VII. XYLENOL BLUE

AND ITS PROPOSED USE AS A NEW AND IMPROVED INDICATOR IN CHEMICAL AND BIOCHEMICAL WORK.

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DURING the course of some studies on the most important of the sulphone-phthaleins synthesised by W. M. Clark and Lubs [1917], the writer was led to synthesise a new sulphonephthalein which has practically the same properties as thymol blue but with the important advantage of being twice as intense and therefore more economical.

Lubs and Acree [1917] in their earlier quests for evidence on the quinone-phenolate theory, now practically firmly established, noticed various relationships between the chemical constitution of the sulphonephthaleins and the corresponding working ranges of utility. Thus the introduction of negative (bromine) groups shifts the working ranges to a more acid region, *e.g.* cresol red with a working range from P_H 7.2 to P_H 8.8 on bromination yields brom cresol purple with a working range from P_H 5.2 to P_H 6.8, a shift of two units for either limit. The same shift occurs on brominating thymol blue to produce brom thymol blue. On brominating phenol red to produce brom phenol blue, a tetra-brominated instead of a dibrominated sulphonephthalein results, and the shift is four units for either limit of the working range.

Whilst then it would appear that negative group-substituents induce fairly regular changes of P_H ranges, another interesting aspect of the matter is presented by the shifting of the ranges towards the more alkaline region, by the introduction into the phenol residue of phenol red of positive groups like methyl and *iso*-propyl as in cresol red and thymol blue, which is a 1-methyl-4-*iso*-propyl-3-phenolsulphonephthalein. Here, however, the well defined acid range of thymol blue complicates matters.

It occurred to me to compare the case of diethyl red and dipropyl red. These are azo indicators and homologues of the well-known methyl red, or more strictly dimethyl red, dimethylaminoazobenzene-*o*-carboxylic acid. The two homologues show the same P_H range, viz. P_H 4.5-6.5, in spite of the increased positive character of the propyl groups. The following show similar anomalous behaviour.

Table I.

1. Methyl Orange: Sodium <i>p</i> -dimethylaminoazobenzenesulphonate	} P_H 3.1-4.4
2. Mono-ethyl Orange: Sodium <i>p</i> -monoethylaminoazobenzenesulphonate	
3. Sørensen's: α -naphthylaminoazobenzene	} P_H 3.7-5.0
α -naphthylaminoazo- <i>p</i> -toluene	
4. Sodium <i>p</i> -benzylanilineazobenzenesulphonate	} P_H 1.9-3.3
5. Sodium <i>p</i> -aminoazobenzenesulphonate	

One would suspect therefore the possibility of a similar anomalous unshifted P_H range when the thymol residue in thymol blue is replaced by *p*-xylenol; the difference here is in the replacement of the *iso*-propyl group in thymol by another methyl radical.

In view of this, it occurred to me, that owing to the smaller molecular weight, the resulting new sulphonephthalein should be used like phenol and cresol reds in 0.02 % solution to produce the same intensity as 0.04 % thymol blue or in strength between the two percentages. We should then have a more symmetrical list in which only the brominated dyes are used in the higher concentration of 0.04 % (or 1.2 % concentrated), alcoholic or aqueous monosodium salt, whenever phenol and cresol reds are chosen in half these strengths. These relative concentrations among the sulphonephthaleins have been recommended largely on the basis of equality of *depth* of colour, as far as can be judged by the eye with the reds and blues, though the reds and blues do not appear of the same *quality*, owing mainly to difference in degree of dichroism.

By condensing the chloride or anhydride of *o*-sulphobenzoic acid with *p*-xylenol, a product was ultimately obtained having the same double P_H ranges as thymol blue and similar colour changes.

EXPERIMENTAL.

The method of preparing 1.4-dimethyl-5-hydroxybenzenesulphonephthalein or xylenol blue is as follows [Chemicals & Bye-products, Ltd., and A. Cohen, 1921]:

A mixture of ten parts of *o*-sulphobenzoic dichloride, or of the acid anhydride; ten parts of fused zinc chloride; and fifteen parts *p*-xylenol, M.P. 74.5°, B.P. 211.5°, is heated in a brine bath for six hours. The melt is heated with forty parts water till disintegrated. It is then filtered hot, washed with hot water, and afterwards with a little alcohol. Next, it is dissolved in excess caustic soda and precipitated with hydrochloric acid whilst being stirred. The mixture is filtered and crystallised from alcohol in the form of a brown solid.

The reactions are:

Samples of the new indicator were titrated against 0.01 *N* NaOH using a micro-burette up to 5 cc. and reading to 0.01 cc.

Sample 1. 0.0205 g. in 150 cc. water (yellowish orange) required 4.90 cc. instead of the theoretical 5.00 cc. or 98 % molecular equivalent alkali.

Two other samples gave identical results.

Carbon dioxide must of course be rigidly excluded in such titrations involving high dilutions.

As in the cases of phenol and cresol reds, a 0.6 % aqueous solution of the monosodium salt can be made by gently boiling, not merely warming, for 3 minutes, 0.6 g. of the solid powdered dye in about 80 cc. water containing 1.47 cc. of normal alkali, and then diluting when cold to 100 cc.

If preferred, a strong alcoholic solution can be made by boiling 0.2 g. of the solid in 100 cc. absolute alcohol.

A 0.02 % solution was prepared by a thirty-fold dilution of the 0.6 % aqueous monosodium salt solution, and added to cordite tubes (or test tubes of hard white glass, unflanged and of uniform diameter) each containing 10 cc. of a standard buffer solution. Using a concentration of 0.05 cc. or 1 drop indicator per cc. buffer solution, the double series P_H 1.2–2.8 and P_H 8.0–9.6 (each in consecutive steps of 0.2 P_H) with xylenol blue, were similar to those with thymol blue added as a 0.04 % solution in the same concentration of 0.05 cc. per cc. The tints were, in fact, rather more distinct with xylenol blue in the alkaline range, appearing a purer blue in the daylight. Further, unlike dipropyl red in the anomalous case mentioned above, xylenol blue does not tend to precipitate out from buffer solutions even after three months' standing. On the contrary, xylenol blue stands in this respect to thymol blue, as the preferred diethyl red stands to dipropyl red.

Like thymol blue, it can be used for the estimation of the P_H of gastric juice and ammoniacal fluids; and in the differential acidimetric and alkali-metric titrations as described by A. B. Clark and Lubs [1918].

In Table II some results are given, obtained with mixtures of hydrochloric and benzoic acids. A 5 cc. micro-burette graduated in hundredths of a cubic centimetre was used, and I have found it convenient to use a special easily constructed titration flask in which a cordite tube is fused horizontally to the side of the flask to facilitate colorimetric comparison with the standard coloured benzoic acid. The cordite tube attachment also serves to stir the liquid when the flask is tilted. Such a flask can be heated without any cracks developing in the joint, provided the heating is done at the side remote from the joint. I wish here to thank one of my colleagues Mr G. H. Wallis for his kindness in constructing this flask.

0.5 cc. of 0.02 % aqueous xylenol blue was added to 50 cc. of an unknown benzoic acid solution, and 10 cc. of this standard kept in a cordite tube. The 0.5 cc. indicator is arbitrary and others may find it better to use more or less, but the less indicator is used the better; the differentiating power of the eye being an inadequately known function of subjective colour differences.

The mixtures titrated had, of course, the same concentration of indicator as the standard.

Table II. *Titration of mixtures of hydrochloric and benzoic acids.*

	Benzoic acid cc. used	1 <i>N</i> HCl cc. used	0.993 <i>N</i> NaOH		<i>N</i> HCl found cc.	G. of benzoic acid per cc. found
			to standard	to blue		
1	10	nil	nil	0.39	nil	0.0047
2	10	3.03	3.06	3.46	3.04	0.0049
3	25	4.04	4.05	5.00	4.02	0.0046

Experiments are in progress on the conditions for brominating xylenol blue.

SUMMARY.

p-Xylenolsulphonephthalein or xylenol blue, a new indicator having two working ranges of utility from P_H 1.2 (red) to 2.8 (yellow) and from P_H 8.0 (yellow) to 9.6 (blue), can be successfully employed in all work for which thymol blue has heretofore been recommended.

The fact, that *p*-xylenol is easily prepared from diazotised *p*-xylidene and that only half as much xylenol blue as thymol blue is required, should render its use eminently preferable.

REFERENCES.

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