation", is no longer strictly identical with the true degree of dissociation. Regarding the matter from another point of view, we see that a determination of the equilibrium constant from the data for the color change would not reveal whether this constant is a simple acid dissociation constant K_{diss}, or a complex constant K'.

The simplified treatment given above may be elaborated to take into account the ionization of both tautomers instead of just one. Noyes[7] has analyzed the situation and has shown that the elaboration does not change the form of the final equation. The consideration of the tautomeric equilibria only modifies the original Ostwald treatment to the extent that the found dissociation constant is a function of the several equilibrium and ionization constants involving the different tautomers. It is what Acree[8] calls the "total affinity constant" or what Noyes[7] calls the "apparent dissociation constant."

It is perfectly proper, therefore, for the sake of convenience, to disregard completely the complications introduced by the actual mechanism of the color change, since analysis shows that the Ostwald treatment gives an adequate picture of the relationships involved. Henceforth in this paper the symbol K will be used without prime or subscript, it being understood that it stands for the apparent, not the true, dissociation constant of the indicator. Similarly, α will be used to represent the degree of color transformation and not the true degree of dissociation.

Equation (3) above, involving the relationship between the degree of color transformation, the hydrogen-ion concentration, and the indicator constant, is the fundamental equation used in this work to measure the indicator constant. It is more convenient, however, in the logarithmic form. Solving equation (5) for $[H']$ gives

$$[H'] = K \frac{1-\alpha}{\alpha} \qquad (7)$$

Taking the logarithm of the reciprocal of both sides of (7) leads to the expression

$$\log \frac{1}{[H']} = \log \frac{1}{K} + \log \frac{\alpha}{1-\alpha} \qquad (8)$$

Making use of the well-known symbol $pH = \log \frac{1}{[H']}$ and introducing the exactly analogous symbol $pK = \log \frac{1}{K}$, equation (8) becomes

$$pH = pK + \log \frac{\alpha}{1-\alpha} \qquad (9)$$

Equation (9) is one of the most useful equations

found in indicator work. It shows that if the pK of an indicator is known, and its α can be measured, then the pH of the solution is determined. Or on the other hand, if α can be measured at a known pH, the pK of the indicator can be determined.

Thus the pK (or the K) of a given indicator is perhaps its most important quantitative characteristic. It is this constant which is determined for each of the seven indicators studied in the present work. Inspection of equation (7) or of equation (9) gives a clue as to the physical meaning of the "indicator constant": K is the $[H^+]$ at which α equals 0.50, that is, at which the indicator is half transformed. In logaritmic form, the pK is the pH at which the indicator is half transformed.

In all of the foregoing analysis it has been assumed that the indicator dissociated as a weak acid. While this is true for a large number of indicators, there are a few which dissociate as weak bases, and at least a few which are amphoteric in nature. It is readily seen that if the dissociation is a basic one, the only change in equation (9) would be the substitution of pOH for pH. But since in aqueous solution $[H^+]$ and $[OH^-]$ are always related through $K_w = [H^+][OH^-] = 10^{-14}$ at room temperature, it follows that the pOH scale is identical in form but reversed in direction to the pH scale, i.e. $pOH = 14 - pH$. It thus becomes very simple and convenient, and quite rigorous, to treat all indicators as if they dissociated as weak acids. This only serves to emphasize again that

the measured K is an apparent (acid) dissociation constant, and that the degree of color transformation α does not need to specify whether the transformation is from acid to base color or vice versa.

It is at first a rather striking experimental fact that all indicators of whatsoever pK have about the same rather small useful range. Why this is so becomes obvious after study of equation (9). For a given pK the range of pH is controlled by the variation of α. Since the latter can vary, practically speaking, only from 0.99 to 0.01, the value of the term $\log \frac{\alpha}{1-\alpha}$ can vary only from 2 to -2, giving a total pH range of only 4 pH units. As a matter of fact, at the extreme ends of the transformation small changes in the value of α are undetectable as color changes except with the very finest optical methods, and then not with much accuracy. As a result the "useful variation" of α lies always between 0.90 and 0.10, so that the "useful pH range" of nearly all indicators is less than 2 pH units.

Methods of measuring indicator characteristics. The useful range of any indicator can be conveniently measured simply by preparing a series of buffer solutions of known pH, the individual members of the series differing by a small constant amount (usually 0.2 pH unit). To equal volumes (e. g. 10 cc.) of each member of the series is added an equal volume (e.g. 0.5 cc.) of the indicator stock solution, which is usually 0.04%.

The limits of the range of solutions wherein color differences are visible to the eye are then recorded. Sometimes it is possible to extend slightly the useful range of an indicator by using a color comparator in the extremes of the range; this however is hardly necessary since enough good indicators are available to cover any desired range by the proper selection. Many ingenious attempts to extend the range of one indicator solution by using a mixture of indicator substances have been made. In these cases the range of one indicator is simply supplemented by the range of another, or several other, indicators.

The method outlined above is that used by Harden in his investigation of the ranges of the halogenated sulphonphthaleins used in the present work.

A number of methods have been devised for determining the indicator constant. Perhaps the simplest is that of Salm[9] who devised a method for determining the pH at which the indicator is half transformed. It consists in preparing two solutions each containing one-half the usual amount of indicator. One solution is maintained at a pH high enough to keep the indicator entirely in its alkaline form and color; the other is at a pH low enough to keep the indicator entirely in its acid form and color. When equal layers of the two solutions are superimposed, the color observed is due one-half to the alkaline form and one-half to the acid form. The superimposed layers are then matched with a similar pair,

one of which contains the full strength of the indicator at a known pH, and the other of which is a water blank. When a match is obtained, the known pH of the matching solution is obviously the pK of the indicator.

A method due to Gillespie[10] is very similar but somewhat more elaborate, and gives more information. A whole series of solutions is prepared containing the acid form of the indicator in amounts ranging from one drop in the first tube to nine drops in the last. An exactly similar series of the alkaline form is prepared in amounts ranging from nine to one drops. These are matched by pairs with a solution containing ten drops of the indicator at a known pH, backed by a water blank. The <u>drop-ratio</u> of the matching pair is thus the color transformation ratio α, so that instead of obtaining pH for $\alpha = 0.50$ alone, as in Salm's method, at least nine points on the whole pH-α curve are obtained and the curve may be drawn. As before, of course, pK is the pH at which $\alpha = 0.50$.

The method of Gillespie has been made much more accurate and convenient by an ingenious adaptation of a color comparator for the matching of samples. The modification consists in the use of three tubes, one above another, on one side of the comparator. The first tube is quite narrow and the third tube quite wide, so that the second medium-width tube can slide either up around the first or down into the third. The upper and lower tubes are fixed in position, so that

the distance from the bottom of the upper tube to the bottom of the lower tube is always the same; but the middle tube is movable vertically so that its bottom may take any position between the other two. If now the movable tube is filled with the acid form of the indicator, and the bottom tube is filled with the alkaline form, and the system is viewed from the top through the upper tube (which may be empty or filled with a blank solution), the color ratio may be varied continuously from $\alpha = 0.00$ to $\alpha = 1.00$ simply by moving the middle tube. Motion of this tube is recorded by a scale usually reading from 0 to 100--i.e. in percent color transformation. A match for a given solution can thus always be conveniently obtained, and the scale reading when matched gives α in percent. A convenient instrument embodying this principle has been designed by J. J. Beaver[11] and is marketed by the Klett Manufacturing Company of New York.

Another principle which has been put to use in the estimation of color-ratios is the "color wedge" of Bjerrum[12]. This is a long rectangular box with glass sides and a diagonal glass partition which divides the interior into two equal wedges. One compartment contains the indicator in its acid form, and the other in its alkaline form. A view through these wedges should imitate the view of a like depth and concentration of the indicator transformed to that degree which is represented by the ratio of wedge thicknesses at the point under observation.

The indicator constant can be most accurately measured, and the greatest amount of information about the color of an indicator can be obtained, by means of spectrophotometric measurements. In dealing with "colored" substances, a spectrophotometer gives a quantitative measure of the color in a detailed way that no other instrument imitates. A discussion of the principles of spectrophotometry and their application to indicators follows.

When radiant energy of any given wave-length travels through a material medium, it may suffer a diminution in its power or intensity. The medium is said to <u>absorb</u> some of the energy. "Transparent colorless" materials are those which do not absorb appreciably any radiant energy in the "visible range" (λ from 400 to 800 mμ.) A "colored" substance is one which does absorb some particular wave-length or wave-lengths in the visible range, so that incident "white" light, containing all visible wave-lengths at full intensity is deficient after travelling through the medium in some of the visible wave-lenghts. The resulting emergent light is hence colored. "Opaque" substances are those which absorb all of the visible wave-lengths so that none of the incident light travels through the medium. (It should be mentioned here that the above classification does not take into account those substances which may be colored by <u>reflection</u> of certain wave-lengths. All indicators are substances which impart color to a

solution, so that this discussion may confine itself to substances which are colored by absorption during transmission.

Consider a homogeneous solution of some absorbing substance contained in a glass cell the end-plates of which are plane-parallel, and neglect for the moment any absorption by the solvent or by the glass. Let the direction of the beam of incident light be perpendicular to the end-plates of the cell. In advancing through an infinitesimal length dl of the solution, the radiant energy of a single wave-length suffers the loss of some fraction of its intensity, I. Within the next equal infinitesimal length the remaining intensity is reduced by the same fraction. That is, the decrease of intensity per element of length is proportional to the intensity of the energy falling on that length. Or,

$$-\frac{dI}{dl} = kI \qquad (10)$$

Let the light incident on the first layer have an intensity of I_o, and that emergent from the last layer have intensity I. Integration of equation (10) between these limits gives

$$-\log_e \frac{I}{I_o} = kl \qquad (11)$$

The ratio $\frac{I}{I_o}$ is that fraction of the incident intensity which emerges, and is called the transmittancy T. Using this, and converting to common logarithms gives, from equation (11)

$$-\log T_\lambda = K_\lambda l \qquad (12)$$

Equation (12) is an expression of Lambert's Law, which states that absorption of light is proportional to the length of the medium through which it travels.

The decline of intensity within any infinitesimal length of medium should be proportional to the number of absorbing particles encountered. This number may be considered to be proportional to the concentration c of the absorbing substance under a given set of conditions. Hence equation (10) may be written

$$-\frac{dI}{dl} = k'cl \qquad (13)$$

Integration of (13) between the limits I_o and I, conversion to common logarithms, and use of the transmittancy, give

$$-\log T_\lambda = K'_\lambda cl \qquad (14)$$

This is Beer's Law, which states that the absorption in a given length of medium is proportional to the concentration of the absorbing species. Lambert's Law is believed to be universally applicable, but there are a number of cases where observed deviations from Beer's Law have not been explained. The λ subscripts in equations (12) and (14) serve to emphasize the fact that the equations are strictly valid only when the wave-length is specified.

An instrument which measures the relative intensity of incident and emergent radiant energy at a given wave-length is called a spectrophotometer. Since we are at present concerned with the absorption of visible light only, the following discussion does not include

instruments designed to measure absorption in ultra-violet or infra-red regions.

In order to study the intensities at a given wave-length, every spectrophotometer makes use of a spectrometer, which is an instrument for dividing the source of light into a spectrum and then selecting a narrow portion or band of the spectrum for use as a "single" wave-length. A spectrometer consists of an ordinary spectroscope fitted with an extra slit at the emergent end as well as the usual slit at the incident end, and with a means of rotating the prism on an axis. The extra slit serves to isolate one narrow portion of the spectrum, and the rotation of the prism moves the entire spectrum across the extra slit, so that any desired portion may be isolated. The rotation of the prism is controlled by a drum which is usually graduated to read directly in wave-lengths, but which must be calibrated from time to time during accurate work.

Optical spectrometers make use of two twin beams of incident light, which have separate paths through the system and are combined by means of a bi-prism in the eye-piece, so that the observer sees both beams side by side and may easily match them. One beam passes through a cell containing the solution being measured, and the other passes through an identical cell containing the solvent alone. Thus the differential effect is that due to the solute alone, conditioned of course by the

presence of the solvent. The beam passing through the solution suffers reduction in intensity, and the amount of this reduction is measured by artificially reducing the intensity of the other beam a known amount until a match is obtained. The means of reduction of intensity varies in different types of instruments. The Keuffel and Esser Color Analyser makes use of a rotating variable sector, the magnitude of whose openings can be ingeniously controlled while the sector is rotating. The scale for the reading of these openings is usually graduated in one hundred parts, so that the reading indicates directly the percent transmission (= 100 T.) The König-Martens type of instrument utilizes a system of Nicol or Wollaston prisms to reduce the intensity of the beam by polarization and rotation, and is usually graduated in angular degrees from which the transmittancy can be calculated by an equation depending on the individual characteristics of the instrument.

Measurements depending upon the use of the human eye as a matching instrument are always open to the objection that the identity of the observer will have some effect on the results. Moreover such measurements are always tiring. These difficulties are overcome in the photo-electric type of spectrophotometer. Here the relative intensities of the two beams are measured by converting their energies into electric currents by use of a photo-electric cell, and measuring the magnitude of the currents with a sensitive galvanometer. As

long as the current produced by the photocell is a straight-line function of the intensity of the incident light, and the deflection of the galvanometer is a straight-line function of the current, the method may be used accurately without resort to a rotating sector. An additional advantage is that only one beam of light is necessary (provided the source can be relied upon as constant over short intervals of time) thus obviating the necessity for bi-prisms and complicated optical systems. At a given wave-length the beam is passed through the blank cell, and the reading on the galvanometer is taken. Then the solution cell is placed in the beam, and the new reading observed. The transmittancy is given by the ratio of the second reading to the first, provided each reading is corrected for the reading when no light passes through the instrument. In practice the readings are repeated in a systematic way, and the averages are used to calculate the transmittancy.

An instrument of this type was used in the present investigation. A Brodhun rotating sector being available, it was incorporated in the system, which greatly increased the convenience and rapidity of the measurements. When the sector is used, the procedure is as follows. With the solution cell in the beam, and the sector full open (100 on scale), the galvanometer reading is noted. Then the solution cell is replaced by the solvent cell, and the sector opening is adjusted until the galvanometer reading duplicates the first reading. The beams are

now "matched", and the sector reading gives the percent transmission.

A cesium photocell was used, the charcteristics of which had been carefully studied. It was found that the relationship between cell current and light intensity was very nearly but not quite a straight line. This is the reason that the rotating sector was used, as a straight-line relationship is unnecessary under this condition.

The instrument used was very much more sensitive in the red-orange region of the spectrum than in the violet end. This was due to two causes: (1) the cesium cell itself is very much more sensitive to red rays than to violet; and (2) the coiled-tungsten-filament source was much richer in red rays than in violet. By using a very narrow slit-width in the red region and opening this as the violet end was approached, inequalities in sensitivity were partially adjusted. Using a wider slit at the violet end did not materially affect the results because of variation in the width of wave-length band, since the dispersion of the prism was so much greater in the violet end than in the red end that selection in the violet end did not need to be very fine. The sensitivity in the violet end was further improved by using filters in this region which removed the red and yellow rays from the source. This prevented "stray light" from falling on the photo-cell and affecting the readings. Any absorption effect of the filter was of course blanked out because the

filter was used in both the solution beam and the solvent beam.

The spectrometer drum of the instrument was calibrated before use by means of a helium tube excited by a high-voltage alternating current. This tube was used as the source of light, without any cells or filters in the system, and it was possible to find the location of a particular line in the emission spectrum of helium by obtaining the reading on the spectrometer drum where the photocell current was at a maximum. The correct wave-length of a number of the helium lines was obtained from tables, and compared with the spectrometer readings. A calibration curve was drawn and used to obtain corrections for readings throughout the spectrum.

When determinations of the transmittancy are made at a number of successive wave-lengths, the results may be charted and a curve drawn through the points. Such a curve is called an absorption curve or transmittancy curve according to the manner of charting. For reasons which will become apparent below, in indicator work it is usually most convenient to plot $-\log T$ against λ.

The indicator constant can be obtained from spectrophotometric measurements as follows. The transmittancy curves for a given indicator in the same concentration at successive pH values covering the range of the indicator are obtained and plotted. At a pH high enough to assure that the indicator is entirely

in its alkaline form, it will be found that there is a certain wave-length where the transmittancy is a minimum (maximum absorption). (This should be tested by obtaining the curve at a still higher value and observing that there is no further change in the value of the transmittancy at that wave-length). The transmittancy index K_λ at this wave-length is obviously a characteristic of the alkaline form only of the indicator, since the acid form is not present. When the pH of the solution is lowered far enough to transform the indicator completely to its acid form, the minimum of the curve is at another, usually widely distant, wave-length. Here the absorbing substance is the acid form only, and hence the index $K_{\lambda'}$ at this new wave-length is a characteristic of the acid form. At any intermediate pH both forms are present, but the fractional amount of the alkaline form present should be equal to the ratio $\frac{K_{\lambda x}}{K_{\lambda m}}$, where $K_{\lambda x}$ is the value of the index at wave-length λ and at the pH in question, while $K_{\lambda m}$ is the index at this wave-length and at the pH where the indicator is fully in its alkaline form. These indices need not be calculated, since they are proportional to $-\log T_\lambda$ in each case, and the ratio is the quantity involved. Now the fractional amount of the form present is just α, the degree of color transformation, so that a means is at hand of finding α at a given pH from the spectrophotometric data.

At the same pH in question the fractional amount of the acid form present can be found in exactly the

same way, using values of $-\log T_{\lambda'}$ corresponding. But this is unnecessary, as the color-transformation so found is just the complement of the first color-transformation (i.e. $\alpha' = 1 - \alpha$) since there are two and only two absorbing forms of the one indicator substance. Indeed in the case of one-color indicators the second determination is obviously impossible. It should be added that even with two-color indicators one of the absorbing species usually has its transmittancy minimum so far displaced in the violet that the accuracy of its measurement is far less than that for the other, and hence usually only one measurement is utilized in the calculations.

The simplicity of the method may be made more apparent by repeating the directions for the calculations, omitting theoretical considerations, thus: At a pH where there is no futher change in transmittancy with pH, select a value of $-\log T$ at or near a minimum in the curve. Call this $-\log T_m$. At an intermediate pH find the value of $-\log T$ at the same wave-length. Call this $-\log T_x$ then the degree of color transformation is

$$\alpha = \frac{-\log T_x}{-\log T_m} \qquad (15)$$

Having determined α at a number of pH's, equation (9) is used to find pK, or α may be plotted against pH, and the value of pH corresponding with $\alpha = 0.50$ gives the pK.

It may be remarked here that the form of the

family of curves obtained by the spectrophotometer provides a confirmation of the theory of indicator action used. The curves all intersect at the same point--within the limits of error. The probability of the occurence of such an "isobestic point"[13] would be very low indeed unless two "colored" substances, and two only, are present in the solution and change of pH has the effect of transforming one of these into the other. In fact, the relationship between the isobestic point and the theory used is such that Clark[14] makes the following remark:

> Nonconformity to the isobestic point should be used as a warning that the argument followed should be modified, and that, in the spectrophotometric method....curves that do not conform to the isobestic point are to be avoided.

EXPERIMENTAL WORK

Preparation and Standardization of Buffer Solutions. The buffer solutions used were the standard mixtures of Clark and Lubs.[15] These consist of the following mixtures: potassium acid phthalate and HCl, potassium acid phthalate and NaOH, primary potassium acid phosphate and NaOH, and boric acid, KCl and NaOH. They cover the range from pH 2.2 to 10.0 in steps of 0.2. Elaborate purification of the salts used was not undertaken in this work; instead the C. P. salts were used as such and the final pH of each individual buffer was carefully measured potentiometrically. A quinhydrone electrode was used for measurements below pH 8.0 and a hydrogen electrode for the rest. All of the measurements were made in a thermostat at 25°C ± 0.03°. Table I gives the results of these measurements.

Table I

pH's of Standard Buffer Mixtures.

Intended pH	Measured pH	Intended pH	Measured pH	Intended pH	Measured pH
2.4	2.40	4.8	4.76	7.2	7.15
2.6	2.60	5.0	4.96	7.4	7.28
2.8	2.82	5.2	5.16	7.6	7.51
3.0	3.00	5.4	5.36	7.8	7.68
3.2	3.20	5.6	5.55	8.0	7.78
3.4	3.40	5.8	5.79	8.2	8.05
3.6	3.60	6.0	5.95	8.4	8.24
3.8	3.80	6.2	6.15	8.6	8.44
4.0	3.99	6.4	6.35		
4.2	4.18	6.6	6.57		
4.4	4.35	6.8	6.75		
4.6	4.57	7.0	6.96		

Preparation of Indicator Stock Solutions. The indicator compounds prepared by Harden had been preserved in small glass-stoppered bottles and were used without further purification. 40 milligrams of each dye were ground in a mortar with sufficient 0.01 N NaOH to form the sodium salt, and the resulting mixture was diluted to 100 cc. This gave a stock solution of each indicator at 0.04% concentration.

Preparation of Individual Indicator Series. To 20 cc. of each buffer used in a series was added 1 cc. of stock indicator solution. In case it was found by trial that the resulting solution was too deep in color for satisfactory spectrophotometric work, it was diluted 1:1 with more of the buffer solution, or occasionally with distilled water. This method of varying the depth of color of the solutions was found more convenient than changing the length of the tube used in the spectrophotometer. Thus the tube length was uniformly 40.0 mm., but the concentration was 0.001% for six of the indicators and 0.002%for the seventh.

The pH range covered for each of the indicator series was determined experimentally by making up the series to at least one step beyond the point where no further color change was visible to the eye on both the acid and the alkaline side.

Spectrophotometric Measurements. The measurements were made on the photo-electric spectrophotometer as indicated in the theoretical section of this paper.

Uniform procedure for each solution was to start in the red end of the spectrum and work down through the violet, and then come back up to the red again, checking the readings. Each of the measurements given is thus the average of two readings. No attempt at exact temperature control was made, but the measurements were carried out in a basement room whose temperature varied but little at any time. Frequent checks on the temperature showed that the variation was not greater than from 25° to 28°C. The complete data for Orthocresoltetrachloro-sulphonphthalein are given as an example in Table II, page 28. The data for all of the indicators are shown plotted logarithmically in Figures 1 to 7, inclusive, pages 29-32, inclusive.

Calculations from the Measurements. The apparent degree of dissociation of each indicator at each pH was calculated by the method given on page 23. The results are summarized in Table III, page 33.

The alphas for each indicator were plotted against pH on a large scale, and the pH for $\alpha = 0.50$ was read from the graph. This gave pK for the indicator. A reduced plot showing all of the indicators is given in Figure 8, page 34. The pK value was also obtained in each case by means of equation (9), page 8. The average of the analytical values was in good agreement with the graphical values in every case. The average between the graphical value and the analytical value is reported in Table IV, page 35. These figures are considered to

Table II

Orthocresoltetrachlorosulphonphthalein

Wave-length mμ.	Slit width mm.	Filter	Percent transmittancy at pH 6.35	6.57	6.75	6.96	7.15	7.28	7.51	7.66	7.78	8.05	8.24	8.44
700	0.1	--	100	100	100	100	100	100	100	100	100	100	100	100
650			100	100	100	100	99.5	99.3	99.7	99.8	99.0	98.8	97.9	96.9
630			99.0	98.7	96.4	96.7	92.3	89.6	88.9	88.4	82.1	78.0	75.2	73.9
620			95.9	95.7	91.6	89.5	82.9	76.2	73.1	71.0	59.5	50.5	45.2	43.1
610			90.5	89.6	83.0	75.8	64.3	53.3	46.6	42.6	29.6	20.5	15.1	13.8
600	0.2	--	82.3	80.0	70.9	56.1	44.2	30.5	22.3	19.8	10.1	5.2	3.2	3.0
590			78.6	74.7	64.0	49.0	34.9	21.5	14.5	12.2	5.3	2.5	1.5	1.4
580			79.4	76.4	66.0	51.6	37.6	24.1	16.7	14.1	6.5	3.1	1.9	1.8
570			81.4	79.9	70.9	57.8	44.5	30.6	22.6	19.8	10.2	5.2	3.2	3.0
550			81.5	82.2	74.7	64.2	52.5	39.3	32.2	28.7	17.0	9.9	6.8	6.6
530			75.2	78.0	72.8	65.6	57.4	46.5	41.7	38.6	27.6	18.4	14.0	13.2
500	0.5	Blue-green	52.5	50.0	48.9	46.6	44.9	40.5	43.1	41.5	34.9	30.9	28.4	28.6
480			25.9	27.2	27.4	27.1	28.0	27.1	30.8	31.6	31.0	32.5	34.3	35.5
460			11.8	13.1	13.8	14.0	15.5	15.8	20.2	21.1	24.0	29.0	34.6	35.9
440			6.9	8.4	9.0	9.2	10.6	11.4	15.5	16.4	20.0	26.9	34.4	35.8
430			6.5	7.9	8.6	8.7	10.3	10.9	15.2	16.1	20.1	27.2	35.6	36.0
420			7.1	8.8	9.5	9.4	11.1	11.9	16.6	17.7	21.7	29.2	38.2	40.0
400			11.0	14.2	15.0	15.0	17.2	17.9	23.5	25.0	28.0	34.6	44.0	46.8

Length of tube--40.0mm.
Concentration of Indicator--0.001%

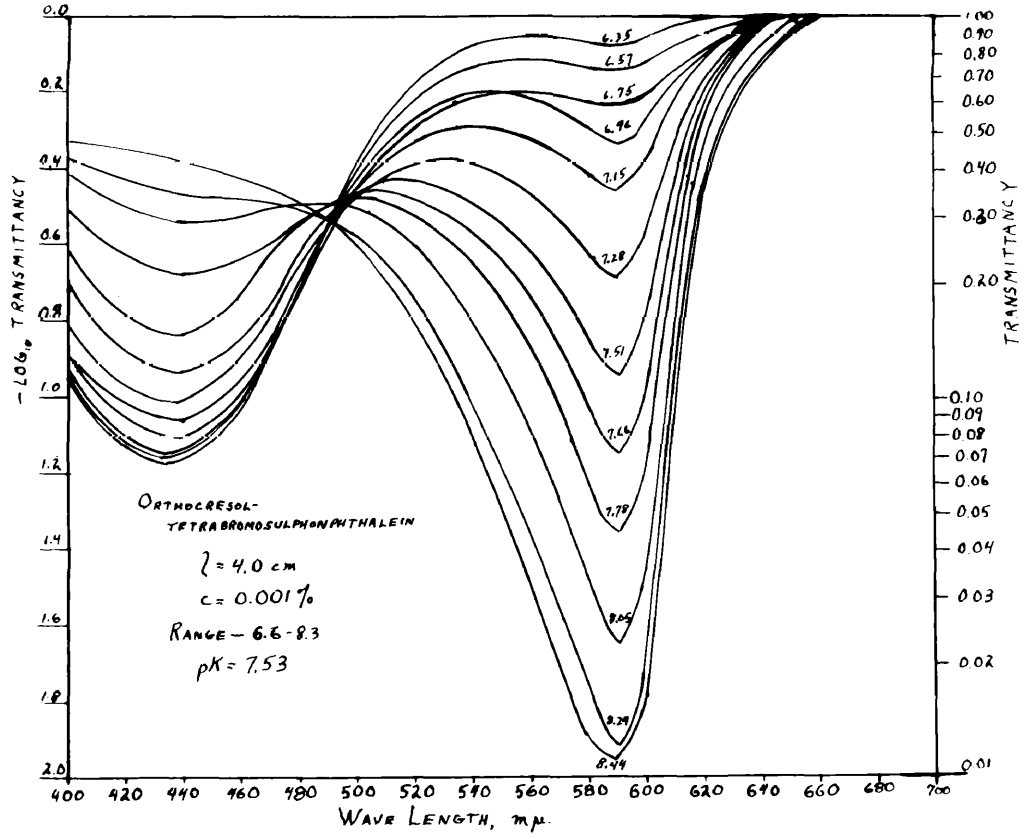


Figure 1.

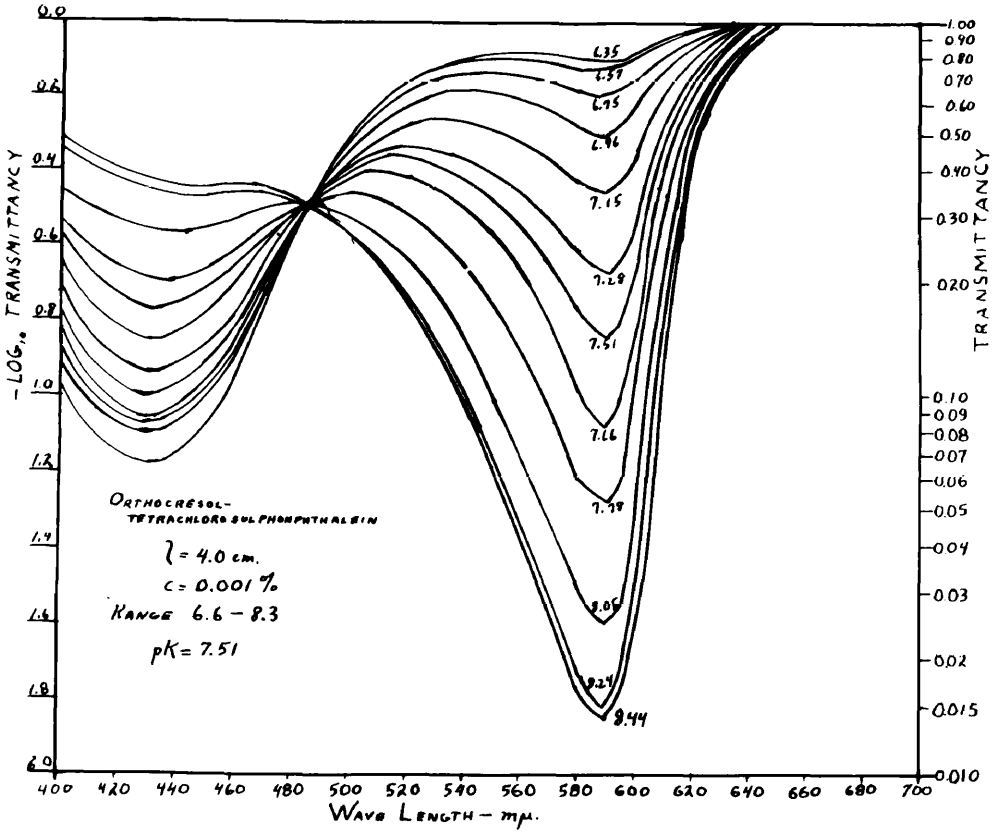


Figure 2.

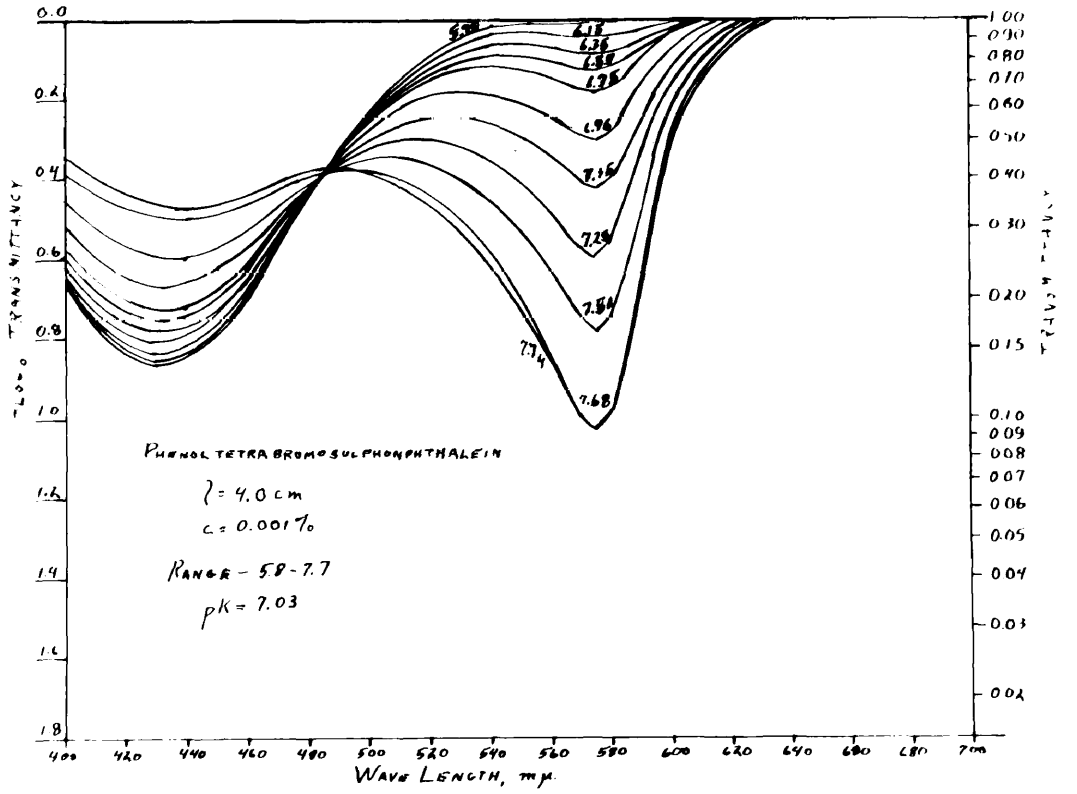


Figure 3.

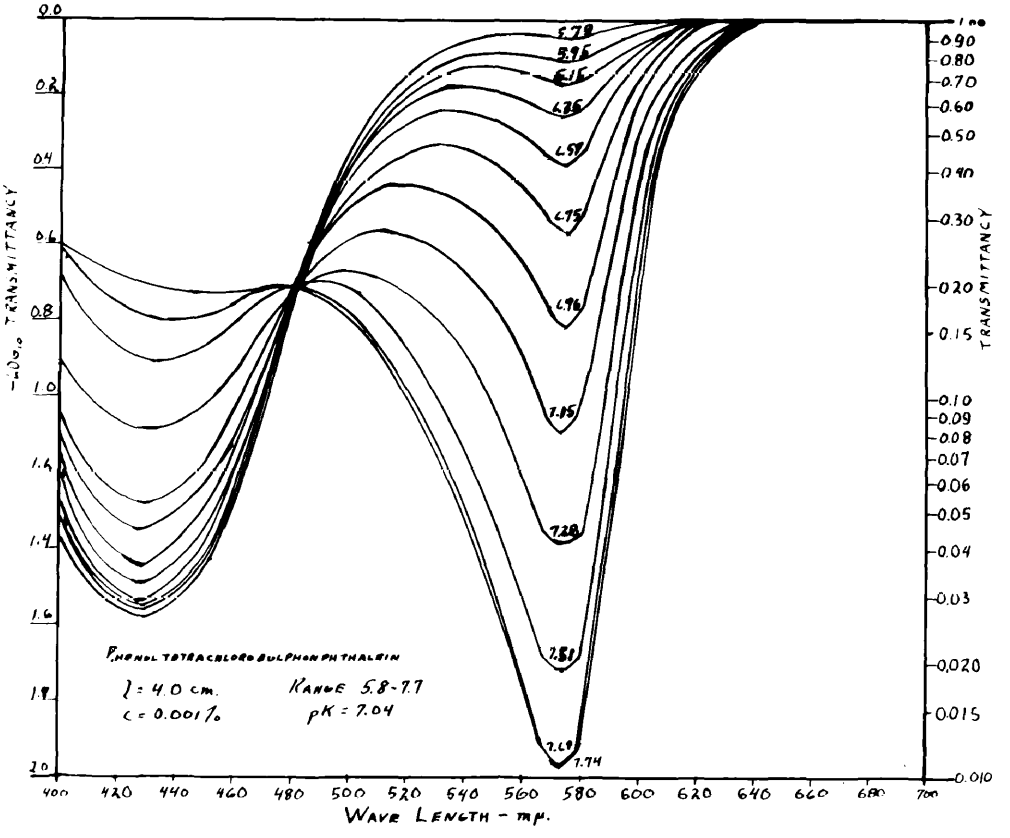


Figure 4.

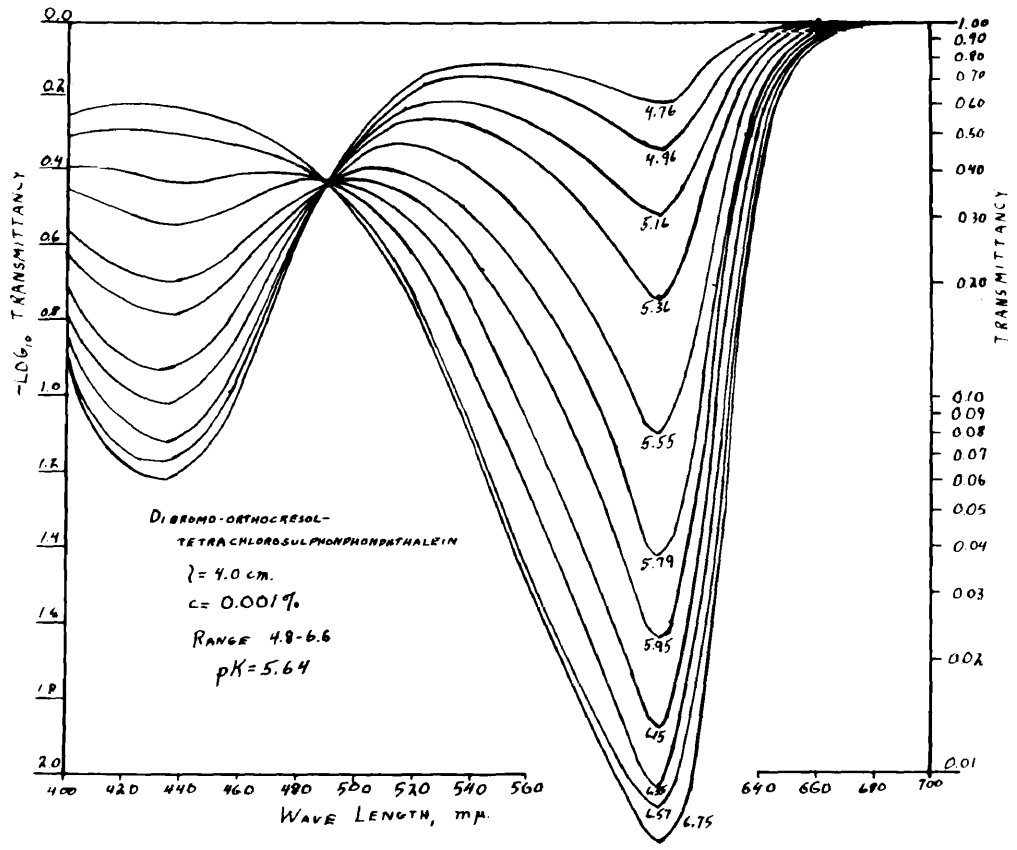

0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
-LOG10 TRANSMITTANCY
400
420
440
460
480
500
520
540
560
640
660
680
700
WAVE LENGTH, mμ.
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
TRANSMITTANCY
4.76
4.96
5.14
5.32
5.55
5.79
5.95
6.15
6.35
6.57
6.75
DIBROMO-ORTHOCRESOL-
TETRACHLOROSULPHONPHONDPATHALEIN
l = 4.0 cm.
c = 0.001%
RANGE 4.8-6.6
pK = 5.64

Figure 5.

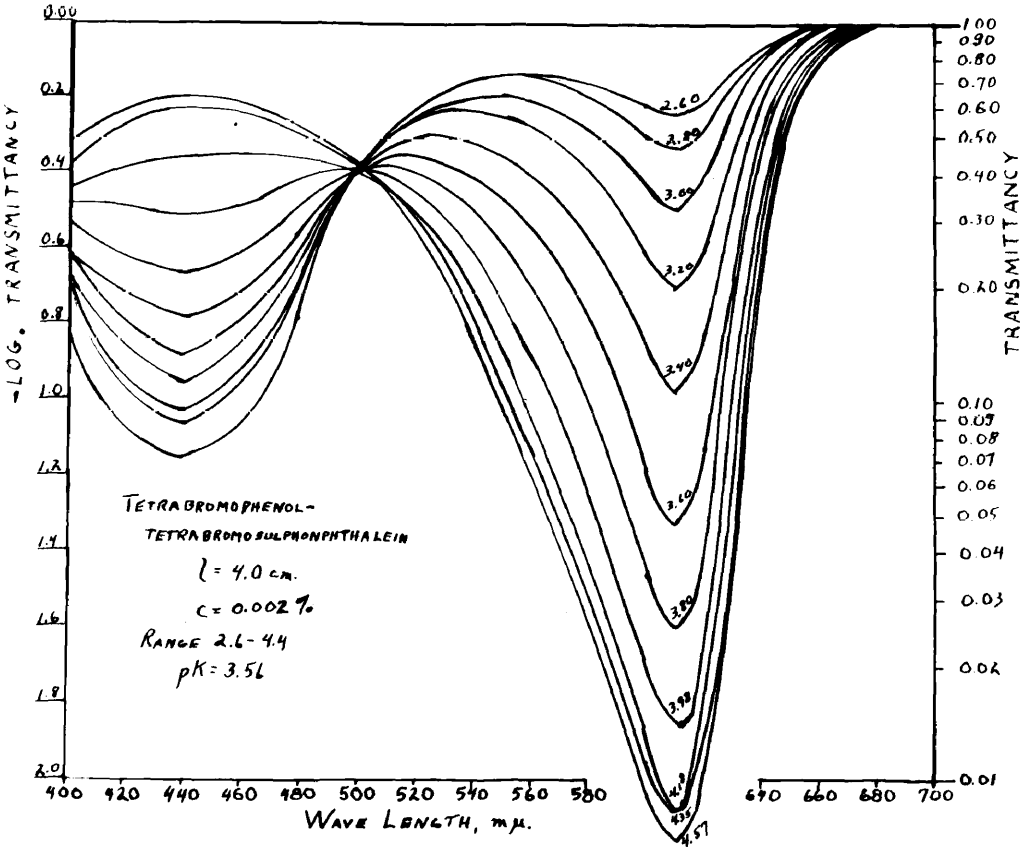


Figure 6.

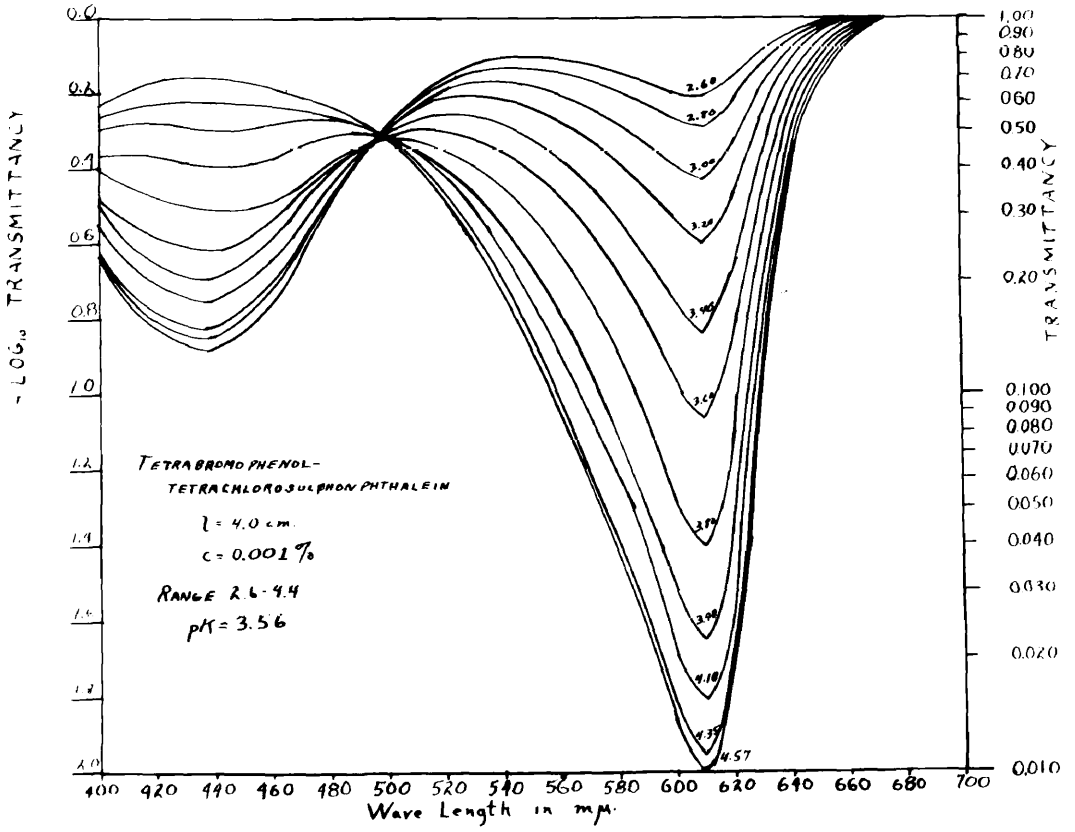


Figure 7.

Table III

Alphas of the Indicators

pH	α for indicator A	B	C	D	E	F	G
2.60	.104	.104					
2.82	.148	.139					
3.00	.216	.201					
3.20	.302	.294					
3.40	.421	.409					
3.60	.526	.562					
3.80	.705	.686					
3.99	.829	.815					
4.18	.912	.915					
4.35	.979	.947					
4.57							
4.76			.086				
4.96			.132				
5.18			.207				
5.36			.297				
5.56			.445				
5.79			.581	.021	.022		
5.95			.669	.058	.051		
6.15			.801	1087	.085		
6.35			.898	.132	.121	.057	.037
6.57			.960	.199	.166	.068	.074
6.75				.289	.294	.105	.123
6.96				.419	.415	.157	.171
7.15				.559	.582	.246	.232
7.38				.769	.764	.360	.349
7.51				.890	.861	.456	.485
7.66				[illegible]		.602	.590
7.78						.687	.696
8.05						.884	.845
8.24						.983	.980
8.44							

A--Tetrabromophenoltetrabromosulphonphthalein
B--Tetrabromophenoltetrabromosulphonphthalein
C--Dibromo-orthocresoltetrachlorosulphonphthalein
D--Phenoltetrachlorosulphonphthalein
E--Phenoltetrabromosulphonphthalein
F--Orthocresoltetrachlorosulphonphthalein
G--Orthocresoltetrabromosulphonphthalein

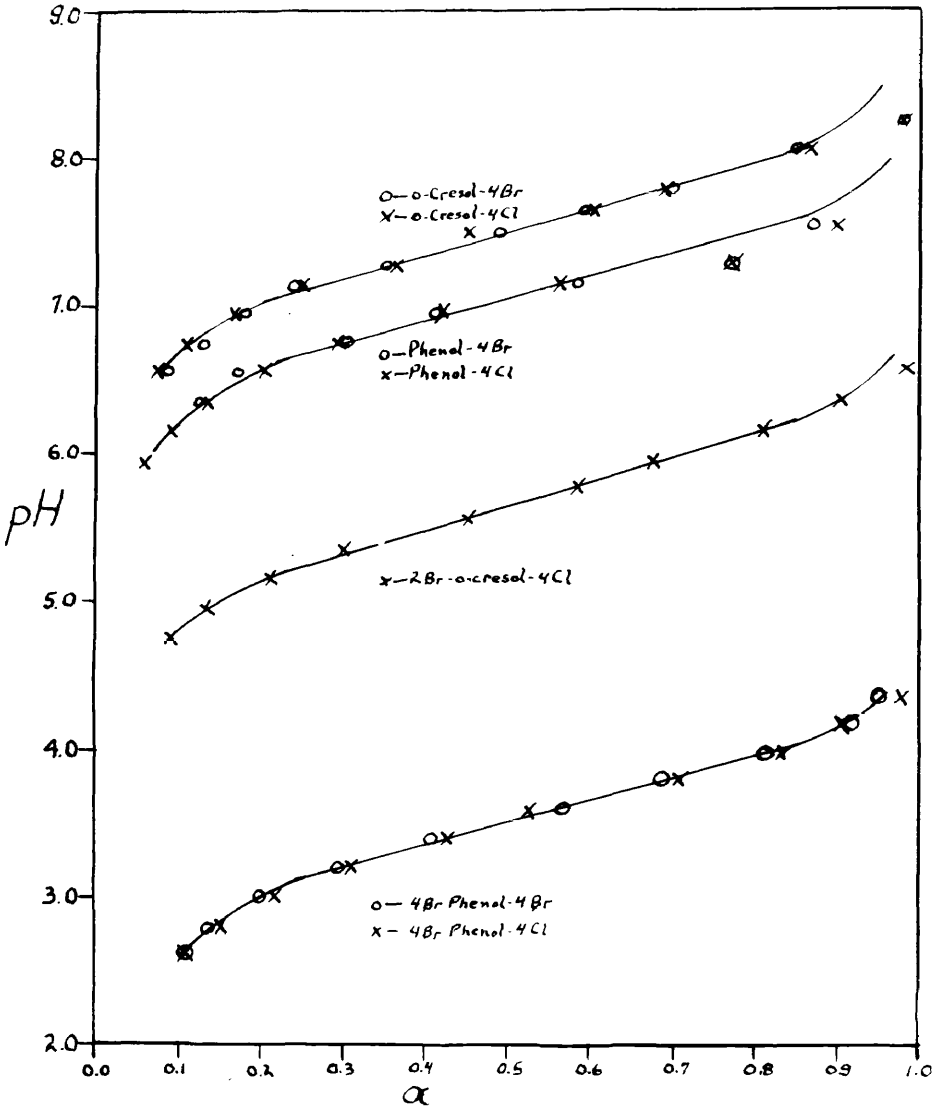

9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
pH
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
α
o—o-Cresol-4Br
x—o-Cresol-4Cl
o—Phenol-4Br
x—Phenol-4Cl
x—2Br-o-cresol-4Cl
o— 4Br Phenol-4Br
x— 4Br Phenol-4Cl

Figure 8.

be accurate to 0.02 pH unit.

Table IV

Indicator Constants

Indicator	pK
Tetrabromophenoltetrachlorosulphonphthalein	3.56
Tetrabromophenoltetrabromosulphonphthalein	3.56
Dibromo-orthocresoltetrachlorosulphonphthalein	5.64
Phenoltetrachlorosulphonphthalein	7.04
Phenoltetrabromosulphonphthalein	7.03
Orthocresoltetrachlorosulphonphthalein	7.51
Orthocresoltetrabromosulphonphthalein	7.53

Discussion of Results. The results bring out the interesting fact that the nature of the halogen atoms attached to the sulphobenzoic acid part of the molecule apparently makes no observable difference in indicator behavior. This fact is emphasized in Figure 8, where one curve serves for a pair of indicators in each of the three cases where corresponding pairs were studied. This behavior was noted by Harden when he measured the indicator ranges of these substances.

Harden observed that for a given pair of indicators, those containing chlorine in the sulphobenzoic acid nucleus had slightly less tinctorial strength than the corresponding bromo- compounds. The reverse was found in this work in two of the three cases, as can be seen by comparing corresponding transmittancies in Figures 1 to 7. It should be noted, however, that the difference in tinctorial strength is small--to the eye much smaller than to the spectrophotometer.

A comparison of the indicator constants of these new sulphonphthaleins with those of the corresponding compounds without halogen in the sulphobenzoic acid nucleus shows that the former are slightly more acid, the difference in indicator constant ranging from 0.5 to 0,9 pH unit.

SUMMARY AND CONCLUSION

The pH range and Indicator Constants of seven members of a series of sulphonphthaleins containing halogen attached to the sulphobenzoic acid part of the molecule have been measured by means of a photo-electric spectrophotometer. The results are summarized below.

Indicator	Conc. (%)	Color Range and Absorption Maxima Acid	Base	pH Range	pK
Tetrabromophenol-tetrachloro-sulphonphthalein	0.001	Yellow-green-blue 440mμ.	610mμ.	2.6-4.4	3.56
Tetrabromophenol-tetrabromo-sulphonphthalein	0.002	Yellow-green-blue 440	610	2.6-4.4	3.56
Dibromo-o-cresol-tetrachloro-sulphonphthalein	0.001	Yellow-green-blue 435	605	4.8-6.6	5.64
Phenoltetrabromo-sulphonphthalein	0.001	Yellow-violet 435	575	5.8-7.7	7.03
Phenoltetrachloro-sulphonphthalein	0.001	Yellow-violet 435	575	5.8-7.7	7.04
o-Cresoltetrachloro-sulphonphthalein	0.001	Yellow-purple 430	590	6.6-8.3	7.52
o-Cresoltetrabromo-sulphonphthalein	0.001	Yellow-purple 430	590	6.6-8.3	7.53

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