cation of ethyl acetate by sodium hydroxide in aqueous solution. The initial molalities of ester and alkali were 0.008 and 0.01, respectively. The molality of the salt solutions varied from 0.02 to 0.5.

Only in the case of solutions of sodium chloride are suitable activity coefficients available for the interpretation of the results. In these instances it was demonstrated that the rate of the reaction is dependent on the molality of the ester and the catalytic activity of sodium hydroxide calculated by means of the expression corresponding to that which has elsewhere been shown to represent correctly the catalytic activity of potassium hydroxide.

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[CONTRIBUTION FROM THE SOILS RESEARCH LABORATORY, ALABAMA AGRICULTURAL EXPERIMENT STATION]

THE ADJUSTMENT OF THE REACTION OF INDICATOR SOLUTIONS AND ITS IMPORTANCE IN DETERMINING THE HYDROGEN ION CONCENTRATION OF SLIGHTLY BUFFERED SOLUTIONS

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Introduction

The determination of the hydrogen ion concentration of slightly buffered solutions has been known to be subject to various sources of errors. It has long been recognized that the hydrogen electrode, as ordinarily used, is unsatisfactory for such solutions. Lately, Beans and Hammett[1] have shown that by taking certain precautions the hydrogen ion concentration of slightly buffered solutions can be determined by the hydrogen electrode, but their electrodes become inactive quickly and the method is not adapted to routine work. Kolthoff and Bosch[2] studied the use of the quinhydrone electrode for such solutions. They found that it can be used with certain modifications for slightly buffered solutions but that it is best suited for use with solutions of moderate buffer capacity. The most generally used method for the determination of the reaction of slightly buffered solutions, however, is the indicator or colorimetric method. It, too, is open to certain objections. The one error most commonly overlooked is probably the reaction of the indicator solutions used. This has recently been emphasized by the work of Stern[3] and of Schlegel and Stueber.[4] During the last few years the writers have observed the importance of this point in working with slightly buffered soil extracts.

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[1] Beans and Hammett, THIS JOURNAL, **47**, 1215 (1925).

[2] Kolthoff and Bosch, *Chem. Weekblad*, **24**, 78–80 (1927); *C. A.*, **21**, 1586 (1927).

[3] Stern, *J. Biol. Chem.*, **65**, 677 (1925).

[4] Schlegel and Stueber, *Ind. Eng. Chem.*, **19**, 631 (1927).

It is the purpose of this paper to present data along this line and to describe a simple method for adjusting the reaction of indicator solutions which has been used in this Laboratory for the last two years.

To What Reaction Should Indicator Solutions Be Adjusted?

Before discussing the methods used in preparing indicator solutions, it seems desirable to consider from a theoretical viewpoint the reaction to which the various indicators should be adjusted. In discussing the use of indicators for determining the reaction of unbuffered material, Taylor[5] states "that the indicator solutions should have a neutral reaction, since any excess acid or alkali will likewise change the *P*H value of the material." While at first thought it might seem that all indicator solutions should be neutral and that a neutral indicator would not affect the *P*H of the solution to be tested, it can readily be proved that such is not the case. If, for example, brom cresol green adjusted to *P*H 7.0 is used for the determination of the reaction of an unbuffered solution of *P*H 4.5, the indicator will tend to change the reaction of the unbuffered solution toward its own reaction. The resultant *P*H reading will be too high, as is shown by the data presented in Table IV.

Michaelis[6] states "that the indicator method can only give correct results if the *P*H of the fluid under investigation is not altered by the addition of the indicator." This condition can only be met when the indicator solution has the same or almost the same reaction as that of the unknown. Any given indicator is used over a rather limited *P*H range designated by Kolthoff as the transition interval. If, therefore, its reaction is adjusted to a *P*H corresponding to the middle of the transition interval, it will be approximately of the same reaction as that of any unknown whose reaction can be determined with that particular indicator. It is obvious, therefore, that instead of adjusting all indicators to neutrality the various indicator solutions should be adjusted to a *P*H corresponding to about the middle of the transition interval. These values for the various indicators used by the writers are: Brom phenol blue, 3.80; Brom cresol green, 4.60; Brom cresol purple, 5.80; Brom thymol blue, 6.80; Phenol red, 7.60.

Methods of Preparing Indicator Solutions

Various methods have been used in preparing indicator solutions but little consideration has been given until recently to the adjustment of their reaction. Some of the early workers, Friedenthal,[7] Salm[8] and Sørensen[9] believed that the acidic and basic properties of indicator solu-

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[5] Taylor, "The A B C of Hydrogen Ion Control," LaMotte Chemical Products Company, Baltimore, Maryland, p. 17.

[6] Michaelis, "Practical and Colloid Chemistry," 2nd ed., p. 47.

[7] Friedenthal, *Z. Elektrochem.*, 10, 113 (1904).

[8] Salm, *Z. physik Chem.*, 10, 341 (1904); 12, 99 (1906).

[9] Sørensen, *Biochem. Z.*, 21, 131 (1919); 22, 352 (1919).

tions would affect the *P*H of unbuffered solutions and, therefore, concluded that the *P*H of pure water or solutions of strong acids or base could not be determined by the colorimetric method.

While Kolthoff[10] does not agree fully with this viewpoint, he believes that with some indicators an error may be introduced if their acidic properties are not considered. In the preparation of sulfonephthalein indicators, however, he does not adjust the reaction of the indicator solutions His method of preparation consists in dissolving 100 mg. of indicator in 20 cc. of warm alcohol and diluting to 100 cc. with water.[11]

Probably the most common method of preparing the sulfonephthalein indicators, used largely in this country for *P*H work, is that recommended by Clark,[12] in which the theoretical amount of base necessary to form the salt is added. The inadequacy of this method when working with slightly buffered solutions has been shown by Stern and by Schlegel and Stueber, with regard to the use of brom thymol blue indicator.

Both Stern and Schlegel and Stueber tested the neutrality of their brom thymol blue indicator with especially prepared conductivity water of *P*H 7.0. The indicator solutions were adjusted by the addition of alkali until the neutral conductivity water tested with the indicators gave a *P*H of 7.0. The difficulty of obtaining conductivity water of *P*H 7.0, however, is a serious objection to their method. Moreover, the method can only be used when the indicator solution is to be adjusted to *P*H 7.0.

Quinhydrone Titration Method.—Stern used the quinhydrone electrode in determining the reaction of his indicator solutions and found that the readings were accurate to within 10 millivolts. Evidently, then, the quinhydrone electrode can be used for the adjustment of the indicator solutions to any desired reaction. It was used by the writers and found very satisfactory. The hydrogen electrode, on the other hand, was found in preliminary work to be unsatisfactory, for a constant potential is not obtained. Apparently this is due to the reducing effect of the hydrogen introduced, for the indicator solutions gradually turn a yellowish-green color as the hydrogen is introduced.

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[10] Kolthoff, "Indicators," John Wiley and Sons, Inc., New York, **1926**, p. 169.

[11] Since this investigation was completed, the attention of the writers has been called to a recent paper by Kolthoff, *Biochem. Z.*, **168**, 110–21 (1926), in which the author states that in working with poorly buffered solutions such as distilled water the neutral salts of the indicators should be used since the indicators are acid in character and give erroneous results if not neutralized. He also points out the fact that the sodium salt solution of indicators made up according to Clark may also give slight errors and concludes that when one desires an absolutely accurate result, he must first approximately estimate the *P*H of the weakly buffered solution and then use a mixture of the indicator acid with its salt so mixed that the *P*H of the mixture approaches that of the solution to be tested.

[12] Clark, "Determination of Hydrogen Ions," 2nd ed., Williams and Wilkins Co., Baltimore, Md., **1923**, p. 80.

The Varying Drop Method.—The method that has been used in this Laboratory for the last two years is based on the principle that if an indicator solution is more acid or more alkaline than the weakly buffered solution to be tested a different hydrogen ion concentration will be obtained when different amounts of the indicator solution are used. The more of the indicator solution added, the more will the indicator have a tendency to change the *P*H of the weakly buffered solution toward its own reaction. It will exert no effect, however, on a strongly buffered solution. Thus, if in comparing the *P*H of a weakly buffered solution with a standard buffer of the same reaction, 3, 5 and 8 drops of indicator are successively added to each and the unbuffered solution changes its *P*H toward the acid side as compared with the standard buffer, it proves that the indicator solution is more acid than the unbuffered solution. Table I gives an illustration of the results obtained by this method.

TABLE I

THE HYDROGEN ION CONCENTRATION OF UNBUFFERED SOLUTIONS WHEN TESTED WITH INDICATOR SOLUTION VARIOUSLY ADJUSTED

Indicator	Drops of indicator solution per 5 cc. of solution tested	Hydrogen ion concn. of an unbuffered soln. with indicators adjusted according to	
		Clark. *P*H	Varying drop method. *P*H
Brom thymol blue	3	6.70	6.80
	5	6.40	6.80
	8	6.30	6.80

The unbuffered solution consisted of distilled water to which a little sodium hydroxide had been added. It will be noted that the more of the indicator solution adjusted according to Clark is added, the lower is the *P*H of the unbuffered solution as compared with the standard buffer. With the indicator solution to which was added more sodium hydroxide the *P*H was the same regardless of whether 3 or 8 drops were used, indicating that the indicator solution had a *P*H of about 6.80. By thus adding alkali or acid to the indicator solutions until slightly buffered solutions tested with it give the same reaction whether 3 or 8 drops are added, the indicator solutions can be adjusted to the desired *P*H. Although this method is slightly more tedious in operation than is the adjusting of the indicator solution by use of the quinhydrone electrode, it enables one to adjust the reaction of indicator solutions without the use of the electrical apparatus needed for the latter method.

Properties of Indicators Prepared by Various Methods

In order to study the effect of using variously adjusted indicator solutions on the *P*H of unbuffered or slightly buffered solutions, the five common indicators used in soils work were each prepared according to the following methods: (1) solution of indicator acid in alcohol according to Kolthoff,

(2) neutralizing according to the theoretical values recommended by Clark, (3) adjusting the indicators to the *P*H values recommended, by means of the varying drop method, and (4) adjustment to *P*H 7.0 by titration, using the quinhydrone electrode. In Table II are given the amounts of alkali used in each method and the resulting *P*H of the solutions as determined by the quinhydrone electrode. It will be noticed that the indicator solutions prepared according to Kolthoff are very acid and range between *P*H 2.51 and 2.80. Adjustment according to Clark also leaves the solutions very acid, varying from *P*H 3.73 to 5.19 for the different indicators. It will be seen that the *P*H values of the solutions adjusted according to the varying drop method are close to the reaction values previously recommended. It will also be noted that with some indicators it took nearly twice as much alkali to adjust according to the varying drop method as it did according to Clark. Adjustment to *P*H 7.0, as would be expected, requires considerably greater amounts.

TABLE II

THE HYDROGEN ION CONCENTRATION OF INDICATOR SOLUTIONS AS ADJUSTED BY VARIOUS METHODS

	Kolthoff method		Clark method		Varying drop method		*N*/20 NaOH required to adjust to *P*H 7.0, cc.
Indicator	*N*/20 NaOH, cc.	H-ion concn., *P*H	*N*/20 NaOH, cc.	H-ion concn., *P*H	*N*/20 NaOH, cc.	H-ion concn., *P*H	
Brom phenol blue	None	2.72	3.0	3.73	3.56	3.96	7.16
Brom cresol green	None	2.80	..	..	3.57	4.53	6.00
Brom cresol purple	None	2.68	3.7	3.76	5.60	5.87	6.80
Brom thymol blue I	None	2.70	3.2	4.97	4.82	7.13	4.60
Brom thymol blue II	...	..	3.2	5.19	4.23	6.78	4.42
Phenol red	None	2.51	5.7	4.29	9.20	7.71	7.98

TABLE III

A COMPARISON OF INDICATORS FROM VARIOUS SOURCES

			Color of indicator solution made up according to		
Indicator	Source	Color of indicator powder	Clark	Varying drop method	Relative tinctorial power
Brom phenol blue	A	Yellowish-orange	Purplish-red	Purplish-red	100
	B	Purplish-brown	Purplish-red	Purplish-red	100
Brom cresol green	A	Yellowish-orange	......	Green	100
	B	Chocolate brown	......	Green	100
	C	Dark brown	......	Green	98
Brom cresol purple	A	Yellowish-brown	Yellowish-red	Wine red	75
	B	Light pink	Wine red	Wine red	100
	C	Pinkish-yellow	Yellowish-red	Wine red	90
Brom thymol blue	A	Pink	Greenish-brown	Bluish-green	87
	B	Chocolate brown	Greenish-brown	Bluish-green	100
	C	Pink	Greenish-brown	Bluish-green	76
Phenol red	B	Crimson red	Yellow	Red	90
	C	Crimson red	Red	Red	100

A comparison was also made of the properties of indicators secured from different sources. These results are given in Table III. It will be seen that there are marked differences in the appearance of the indicator powders as well as in the color of the indicator solutions made up according to Clark. The color differences of the solutions were found to be related to the hydrogen ion concentration. Thus it was found that the phenol red from source B, which had a yellow color, required 3.5 cc. of $N/20$ sodium hydroxide in excess of that recommended by Clark to bring it to the desired reaction, while that from source C, which was red in color, required only 0.9 cc. Similar variations in the amount of alkali required by indicators from different sources to bring them to the desired reactions were also found for the other indicators.

The relative tinctorial powers of the same indicator from various sources are also given in the last column of Table III. These values were obtained by adding equivalent amounts of each indicator solution to similar amounts of a buffer solution whose P_H was near the middle of the transition interval and by comparing the relative strength of the color produced by means of a colorimeter. It will be noticed that there are marked differences in tinctorial power, which fact emphasizes the importance of using the same lot of indicator in making up solutions to be used for the standard buffers and the unknown solutions.

Errors Due to Method of Preparation or Reaction of Indicator Solutions

Soil extracts were prepared according to the collodion sac method,[13] and their hydrogen ion concentrations determined by using varying amounts of the variously adjusted indicator solutions. The hydrogen ion concentration of the soil suspensions was also determined by means of the hydrogen electrode. The data are reported in Table IV.

It will be noted that the P_H values obtained by the colorimetric method varied greatly depending on how the reaction of the indicators had been adjusted and on the number of drops of indicators added. In all cases there was no difference in the P_H values obtained when different amounts of the indicator solutions adjusted according to the varying drop method were used, but a considerable difference when an indicator solution adjusted according to the other methods was employed. As would be expected, the greater the difference between the reaction of the indicator solutions and that of the solution to be tested, the greater is the error; and the more improperly adjusted indicator solutions used, the greater is the error with these slightly buffered solutions.

The amount of indicator usually recommended is about 10 drops per 10 cc. of solution, which corresponds to 5 drops for 5 cc. Since 3 drops have been found sufficient to give good color differences, this amount is rec-

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[13] Pierre and Parker, *Soil Science*, **23**, 13 (1927).

TABLE IV

THE HYDROGEN ION CONCENTRATION OF SOIL EXTRACTS AND WEAKLY BUFFERED SOLUTIONS DETERMINED BY THE USE OF INDICATOR SOLUTIONS PREPARED BY VARIOUS METHODS

Extract of soil or slightly buffered soln.	Specific resistance of extract (23–25° C.) ohms	H-ion concn. (H electrode) P_H	Indicator		H-ion concn. obtained with indicators variously prepared			
					Methods of adjustment			
			Name	Drops per 5 cc.	Kolthoff,[a] P_H	Clark. P_H	Varying drop method. P_H	Neutrality method. P_H
Sandy muck	6195	3.99	BPB	3	3.85	4.05	4.05	4.10
				5	3.85	4.05	4.05	4.15
				8	3.80	4.05	4.05	4.25
Sassafras loam	10,280	4.61	BCG	3	4.45	..	4.60	4.80
				5	4.30	..	4.60	5.00
				8	4.15	..	4.60	5.05
Norfolk sandy loam	38,664	5.60	BCP	3	5.30	5.45	5.60	5.60
				5	5.15	5.45	5.60	5.70
				8	4.95	5.40	5.60	5.75
Greenville sandy loam	23,184	5.89	BCP	3	5.60	5.80	5.85	5.95
				5	5.40	5.75	5.85	6.00
				8	5.20	5.70	5.85	6.05
Norfolk sandy loam (washed)	45,140	5.60	BCP	3	<5.20	5.45	5.60	5.80
				5	<5.20	5.40	5.60	5.85
				8	<5.20	5.30	5.60	5.95
Distilled water	495,000	..	BCP	3	<5.20	5.20	5.60	5.85
				5	<5.20	<5.20	5.60	5.95
				8	<5.20	<5.20	5.60	6.05
Distilled water and NaOH	72,370	..	BTB	3	6.30	6.75	6.90	6.90
				5	<6.20	6.60	6.85	6.90
				8	<6.20	6.55	6.85	6.90

[a] Since the indicator solution made up according to Kolthoff is 2.5 times as concentrated as the others, 1, 3 and 5 drops were used instead of 3, 5 and 8, respectively. Due to the Kolthoff solution being made up in 20% alcohol, however, it took 38 drops as compared with 26 drops of the other solutions to make 1 cc. Therefore, 3 and 5 drops, respectively, are approximately equivalent to 5 and 8 drops, respectively, of the other solutions.

ommended as being less likely to cause errors from the use of unadjusted indicator solutions than would larger amounts. The data also prove definitely that the reaction of all indicators should not be adjusted to P_H 7.0 but rather to a P_H corresponding to about the middle of the transition interval. It will also be noted that the hydrogen ion concentration of the soil suspension determined with the hydrogen electrode checks well with that obtained by the colorimetric method, using indicators adjusted by the varying drop method.

The Storage of Indicator Solutions

The question as to how long indicator solutions can be stored has been raised by several investigators. Schlegel and Stueber found that the storage of brom thymol blue in ordinary glass bottles renders the solution alkaline due to the solution of alkali from the glass. They also point out that the coating of the inside of the bottles with paraffin may also lead to a change in the reaction of the stored solutions due to the fact that ordinary paraffin has a distinctly acid reaction. By using Pyrex glass bottles these authors. found that brom thymol blue solutions showed no changes in reaction even after prolonged periods of storage, provided the bottles were stoppered to exclude air. If, on the other hand, the bottles were frequently opened the indicator solutions were found to become progressively more acid. Both Stern and Kolthoff also found that neutralized indicator solutions turn somewhat acid on standing.

In order to obtain definite data regarding the effect on the hydrogen ion concentration of indicator solutions stored in different kinds of flasks, the following experiment was conducted. The four indicator solutions used in this study were stored in each of the following kinds of flasks: (1) paraffin coated flasks, (2) thoroughly weathered Pyrex flasks and (3) thoroughly weathered reagent bottles of ordinary glass. The bottles were weathered by heating in dichromate solution for a period of one hour, after which they were heated with successive portions of distilled water and thoroughly washed. The indicator solutions, the *P*H values of which had been determined, were placed in these bottles and the bottles stoppered. After a period of nine months the *P*H value of each was again determined. The results are given in Table V.

TABLE V

THE CHANGE IN HYDROGEN ION CONCENTRATION OF INDICATOR SOLUTIONS AFTER A NINE MONTH'S STORAGE IN VARIOUS KINDS OF FLASKS

Indicator	H-ion concn. before storage, *P*H	H-ion concn. after storage		
		In paraffin coated flasks, *P*H	In weathered Pyrex flasks, *P*H	In weathered reagent bottles of ordinary glass, *P*H
Brom cresol green	4.70	4.59	4.54	7.23
Brom cresol purple	5.50	3.92	4.05	6.51
Brom thymol blue	6.88	6.76	6.59	7.59
Phenol red	7.84	7.44	7.65	7.95

It will be seen that storage in ordinary glass bottles decreases the hydrogen ion concentration of all the solutions materially, due no doubt to the solution of the glass. Storage in Pyrex or paraffined flasks rendered all the solutions more acid. In the case of the solutions of brom cresol green, brom thymol blue and phenol red the change was small, but a large increase in acidity resulted with the brom cresol purple indicator solution. It is also probable, as pointed out by Schlegel and Stueber, that if the flasks had been opened from time to time a greater increase in acidity would have been noted. The importance, then, of determining and adjusting the reaction of indicator solutions stored even in Pyrex bottles becomes evident if these indicators are to be used with slightly buffered solutions. This is especially true with the brom cresol purple indicator solution.

Conclusion

From these data it is evident that in order to obtain accurate results with slightly buffered soil extracts or unbuffered solutions of any kind, the indicator solutions should be adjusted to a hydrogen ion concentration corresponding to a *P*H near the middle of the transition interval. While most soil extracts and most of the various kinds of solutions tested by the colorimetric method are well enough buffered not to be affected by the use of indicator solutions made up according to Clark, the proper adjustment of indicator solutions is no doubt of considerable importance in many lines of work where the hydrogen ion concentration of slightly buffered solutions is determined. The old practice, for example, of diluting strongly colored solutions until the color or turbidity no longer interferes, results in slightly buffered solutions which upon testing with unadjusted indicators may give erroneous results. Schlegel and Stueber have shown the importance of properly adjusted indicator solutions for use in sugar refinery practice. Erroneous results would undoubtedly also be obtained in determining the *P*H of many natural waters or in *P*H control work in water purification.

Regarding the methods of adjustment, two simple methods are available; the varying drop method and the quinhydrone titration method. The latter is very rapid and convenient but requires the electrical apparatus necessary for electrometric determinations. The former, on the other hand, can be used without this equipment, is simple and gives accurate results.

Summary

A critical study was made of the various methods used for preparing and adjusting the reaction of indicator solutions and a simple method, proposed by the authors, is described. The use of the quinhydrone electrode was also found satisfactory for this purpose. Data are presented on the *P*H of indicator solutions adjusted by the various methods and on the effect of using such solutions in determining the *P*H of weakly buffered soil extracts and other solutions. It is shown that the use of indicator solutions made up without neutralization, of solutions adjusted to *P*H 7.0 and of those made up according to Clark give erroneous results with slightly buffered solutions. For such solutions the indicator solutions are shown to require adjustment to a *P*H corresponding to that of about the middle of the transition interval.

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