THE SALT ERROR OF INDICATORS CAUSED BY STANDARD ALKALINE BUFFERS THEMSELVES.

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A series of well known buffer solutions has been standardised by means of the hydrogen electrode and their true alkalinity thus determined (see, for example, Clark[1]). It is then assumed that the colour of an indicator in one of these buffer solutions corresponds to the true alkalinity of that buffer solution; in other words the colour should be the same as that shown by the indicator in a solution of pure sodium hydroxide of the same alkalinity. We have found that, in many cases, this is not true and it will be shown that very serious discrepancies arise if this unexpected source of error is not taken into account. We have therefore tested the commercially available indicators which are advertised as suitable for use in the range of alkalinity in which we were particularly interested; namely, 0.01 to 0.0001 N. Only two of the simple, and one of the mixed indicators, out of the twenty tested, read correctly at 18°C. This is a higher range of alkalinity than that usually studied by biologists and many of the common indicators are inapplicable. We have carried out no tests on the acid side but it is evident that the same source of error must be looked for there. Indeed Kolthoff[2] found that tetrabromophenol sulphophthalein in phthalate-acid buffer begins to change in colour when pH is 2.8, while in pure hydrochloric acid, the same change begins when pH is 3.5. Since the present work was completed Kolthoff has published a further paper[3] in which he has come to similar conclusions as ourselves, using

[1] Clark, W. M., The determination of hydrogen ions, Baltimore, 2nd edition, 1922.

[2] Kolthoff, I. M., *Rec. trav. chim.*, 1922, xli, 62.

[3] Kolthoff, I. M., *Rec. trav. chim.*, 1925, xliv, 275.

borate, phosphate, and acetate buffers. The colours shown by indicators in dilute solutions of buffers differed from the colours shown in ordinary, more concentrated buffers of the same pH as measured by the hydrogen electrode. This corroborates our findings by a wholly independent method.

We have also standardised with pure sodium hydroxide the colours given by various indicators at 90°C. Here the modern jargon, which speaks of pH instead of alkalinity is not helpful. With four indicators, (thymol blue, phenol violet, methyl thymol blue (approximately) and *o*-cresol phthalein (approximately)) the so called pH appears the same in cold and in boiling solutions of pure sodium hydroxide, which indicates that the apparent acidity is unaltered; or, in the stricter language of the chemist, the alkalinity has really altered about 100 fold because the dissociation of the water is correspondingly altered. The distorted result is due to the indicator.

With five indicators (phenolphthalein, phenol thymolphthalein, thymol violet, tropeolin O and alizarin yellow G) neither pH nor alkalinity appeared the same at 18° and 90°C. Only three (namely alizarin yellow G, tropeolin O, and thymol violet) have the same colour at 18°C. in pure sodium hydroxide solution and in buffer of identical alkalinity and therefore identical pH. Even with these we have found that the colour is dependent upon the concentration of the indicator. It is a surprising fact that the colour exhibits a constant maximum within a certain concentration of indicator and the colour is diminished if too much or too little indicator is added.

EXPERIMENTAL.

A very simple method of standardisation has been used throughout, namely direct comparison of the colours given by various indicators in standard buffer solutions by matching with the colour produced in dilute solutions of pure sodium hydroxide of known concentration and therefore known alkalinity. In all cases the buffer solution was kept at 18°C. whereas the sodium hydroxide solutions were employed at 18° and also at 90°C. This enables colours obtained at 90°C. to be measured by means of buffers at room temperature and interpreted in terms of true alkalinity. The colour in a solution of pure sodium hydroxide must be taken as normal and the procedure measures

directly the amount of salt error which must be allowed for in the buffer solution itself, since it is found that the colour in the solution of pure alkali is often not the same as that corresponding to the alkali in the buffer as standardised by the hydrogen electrode.

Indicator.	Concentration of solution.	Amount used per 10 cc. solution.
		drops
Alizarin yellow G.	0.01 per cent in water.	10
Universal indicator.		10
o-Cresol phthalein.	0.02 per cent in alcohol.	10
Haematoxylin.	0.2 per cent in 20 per cent alcohol.	5 (0.15 cc.)
2:5 Dinitrohydroquinone.	0.2 per cent in 90 per cent alcohol.	10 (0.2 cc.)
α-Naphthol benzoin.	1.0 per cent in weak aqueous alkali.	5
Thymol blue.	0.04 per cent of mono-sodium salt in 20 per cent alcohol.	10 or 12
or	0.04 per cent in water.	25
Phenolphthalein.	0.1 per cent or 1.0 per cent in alcohol.	Varied.
α-Naphthol phthalein.	0.02 per cent in alcohol.	10
α-Naphthol sulphon phthalein.	0.02 per cent in alcohol.	10
Tropeolin O.	0.02 per cent in water.	10
Brilliant yellow.	0.1 per cent in 20 per cent alcohol.	5 (1.4 cc.)
Methyl thymol blue.		10
Phenol violet.		10
Phenol thymol phthalein.		20
Thymol violet.		3
Iodeosin.		3
Thymol phthalein.		10
Phenol tetrachlorphthalein.		1 (0.06 cc.)
Phenol tetrabrom phthalein.		

If sufficient care is taken to ensure the purity of the aqueous sodium hydroxide there can be no uncertainty as to its true alkalinity. As one check, solutions were observed at 18°C. before and after heating to 90°C.; the colours before and after being always found the same.[4] N/10 sodium hydroxide was made up from carbon-dioxide-free drippings from metallic sodium and freshly boiled out conductivity water and the solutions were made in Jena glass. Solutions were prepared immediately before use by dilution with freshly boiled out conductivity water. Hydrochloric acid prepared from constant boiling acid[5] was used to standardise the sodium hydroxide.

[4] Except in the case of haematoxylin.

[5] Hulett, G. A., and Bonner, W. D., *J. Am. Chem. Soc.*, 1909, xxxi, 390.

Two types of buffer mixture were used, Sörensen and Palitzsch's glycine/sodium hydroxide and borax/boric acid (see Clark[6]). The glycocoll, sodium chloride, borax, and boric acid were Kahlbaum's purest. After addition of indicator buffers were not usually kept in use longer than a day and never longer than 3 days, to avoid error due to fading. Colours were matched by eye, under a Sherringham daylight lamp.

The indicator was added in the amount stated below to 10 cc. of each of the solutions contained in test-tubes of equal bore. Resistance glass was used for the solutions of sodium hydroxide.

Calculation of Results.

Sodium hydroxide is completely dissociated in very dilute solution; the pH value was calculated from the normality

$$\mathrm{pH} = \log \frac{1}{\mathrm{H}} = \log K_w/\mathrm{OH}$$

using the following values for K_w.

K_w 18°C. $= 0.72 \times 10^{-14}$ (Sörensen).
K_w 20°C. $= 0.80 \times 10^{-14}$ (interpolated).
K_w 90°C. $= 53.3 \times 10^{-14}$ (Lorenz and Böhi).

Direct measurements of the hydrogen exponents of the buffer mixtures were not made and are not required for a relative comparison of the behaviour of various indicators. However, the buffer mixtures were prepared with sufficient care to justify the adoption of the pH values determined by Walbum[7] and Palitzsch.[8] The degree of accuracy attempted was pH 0.2. The results are collected in Tables I and II for the Sörensen standard glycine buffers and in Table III for borate buffers. In the first column is given the pH of the standard buffer as tabulated in Clark[1] and in the second column the concentration of hydroxyl ion that is calculated therefrom; these standard values come from the hydrogen electrode. In the following columns are given the normalities and true pH values of solutions of sodium hydroxide which with various indicators give the same colour as the the standard buffers. If there were no salt error due to the buffers themselves these numbers should all be identical with the values in the first and second columns. It will be noted that in some cases the discrepancies in pH exceed one unit; that is, an error of 1000 per cent.

[6] Clark,[1] pp. 111, 115.
[7] Walbum, in Clark,[1] p. 111.
[8] Palitzsch, in Clark,[1] p. 115.

Results with Glycine Buffer Mixtures.

From the sodium hydroxide values in Table I, or more obviously from the graphical representation of these values, it is evident that the nine indicators investigated fall into two groups.

To one group belong thymol violet, tropeolin O and alizarin yellow G. These indicators show little or no discrepancy between sodium hydroxide pH values and electrometric values for the buffers; that is, these indicators are free from any salt error caused by the constituents of the buffers themselves.

In the second group of indicators the discrepancy is large, that is to say there is a considerable difference in colour between sodium hydroxide solutions and the buffer solutions of similar true pH value. In the buffer solutions the indicator is further transformed to its alkaline colour than in the sodium hydroxide. This means that in measuring an unknown solution of small neutral salt content the observed alkalinity would be too low. Regarding sodium hydroxide as our point of reference, the mean discrepancies in colour amount to 0.79 units of pH for universal indicator; 1.19 pH for thymol blue; 1.0 pH for *o*-cresol phthalein; 0.7 pH for methyl thymol blue; 0.5 for phenol thymol phthalein.

In Table II are given the sodium hydroxide values for 90°C. With the exception of 0.0096 N sodium hydroxide with universal indicator, and the two sodium hydroxide solutions with α-naphthol benzoin, the result of heating the sodium hydroxide is a decrease in the characteristic colour.

In the introduction four indicators were mentioned whose colour is the same in solutions of sodium hydroxide at 90°C. as in glycine buffers of the same numerical pH value at 18°C., although the true alkalinity of the two solutions is very widely different. The following additional five indicators may likewise be used at 90°, again with the proviso that these errors be allowed for since here the sodium hydroxide solutions at 90° which have the same colour as the buffer at 18° have neither pH nor alkalinity in common: phenolphthalein, phenol thymol phthalein,[8] thymol violet,[9] alizarin yellow G, and tropeolin O.

Several indicators not included in the tables may be briefly referred

[9] Colour fades quickly.

TABLE I.

Concentrations and pH Values of Sodium Hydroxide Solutions Giving the Same Colour As Glycine Buffers at 18°C.

Buffer. pH	Buffer. C_{OH}	Thymol violet.	Tropeolin O.	Alizarin yellow G.	Universal indicator.	α-Naphthol benzoin.
12.86	0.051	0.046*	—			
		12.82				
12.67	0.034	—	0.039			
			12.74			
12.40	0.018	0.027	0.021			
		12.50	12.47			
12.10	0.0073	0.011	0.011	0.0096		
		12.18	12.18	12.12		
11.57	0.0027	0.0056		0.0065		
		11.89		11.96		
					0.0096	
11.25	0.0014	—		—	12.11	
			Phenol thymol			
11.07	0.00085	0.00098	phthalein.	0.00098		0.0017
		11.13		11.13		11.22
10.48	0.00022	—	0.010		—	0.000005
			12.10			8.86
10.42	0.00020	—	—		0.001	
					11.11	
10.14	0.000099	0.0001	0.00033	Methyl thymol	—	
		10.10	10.62	blue.		
10.09	0.000098			0.00033	0.0006	
				10.62	10.87	
9.71	0.000036				—	
				o-Cresol		Thymol
9.66	0.000036			phthalein.	0.00025	blue.
					10.50	
9.36	0.000016			0.0000997		0.000197
				10.14		10.44
8.93	0.000006					0.0000997
						10.14
8.58	0.0000028					

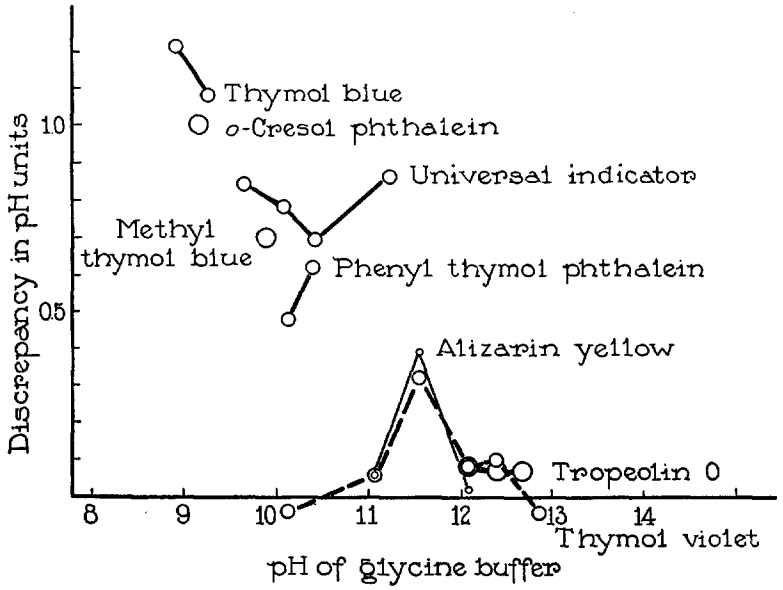
*In each case the first number refers to concentration, the second to pH.

TABLE II.

Concentrations of Sodium Hydroxide Solutions at 90°C. Giving the Same Colour As Glycine Buffers at 18°C.

Buffer. pH	= C_{OH}	Thymol violet.	Tropeolin O.	Alizarin yellow G.	Universal indicator.	α-Naphthol benzoin.	Phenol thymol phthalein		
12.40	0.018	0.046–	—						
12.10	0.0073	—	0.046–0.031						
11.57	0.0027	0.027	0.024						
11.31	0.0026	—		0.0096					
11.25	0.0014	—		—	0.0096				
11.07	0.00085	0.0096	Methyl thymol blue.	0.0065	—	0.0017			
10.48	0.00022	0.0056			—	0.000005 Phenol violet.	0.01	*o*-Cresol phthalein.	Phenol-phthalein.
10.14	0.000099	0.00098	0.01		—	0.00912	0.001		0.0010
10.09	0.0001	—	—		0.0010		—	—	—
9.71	0.000036	—	—	0.00082	—	0.0039–0.00099	—	0.000143	0.00072
9.66	0.000036	—	—	Thymol blue.	0.0007	—	—	—	—
9.36	0.000016	0.0001	0.00167	0.00066	0.0005	—	—	0.00082	0.00049
8.93	0.000006		0.001 0.00042	0.00039	—	0.0005	—	0.00030	0.00030
8.88	0.000006			—	0.00029		—	—	—
8.58	0.0000028			0.0001			0.00033	0.00020	—

to. Haematoxylin gave good colours with the buffer solution but changed after about 4 hours; however, the colours obtained with solutions of pure sodium hydroxide (and likewise with soap, or soap and alkali) were unlike the colours in the buffer solutions. The colours obtained with thymol phthalein, α-naphthol phthalein and α-naphthol-sulphon phthalein and especially tetrabromophenol phthalein were transient. The last mentioned indicator pH 8 to 12 like brilliant yellow 8.6 to 9.7, phenol tetrachlorphthalein 8.9 to 9.7, iodeosin 10.1



GRAPH 1. The discrepancies between the true pH's of standard glycine buffers (Sörensen) and of solutions of pure alkali which give the same colour with various indicators.

to 12.9, and 2:5 dinitrohydroquinone 8.6 to 12.4, scarcely changed in colour over the range indicated.

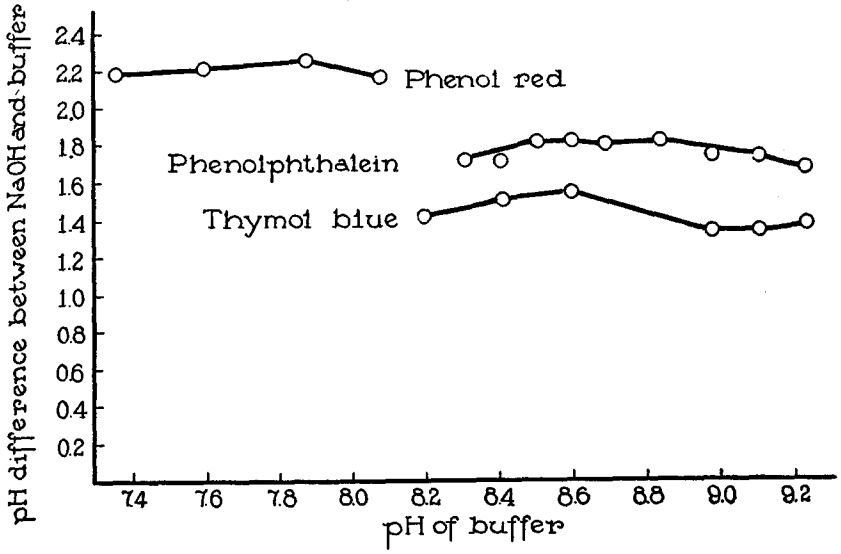
Results with Borate Buffers.

All the three indicators investigated show discrepancies of the kind found with glycine at 18°, but here with borate buffers they are far worse: phenolphthalein 1.8 units of pH, thymol blue 1.4 pH, phenol red 2.2 pH (see Graph 2). The buffer solutions are always of a more

alkaline colour than the sodium hydroxide solutions. The error in measuring alkalinity with phenol red exceeds 100 fold if this source of error is neglected.

Phenol red is the only one of the indicators investigated requiring a smaller concentration of sodium hydroxide at 90° than at 18° to produce the same colour.

The ratio of the concentration of sodium hydroxide required at 90° to that required at 18° to produce the same colour with phenolphtha-



GRAPH 2. The discrepancies between the true pH's of standard borate buffers (Sörensen) and of solutions of pure alkali which give the same colour with various indicators.

lein varies between individual readings from 1.38 to 2.0 the mean value being 1.5. The same ratio for thymol blue varies between 1.25 and 3.3 the mean being 2.5. The two values obtained for the ratio with phenol red were 0.8 and 0.46.

These measurements, even though they do not claim the highest accuracy, do at least show the fact that statements of the "effective ranges" of indicators are incomplete without mention of the specific solution in which they were determined. For example, phenolphthalein is pale pink in a buffer solution of pH 8.31, but in sodium hydrox-

ide it does not attain the same degree of colour until the pH is 10.13. The data for thymol blue illustrate the same point. With the borate buffers this indicator has just begun to change when the pH value

TABLE III.

Concentrations of Sodium Hydroxide Solutions at 18° and 90°C. Giving the Same Colour As Borate Buffers at 18°C.

Buffer.		Phenol phthalein.		Thymol blue.		Phenol red.	
pH	= C_{OH}	18°C.	90°C.	18°C.	90°C.	18°C.	90°C.
9.24	0.000012	0.00058	0.00080	0.000296	—		
		10.91		10.61			
9.11	0.0000092	0.00050	0.00065	0.000197	0.00061		
		10.84		10.44			
8.98	0.0000065	0.00038	0.00059	0.000148	0.000489		
		10.72		10.31			
8.84	0.0000051	0.00033	0.00046	—	0.000396		
		10.66					
8.69	0.0000036	0.000285	0.00038	—	0.00025		
		10.59					
8.60	0.0000029	0.00024	0.00033	0.000099	—		
		10.52		10.14			
8.51	0.0000023	0.00019	0.000285	—	—		
		10.42					
8.41	0.0000018	0.00012	0.00024	0.000058	0.00012		
		10.22		9.91			
8.31	0.0000015	0.000097	0.00019	—	0.00010		
		10.13					
8.20	0.0000011	—	0.00012	0.000030	—	—	0.000124
				9.61			
8.08	0.00000087	—	0.000073	—	0.000058	0.000124	0.000099
						10.24	
7.94	0.0000006					—	0.000062
7.88	0.00000055					0.000099	0.000046
						10.14	
7.78	0.00000042					—	—
7.60	0.00000029					0.000046	—
						9.80	
7.36	0.00000016					0.000025	—
						9.54	

reaches 8.08, which corresponds with the range as usually given pH 8.0 to 9.6. However, at a point near the middle of the colour change, two concentrations of sodium hydroxide were obtained which matched

members of both the borate and glycine series. Since the colours were the same, the indicator must have been transformed to the same degree in all three solutions, and yet their pH values are by no means identical.

pH of Similarly Coloured Solutions at 18°C.

NaOH	Thymol blue.	
	Glycine.	Borate.
10.14	8.93	8.60
10.44	9.36	9.11

Kolthoff[10] has placed side by side with the ordinary ranges of the indicators (presumably in buffer solution) at 18°, the ranges found at 100° in pure dilute acid or alkali. The apparent shift in range is therefore due to the sum total of the effects of salt and temperature, and since these are in opposition to one another the real shift in range is much greater than that observed by Kolthoff. For example, his data for phenolphthalein are:

Range at 18°.		Range at 100°.	
pH	pOH	pH	pOH
8.3–10.0	5.9–4.2	7.9–9.0	4.1–3.2

Our values for the limits of the range determined at both temperatures in sodium hydroxide solution are (from Table III):

In NaOH at 18°. $K_w = 0.72 \times 10^{-14}$)		In NaOH at 90°. ($K_w = 53 \times 10^{-14}$)	
pH	pOH	pH	pOH
Approximately 9.9 to >11.1	4.3 to <3.1	8.3 to >9.2	4.0 to <3.0

The buffer at 18° corresponding in colour (very pale pink) with these sodium hydroxide solutions at 90° had pH 8.1 which agrees with Kolthoff's value 8.3 in the buffer at 18°. There is also agreement

[10] Kolthoff, I. M., *Rec. trav. chim.*, 1921, xl, 775.

between Kolthoff's value of pH 7.9 at 100° and our value of pH 8.3 at 90°, when the difference of 10° in temperature is taken into account, since increase of temperature decreases the pH. There is, however, a discrepancy of pH 1.6 between our result for sodium hydroxide at 18° and that of Kolthoff, presumably due to salt error of the buffer used by the latter at 18°. Incidentally, the values found in sodium hydroxide solution show a large change in pH and only a small change in pOH which is at variance with Kolthoff's suggestion that "Les indicateurs qui sont eux-mêmes des acides faibles sont presque tout aussi sensibles aux ions hydrogène à température plus élevée qu'à la température ordinaire. Ceux qui sont des bases faibles deviennent moins sensibles aux ions hydrogène mais gardent à peu pres la même sensibilité aux ions hydroxyle." Phenolphthalein was regarded by Kolthoff as being in accordance with this since the pH range as he recorded it did not change much with rise in temperature, but this was due to his comparing the colour with buffer at 18° instead of with dilute alkali.

For two other indicators, thymol blue and alizarin yellow G it is possible from our data to get some idea of the change of the lower limit of the range in pure sodium hydroxide at 90°.

Thymol blue (in borate buffer pH 8.08).			
NaOH at 18°.		NaOH at 90°.	
pH 9.5	pOH 4.7	pH 8.0	pOH 4.3
Alizarin yellow G (in glycine buffer pH 11.07)			
NaOH at 18°.		NaOH at 90°.	
pH 11.1	pOH 3.1	pH 10.1	pOH 2.2

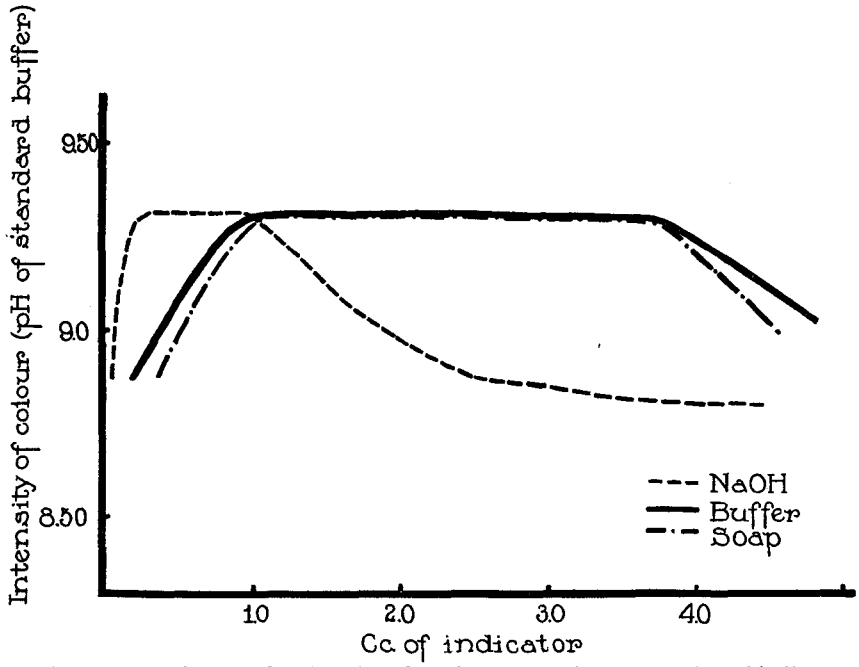
These two indicators are not in Kolthoff's table; they show a large change in both pH and pOH with change in temperature.

The changes in apparent pH as shown by indicators at 70° were found by Kolthoff by heating buffer solutions whose temperature coefficients had previously been determined by hydrogen electrode (Walbum). The method used by the authors, namely that of heating sodium hydroxide solutions and comparing with constant buffers at

18° standardised against the dilute sodium hydroxide at 18° gives values for the changes in apparent pH as shown by the indicators at 90° which are one or two complete units of pH greater than Kolthoff's values; 0.5 of this is due to the change in K_w between 70° and 90°.

Effect of Varying Concentrations of Indicator.

The most satisfactory concentration of indicator is the smallest which will produce a maximum colour in the solution, since at this



GRAPH 3. Diagram showing that there is a range of concentration of indicator producing maximum colour which falls off again if too much indicator is added.

point only is the full alkalinity of the solution indicated. It is, however, important that different amounts of indicator are necessary for the attainment of maximum colour in unlike solutions. Graph 3 illustrates this fact. Consider the three solutions sodium hydroxide, glycine buffer pH 9.31, and a soap solution, which all eventually come to the same maximum colour. Using 0.1 per cent phenolphthalein it was found that 0.25 cc. of indicator was required to produce maximum

colour in 10 cc. of sodium hydroxide solution (0.00026 N). Between 0.9 and 1.0 cc. was required to bring the buffer solution to the same colour.

Experiments with soap solutions were made with another indicator, *o*-cresol phthalein 0.02 per cent. These showed that the soap solution requires the same concentration of indicator as the buffer. The gradual appearance of colour, accompanying a gradual increase in the indicator concentration, was followed by comparing the sodium hydroxide, buffer, or soap solutions, in turn, with a series of buffer standards in which the maximum colour was developed. It was found that the colour of the soap solution increases more rapidly than that of the buffer until the maximum intensity is reached when the two colours become identical.

TABLE IV.

Changes in Apparent pH As Shown by Indicators As Found by Kolthoff by Heating Buffer Solutions to 70°, and As Found by the Authors on Heating in Dilute Hydroxide Only, from 18° to 90°.

Indicator.	Kolthoff.	Authors.
Phenolphthalein	−0.4 to 1.0	−1.6 to 1.7
Phenol red	−0.3	−2.0 to 2.2
Thymol blue	−0.35 to 0.45	−1.3 to 1.5

An excess of indicator causes the colours to fade, that of the soap decreasing more rapidly than that of the buffer. On the other hand, comparing the rate of appearance of colour in sodium hydroxide and buffer solutions, it is seen that the sodium hydroxide reaches its maximum colour first, when the concentration of indicator is 0.25 cc. When 0.9 to 1.0 cc. has been added to the buffer the maximum colour is attained, but if 1.0 cc. is added to the sodium hydroxide the colour has already begun to fade. Hence in our further work we have found it advisable to use maximum colours. By using this precaution and availing ourselves of the standardisations described in the present paper we have found several indicators which give values for the hydrolysis of soap solutions at 90°C. in agreement with previous values determined in this laboratory by such methods as hydrogen electrode and rate of catalysis. The results will form the subject of a separate

communication, but it may be mentioned that the universal indicator gives quite misleading results when used with soap solutions.

SUMMARY.

1. It is found that with many indicators there is a big discrepancy between the true alkalinities or pH values of solutions of pure sodium hydroxide and of standard alkaline buffers which give the same actual colour. This discrepancy must be ascribed to salt error caused by the buffer itself and exceeds in the most extreme case two whole units of pH; that is, an error of 100 fold in determining alkalinity. In only three cases, namely alizarin yellow G, tropeolin O, and thymol violet, was this error inappreciable. Most of the thirty indicators tested were found for various reasons unsatisfactory in the alkaline range studied.

2. Several indicators show a maximum depth of colour after sufficient indicator has been added, but above a certain concentration further addition of indicator diminishes the colour again.