

**A STUDY OF THE RELATIVE VALUE OF LACMOID,
PHENACETOLIN AND ERYTHROSINE AS IN-
DICATORS IN THE DETERMINATION OF
THE ALKALINITY OF WATER BY
HEHNER'S METHOD.**

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I^N the determination of the alkalinity of water for sanitary and technical purposes, Hehner's method offers theoretically as scientific and accurate a process as could be desired. The accuracy of the method as it is usually carried out is lessened, however, by the character of the indicators which one is obliged to employ. The color changes of these indicators are unfortunately rather indistinct and therefore introduce into the determination the personal equation of the operator.

The titration with sulphuric acid of the calcium and magnesium carbonates dissolved in water, as this method directs, necessitates the use of indicators which either are not susceptible to the carbonic acid liberated in the reaction, or to its removal as soon as formed, when using indicators which are sensitive to this acid.

Quite a number of indicators have been advocated by different writers and for the most part they fail to agree as to the one best suited for this determination. Hehner¹ in his original notes upon the method recommends the use of phenacetolin. Thomson² prefers lacmoid and considers phenacetolin indistinct in its color changes, requiring too much skill for its successful use by the ordinary analyst. Draper³ considers either lacmoid or carminic acid as well suited for this determination, but favors the latter indicator as being somewhat less susceptible to carbonic acid. Methyl orange has also been employed by some analysts with considerable success.

Erythrosine⁴ was first proposed as an indicator by Mylius⁵ and Foerster, but the writer is not aware that it has ever been com-

¹ *Analyst*, 8, 77.

² *Chem. News*, 47, 123, 185.

³ *Ibid.*, 51, 206.

⁴ Sutton's Volumetric Analysis, §14, p. 39.

⁵ *Ber. d. chem. Ges.*, 24, 1482; also *Chem. News*, 64, 228.

monly used for this determination, and as its use requires a somewhat different procedure from that employed with the ordinary indicator, it will be described in detail beyond.

Having occasion to employ Hehner's method for a long series of alkalinity determinations, a number of experiments were undertaken with a view to determine the relative value of the three indicators, lacmoid, phenacetolin, and erythrosine, and if possible to fix definitely upon the color change which corresponded with the completion of the reaction. Considerable attention was paid to the technique of the titration, and especially to the improvement of the original method of Mylius and Foerster when erythrosine was employed as an indicator. A detailed description of the process as carried out with the several indicators may therefore be of interest.

SOURCE, COMPOSITION, AND PREPARATION OF THE THREE INDICATORS.

Lacmoid and phenacetolin, as described by Sutton and other writers, are prepared as follows :

Lacmoid is derived from resorcin by heating with sodium nitrite at a temperature of 110° to $120^{\circ}\text{C}.$, dissolving the product in aqueous ammonia and reprecipitating with hydrochloric acid. It is then washed free from acid and dried. The varying shades of color of the alcoholic solution of different samples of this substance are probably due to the thoroughness with which the last traces of acid are removed. For titration work a solution is prepared by dissolving two grams of the substance in one liter of fifty per cent. alcohol.

Phenacetolin is prepared by boiling together for several hours phenol, acetic anhydride, and sulphuric acid. The resulting product is washed with water to free from acid, and dried. Two grams of the substance are dissolved in one liter of strong alcohol for use as an indicator.

Erythrosine or iodeosine is a product of resorcin-phthalein or fluorescein. The halogen substitution-products of the latter form a series of fluorescing dyes among which is the well-known dye eosine. Eosine, the potassium salt of tetra-bromo-fluorescein ($\text{C}_{20}\text{H}_6\text{Br}_4\text{O}_5\text{K}_2$), has its counterpart in the iodine substitution-compound tetra-iodo-fluorescein or erythrosine ($\text{C}_{20}\text{H}_6\text{I}_4\text{O}_5$).

Mylius and Foerster, who made an extended study of this substance, describe the commercial product, which is used in cotton printing and paper staining, as a brick-red powder soluble without fluorescence, but with a rose color in dilute alkalis. The above investigators purified their product for the purposes of their experiments, but the sample of dye used in this work showed no impurities which in any way interfered with the experiments undertaken and it was therefore used just as it was obtained on the market.

The sample employed in the writer's experiments was apparently the sodium salt of erythrosine. It was practically insoluble in ether and chloroform, but soluble in water with a slight fluorescence, the latter property increasing with the dilution, and slightly soluble in aqueous ether. An aqueous solution of erythrosine containing one-tenth gram in one liter of water is prepared for use as an indicator.

TECHNIQUE OF THE DETERMINATION WITH THE SEVERAL INDICATORS.

Lacmoid.—One hundred cc. of the solution to be tested are introduced into a six-inch porcelain evaporating dish, and placed on a stand over a free flame. The liquid is heated until bubbles begin to break at the surface and then the dish is quickly removed. The temperature of the water when titration commences should be about 75° C. One-half cc. of the indicator solution is next added and then sulphuric acid (fiftieth-normal) until the solution shows no change in color as a drop of the acid strikes the surface of the liquid and sinks to the bottom of the dish. After the addition of the acid drop by drop toward the end of the titration, careful stirring should follow as the diffusion does not appear to be very rapid. This gives a uniform reddish or purple color to the liquid which becomes more marked immediately under and around the acid as it sinks into the water.

Phenacetolin.—The preliminary portion of the operation with this indicator is the same as for lacmoid. One-half cc. of the indicator is added and the titration carried on as the preceding one until no change in color occurs immediately about a single drop of the acid as it strikes the surface of the liquid and sinks to the bottom of the dish.

Erythrosine.—Mylius and Foerster in their study of erythrosine prepared an aqueous ether solution of iodeosine for their indicator solution. This indicator when shaken with the alkaline solution to be tested imparts a rose color to the latter. A slight excess of acid, upon neutralization of the alkali, discharges the rose color, leaving the aqueous solution colorless.

The disadvantages arising from this ethereal solution led the writer, after considerable experimenting, to abandon its use and in its place was substituted an aqueous solution of the erythrosine. Beside this, chloroform was employed in the titration, the latter acting in a manner similar to the aqueous ether. As a result of this experimental work the following modified process was devised.

The titration is made in a vessel which can be shaken and preferably in a 250 cc. white-glass flat-stoppered bottle. One hundred cc. of the water are placed in the bottle with two and five-tenths cc. of the aqueous solution of the erythrosine and five cc. of chloroform. They are well shaken together and the acid added in small quantities, followed each time by a thorough shaking of the bottle. The rose color of the water toward the end of the titration becomes less marked and upon the addition of a very slight excess of acid the color disappears completely. The chloroform disseminated through the liquid produces a milky appearance on account of the frequent shaking; but this is no especial hindrance to determining the end-point, although one can wait until the chloroform¹ settles to the bottom of the bottle if desired. A white paper held at the back of the bottle facilitates the detection of any trace of color remaining as the titration approaches the end-point.

For the calculation of results the value of the approximate fiftieth-normal sulphuric acid, standardized against a fiftieth-normal sodium carbonate solution, is first determined and the amount of acid used up by the water tested is expressed in terms of calcium carbonate. In accurate work it is advisable for reasons which will be pointed out later, to employ a correction for the indicator. This may be done by using a measured volume of the indicator and determining the amount of acid required to effect the color change in a blank determination with distilled

¹ The chloroform residues may be saved, washed with dilute potassium hydroxide solution and then with water. The chloroform may then be distilled and used again.

water. The volume of acid thus found is deducted from all readings before converting them into terms of calcium carbonate.

THEORY OF THE ACTION OF THE SEVERAL INDICATORS AND A DISCUSSION OF THE END-POINTS.

The action of the different indicators briefly considered from the standpoint of the present theory of solution will perhaps bring out the reason for certain difficulties connected with their use.

Lacmoid and phenacetolin are moderately acid indicators which are intermediate between methyl orange, a rather strongly acid indicator, and phenolphthalein, a weakly acid indicator. In the presence of the alkaline carbonates of lime and magnesia, the ionized lime and magnesia salts of the indicator used are first formed. Upon adding a strong acid like sulphuric, a fairly sharp color change is obtained when the carbonates present have become neutralized. This color change is the result of the action of the free hydrogen ions, in the slight excess of acid added, upon the dissociated salt of the indicator. The change from one color to another is coincident with the conversion of the dissociated salt of the indicator, with the color due to its free complex ion, to the acid form of the indicator, having in its non-ionized or acid form another color or as in the case of phenolphthalein, none at all.

The reason for the indistinct color changes in the case of lacmoid and phenacetolin is that the non-ionized molecules of both these indicators are colored themselves, in the case of lacmoid a red or purple and in that of phenacetolin a yellow. The ionized salts are blue and pink respectively. The passage of the blue to the purple in the case of lacmoid and the pink to the yellow in the case of phenacetolin, is somewhat confusing to the eye on account of the mingling and blending of the colors. In this way the end-point is rendered less sharp than it would be if the non-ionized molecules of the two indicators were colorless as in the case of phenolphthalein. Like methyl orange they are more or less dissociated in solution, even in the absence of bases and the colors of the complex ions become apparent. For this reason the amount of indicator added in the titration, especially in using dilute acid solutions, should be carefully controlled, and in very

accurate work measured. The amount of acid necessary to bring about the requisite color change can then be carefully determined in a blank experiment.

Another difficulty which arises in the use of lacmoid and phenacetolin is their susceptibility to carbonic acid. As this is formed in the course of the reaction between the sulphuric acid and the carbonates of the bases present, it must be removed by heating the solution. While carbonic acid is one of the very weak acids, yet its acid character is appreciably greater than that of the indicators in question and therefore interferes with the true end-point, unless it is practically all eliminated from the solution as soon as formed.

To consider the end-points with lacmoid and phenacetolin from a practical point of view in titrating for carbonates in water, the question may very properly be asked, When is the neutralization of the carbonates complete? It seems to the writer that the unsatisfactory results obtained with this process are largely due to a lack of knowledge of the true end-point. Obviously the only means of fixing this point is by working with known solutions of sodium, calcium, and magnesium carbonates and checking them against each other.

As has been stated in a preceding paragraph the true end-point determined by working with known solutions is, in the case of lacmoid, the point where no change in color can be detected as a drop of the acid enters the liquid. The change in the color of the whole liquid should properly have ceased to be appreciable for three or four drops, possibly, preceding the true end-point, but if very carefully watched a drop of the acid, as it enters and sinks to the bottom of the dish, will produce about it a brighter reddish or purple color than that of the surrounding liquid. When this phenomenon can no longer be detected the titration is complete.

The same conditions hold true for phenacetolin, and the golden color produced about a drop of the acid as it sinks to the bottom of the dish should be evidence of the incompleteness of the titration and acid should continue to be added until this color change is no longer produced. These end-points require care and skill and some practice in order to be recognized, but these

are difficulties which are not so serious as to bar the use of these indicators in accurate work.

Erythrosine on account of the peculiar manner in which it is used demands a somewhat different explanation of its action. Mylius and Foester state the following facts regarding erythrosine, many of which have been confirmed by my own experiments :

Iodeosine is soluble to a slight degree in water with some rose color, the same as with its solution in alkalis. The red color, of an aqueous alkaline solution of the dye remains even after addition of quite an excess of acid, hence its unsuitability for use directly as an indicator. By adding a strong acid to an aqueous solution of the dye an orange yellow precipitate is formed called by them the 'anhydrous iodeosine'. The red color in solution, they state, is produced by formation of a hydrate, as in evidence they say the more water added to an alcohol or acetone solution, the redder the solution becomes.

The red solution of iodeosine hydrate in water may be decolorized by an excess of ether, although aqueous ether is more of a solvent for the hydrate than the water. Yet water will take up color when shaken with an ethereal solution of iodeosine.

Mylius and Foester evidently intended to explain the facts stated above by the theory of the formation of hydrates in solution, but they pointed out, however, that the phenomena could also be explained by the electrolytic theory of dissociation. It would seem a more plausible explanation of the phenomena to associate them with the latter theory and after a somewhat fuller explanation than that previously given of what takes place when using erythrosine as an indicator, the phenomena in question will be considered from that point of view.

The addition of the aqueous solution of erythrosine to a water, containing alkali in solution, intensifies the color quite a little. The deep rose color remains fairly bright until nearing the end of the titration when it fades away, becoming a rather faint orange. With a slight excess of acid, however, this disappears also. The chloroform, which at first was colorless, shows a brownish tint at the end of the titration and the water solution above appears colorless.

It would seem then that the chloroform, or ether as Mylius and

Foester used, is an essential part of the process, since without it no sharp end-point is possible. Erythrosine is evidently a rather strongly acid indicator from the fact that to an alkaline solution a large excess of acid must be added in order to form the non-ionized iodeosine molecule. In aqueous solution without the presence of alkaline bases it is also, to a certain extent, in the ionic condition, as shown by the red color of the solution due to the free complex ion. If, however, ether or chloroform are present, when using erythrosine as an indicator, the greater solubility of the substance in these solvents cause it to be dissolved out of the aqueous solution as soon as its non-ionized molecule is formed. When thus removed the neutralization of the alkali is immediately apparent and therefore a sharp end-point is obtained.

In an ether or chloroform solution the iodeosine is probably not dissociated at all and so only the color of its non-ionized molecule is apparent. In an alcohol solution an appreciable dissociation results which increases as the proportion of water to alcohol increases.

The phenomena stated by Mylius and Foester regarding the decolorization of aqueous iodeosine solutions with a large excess of ether and the imparting of color to water by ethereal solutions of iodeosine, can probably be explained by the laws relating to the extraction of a dissolved substance from one solvent by means of another, the solvents being non-miscible, and the extent of ionization and non-ionization of the substance in the two solvents.

The readiness with which the chloroform takes up the erythrosine in its non-ionized condition from the water, as soon as it is formed, is undoubtedly explained in a similar manner. The principle involved, it would seem, might be applied to other substances capable of acting as indicators and thus materially increase the number of compounds which could be used in this capacity. Carbonic acid under normal conditions has no appreciable effect on this indicator, thus placing it beside methyl orange in this respect, although in its chemical composition it differs very little from phenolphthalein, which is extremely sensitive to carbonic acid.

COMPARISON OF THE THREE INDICATORS IN TITRATION OF THE CARBONATES OF SODA, LIME, AND MAGNESIA.

In order to fix definitely upon the proper end-point when using lacmoid or phenacetolin as indicators and also to compare the results obtained with those when employing erythrosine, a series of titrations were made using known solutions of the carbonates of sodium, calcium, and magnesium. The solutions were prepared with considerable care and the amount of the salt dissolved determined gravimetrically. The titration work was carried on by two different observers. All the titrations were made in a volume of 100 cc. The amount of carbonate present was varied in the lime solutions, three dilutions being used, while with the soda and magnesia only one was employed.

The figures in the following table refer to the number of cubic centimeters of sulphuric acid required to neutralize five cc. of a fiftieth-normal sodium carbonate solution, after diluting to one hundred cc. with distilled water and titrating with the aid of the different indicators in the manner previously described.

STANDARDIZATION OF THE SULPHURIC ACID AGAINST A FIFTIETH-NORMAL SODIUM CARBONATE SOLUTION.

	Lacmoid.	Phenacetolin.	Erythrosine.
Mean of ten results	5.04	5.06	5.15
Maximum	5.20	5.10	5.20
Minimum.....	5.00	5.00	5.10
Percentage strength of acid in terms of fiftieth-normal sulphuric acid.....	99.1	98.8	97.0†

The correction for each indicator, which has been deducted in the above table, is as follows :

One-half cc. of the lacmoid solution requires four-tenths cc. of the sulphuric acid to produce the end-point ; five-tenths cc. of the phenacetolin solution requires 0.25 cc. of acid and two and five-tenths cc. of the erythrosine solution with five cc. of chloroform requires two-tenths cc. of acid.

SUMMARY OF RESULTS OBTAINED WITH KNOWN SOLUTIONS OF CALCIUM CARBONATE.

Group I.

Calcium carbonate.	Lacmoid.	Phenacetolin.	Erythrosine.
Parts present in solution ¹	67.8	67.8	67.8
Parts found, average of fifteen results	67.6	66.9	65.8
Percentage found of amount present	99.7	98.7	97.0

¹ Amounts expressed in parts per million.

Group II.

	Lacmoid.	Phenacetolin.	Erythrosine.
Parts present in solution.....	40.7	40.7	40.7
Parts found, average of fifteen results	40.0	40.2	40.4
Percentage found of amount present	98.2	98.7	99.2

Group III.

Parts present in solution.....	27.1	27.1	27.1
Parts found, average of fifteen results	26.4	26.3	26.8
Percentage found of amount present	97.4	97.0	98.8
Percentage found, average of forty-five results	98.4	98.1	98.3
Maximum number of parts variation from amount present.....	3.0	3.0	1.5

SUMMARY OF RESULTS OBTAINED WITH A KNOWN SOLUTION OF MAGNESIUM CARBONATE.

Amount of MgCO₃ Present in all Titrations = 36.5 parts.

Magnesium carbonate.	Lacmoid.	Phenacetolin.	Erythrosine.
Parts found, average of five results.	35.9	35.9	36.2
Percentage found of amount present	98.3	98.3	99.1
Maximum number of parts variation from amount present	1.0	1.0	0.5

From the preceding data it is quite apparent that any one of the indicators offers but little superiority over either of the other two. Erythrosine gives more uniform results and a larger percentage of the carbonates actually present with low amounts than with high amounts, while the reverse seems to be true of lacmoid and phenacetolin.

The only conclusion which can be drawn from the above data is that with a knowledge of the true end-point with lacmoid and phenacetolin and with proper care and skill all of the three indicators are suitable for Hehner's method, and that, if uniformity of results within very narrow limits is desired, the preference should be given to erythrosine. The average error of determination when employing any one of these three indicators ought not in good work to exceed ± 1.5 per cent.

Thomson has very fully described the bases capable of being determined with lacmoid and phenacetolin and the salts interfering with their action. Mylius and Foester made use of erythrosine for the study of the neutrality of salts, and certain facts which they brought out are of interest in connection with this determination.

The alkalies, alkaline earths, salts of cadmium, manganese, nickel, and cobalt are alkaline to erythrosine. The oxygen salts of iron, aluminum, and chromium behave like free acids to this indicator. It is not suitable for the titration of organic acids; and even oxalic acid cannot be used. Ammonia, however, may be titrated with it. Erythrosine may be used with fairly turbid waters and also with colored waters. In this respect it is much better than lacmoid or phenacetolin, both of which tend to give too high results with such waters.

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SOME NOTES ON THE ESTIMATION OF CARBOHYDRATES.

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IN common with agricultural chemists in many parts of the country we have been interested in the direct determination of the carbohydrates of grains, foods, etc., in place of the method of subtracting from one hundred all other constituents and calling the remainder " nitrogen-free extract."

It seemed to us, as it was necessary to take advantage of the cupric reducing power for the estimation of the carbohydrates or their hydrolyzed products, that the first matter of importance in studying these methods was a determination of the simplest means for estimating the quantity of copper precipitated.

For the determination of copper electrolytically, if many determinations are to be made at a time, the platinum apparatus becomes very expensive. If for this method, which when properly conducted gives excellent results, we can substitute a volumetric method giving equally good results, it would seem desirable to do so. A volumetric method for the estimation of the precipitated cuprous oxide is much to be preferred to any gravimetric method, provided that it gives results of the same degree of accuracy. We offer the following method which in our hands has given excellent results :

100 cc. of Fehling's solution are diluted with 100 cc. of water and heated in a boiling water-bath. A known amount of sucrose or starch contained in carefully analyzed samples which are as nearly as possible like the substance under analysis, is inverted.