

PERMANENT STANDARDS FOR THE DETERMINATION OF
HYDROGEN-ION CONCENTRATION.

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The advantages claimed for the colorimetric over the electrometric method of p_H determination are simplicity and rapidity of manipulation. This, however, is true only if all the buffer and indicator solutions are at hand: but the difficulty lies in the fact that these solutions are unstable, some deteriorating within three weeks. They take rather considerable time to prepare properly; the NaOH solution is especially difficult to prepare, and must be stored in paraffin-lined bottles. For accurate work these solutions should be checked against the hydrogen electrode. Various methods have therefore been devised for obtaining permanent sets of standards for the different indicators. But the majority of these are merely relatively permanent.

The standards which are found on the market to-day as part of p_H testing sets or comparators consist of buffer solutions containing the various indicators and kept in sealed ampoules under sterile conditions. They are guaranteed by the manufacturers for not more than six months, as the indicator dyes slowly fade in these solutions. In fact, color standards below p_H 3.0 are not included in these sets since they deteriorate in a very short time.

Haskins (1) has prepared standards which he claims do not fade for nine months. They are made with amaranth and paranitrophenol, dyes which are but slightly affected by the buffers in which they are placed. His range of standards, however, is limited to p_H 4.5-7.5, corresponding to colors produced by methyl red, rosolic acid and neutral red. Another disadvantage of his standards is that they are prepared in uneven increments, varying from 0.1 to 0.45 p_H .

Clark (2) has prepared a color chart for eight of his indicators, but due to inherent differences in printed colors and colored solutions, only rough comparisons can be made. It is in fact merely intended as an aid in establishing a color memory.

Gillespie (3) has devised a drop-ratio method for two-color indicators based upon a dissociation curve and not involving the use of buffers. His standards, however, are not permanent for more than three months.

Michaelis, Gyemant and Krüger (4) have a somewhat similar method for one-color indicators. Since, however, their alkaline solutions are unstable, Windisch (5), and later Kolthoff (6) have used potassium chromate and dichromate as comparison solutions. Although these solutions are permanent, the method itself has never found favor in routine testing since it is open to serious error in the hands of workers who are not thoroughly familiar with the theory involved.

A device based upon the principles of Gillespie's method is the double wedge colorimeter of Bjerrum (7), later improved by Kolthoff (8). This consists of two transparent wedges cemented together with Canada balsam, one filled with the fully transformed indicator in alkaline solution, the other with the indicator in acid solution. By means of a screen, lamp and scale the entire transitional range of the indicator may be compared with the solution to be tested. The alkaline solution, however, fades after a few months.

The method which has been almost universally adopted because of its simplicity is that in which a suitable indicator of definite concentration is added to a

specific volume of the solution under examination, and the color obtained is directly compared with a series of standards. It is with this method that the author was primarily concerned in the search for permanent standards.

Thus far there have been but two types of permanent standards used—colored glasses, and stable inorganic salt solutions. Sordén (9) was among the first to use colored glasses for p_H standards. In 1924, Badollet, Hamilton and Walton, Jr. (10) patented a method for obtaining permanent colors in glass. There are, however, two disadvantages in the use of glass standards—one is the difficulty of standardization; the other is the error involved in light absorption, due to the difference in thickness and in the nature of the material in glass and solutions.

Kolthoff (11) has prepared blends of ferric chloride and cobalt nitrate solutions, matching the colors produced by neutral red, methyl orange, methyl red and tropeolin OO. He has not, however, attempted to prepare a complete set of standards, leaving gaps between p_H 4.5–5.2 and 5.8–7.0. His increments are rather uneven, varying from 0.02 to 0.3 p_H .

It was the author's desire to secure a complete set of standards for the Clark and Lubs' indicators since these are so widely used. The difficulty lay in obtaining inorganic salt solutions which could be blended to obtain the deep reds and blues of the brilliant sulphophthalein indicators. However, by using small amounts of the indicator solutions, a sufficient number of hues were matched to obtain a complete series of standards for the range 1.2–9.0 p_H , allowing for a convenient degree of overlapping.

The standard solutions used were the Army (12) "Co-Fe-Cu" fluids. These consist of three solutions which can easily be standardized volumetrically: a $N/2$ cobalt chloride, containing 59.497 Gm. $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ per liter of 1% HCl; a $N/2$ ferric chloride, containing 45.054 Gm. $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ per liter of 1% HCl; and a $N/2$ cupric chloride, containing 42.630 Gm. $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ per liter of 1% HCl. In connection with the copper solution it might be added that the original solution was a $N/2$ copper sulphate, but in order to have all the anions alike, the copper chloride was substituted. Fortunately, the color values of the $N/2$ chloride and sulphate solutions are identical, so that this change in the standard solutions has no effect upon our color researches of the past.

As to permanency, these solutions have been found to stand up without change for more than ten years. The HCl in the concentration present, about $N/4$, acts as a preservative, but has no effect upon the color value of the three solutions. A separate investigation has shown that concentrations of HCl up to $N/2$ may be used. Above this concentration the colors change in shade and intensity.

In selecting the indicators, those recommended in the International Critical Tables (13), (1926) were adopted with a few exceptions. Bromcresol purple and bromphenol blue were not used because of their marked dichroic effects (*viz.*, their property of exhibiting different colors in different light, due to the difference in the quantity and intensity of the light absorbed). The bromphenol blue range was covered by methyl orange, and the bromcresol purple range by chlorphenol red.

In preparing the indicator solutions some workers prefer alcoholic solutions and others aqueous solutions in which the acidity of the dye has been exactly neutralized. The advantage of alcohol is of course protection against mold

growth and ease and rapidity of solution, but the use of unneutralized dye may cause serious error in the determination of p_H in non-buffered solutions. In some cases the alcohol may cause precipitation of material in solution.

The only disadvantage of the neutralized aqueous indicator is its tendency toward mold formation. The author has been able to avoid this by using a 20% alcoholic solution of the dye to which enough standard alkali has been added for neutralization. As but 0.2–0.5 cc. of this indicator solution is used in the p_H determination, the slight amount of alcohol has practically no effect upon the solution examined.

As to the concentration of indicator used, there seems to be no uniformity, most workers using both different percentages of dye and different amounts of indicator solution. With a view toward standardization, the 0.04% strength directed in the International Critical Tables (14) was adopted, the amount used in each case being dependent upon the color intensity of the indicator.

All of the indicators used were two-colored. Their advantage is that there is a change of shade with every shift in p_H , while in the one-colored indicator a change in p_H is accompanied by a change in intensity of the color. The advantage is obvious when one considers that a worker is using his own indicator solution, but basing his comparisons upon standards worked out by someone else. A slight difference in the dyestuff, concentration of dye, or in the amount used, which is very probable when but 0.2 to 0.5 cc. is employed, will not have any material effect upon the shade of a two-color indicator, but will have a decided effect upon the intensity of a one-color indicator.

In the following table is listed the amount of standard alkali used for neutralizing the acidity of the dye. A 0.04% solution is used throughout. 0.1 Gm. of the dye is dissolved in 52 cc. of neutral alcohol (95%), the designated amount of alkali added, and the solution made up to 250 cc. with distilled water. It is advisable to distil both the alcohol and the water over barium hydroxide.

TABLE I.

Indicator.	Cc. of N/100 NaOH.
Metacresol Purple (acid and alkaline range).....	26.5
Thymol Blue (acid and alkaline range).....	21.5
Methyl Orange (sodium salt of helianthin).....
Bromocresol Green.....	14.5
Methyl Red.....	37.0
Chlorphenol Red.....	23.5
Bromothymol Blue.....	16.0
Phenol Red.....	28.5
Cresol Red.....	26.3

In the following tables, Lovibond values of both the buffer standards and corresponding "Co-Fe-Cu" blends are given for comparison. In some cases slight differences in color matches will be noted. This is due to the fact that the Lovibond determinations were made by reflected light while the actual comparisons of buffer standards and blends were made by direct light, which is the usual method employed for colorimetric p_H determinations

The Lovibond glasses were of the following series: Yellow—No. 510; Blue—No. 1180; Red—No. 200. All readings were made in 1/2-inch glass cells, by clear northern daylight.

The "Co-Fe-Cu" standard blends may be preserved in colorless 1/2-ounce g. s. french square bottles. These may be procured of even dimensions, and the solution to be tested may be directly matched against the standards in these bottles.

In all cases the specified amount of indicator solution is added to 10 cc. of the solution under test. Comparisons are made at 20° C.

TABLE II.—METACRESOL PURPLE (*m*-CRESOLSULPHONPHTHALEIN) (ACID RANGE).

Use 0.3-cc. indicator.

p_H .	Lovibond readings of buffer standards.			Matching blends (cc.).				Lovibond readings of blends.		
	R.	Y.	B.	Co.	Fe.	Cu.	H ₂ O.	R.	Y.	B.
1.2	8.4	0.4	...	9.0	...	1.0	...	8.3	0.6	...
1.4	6.4	0.7	...	6.5	0.1	...	3.4	6.4	0.8	...
1.6	5.6	1.2	...	5.5	0.2	...	4.3	5.6	1.3	...
1.8	4.7	1.7	...	4.4	0.5	...	5.1	4.8	1.7	...
2.0	4.2	2.3	...	4.1	1.3	...	4.6	4.3	2.4	...
2.2	3.2	3.0	...	2.8	2.1	...	5.1	3.3	3.0	...
2.4	2.7	3.5	...	2.3	2.7	...	5.0	2.7	3.6	...
2.6	2.1	4.0	...	1.7	3.3	...	5.0	2.0	4.0	...

TABLE III.—THYMOL BLUE (THYMOLSULPHONPHTHALEIN) (ACID RANGE).

Use 0.5-cc. indicator.

p_H .	Lovibond readings of buffer standards.			Matching blends (cc.).				Lovibond readings of blends.		
	R.	Y.	B.	Co.	Fe.	Cu.	H ₂ O.	R.	Y.	B.
1.6	5.3	0.4	...	5.3	4.7	5.3	0.4	...
1.8	4.4	1.2	...	3.9	0.3	...	5.8	4.4	1.2	...
2.0	3.7	1.6	...	3.2	0.8	...	6.0	3.8	1.6	...
2.2	2.3	2.4	...	2.2	1.8	...	6.0	2.2	2.5	...
2.4	2.1	2.8	...	1.9	2.2	...	5.9	2.1	2.8	...
2.6	1.9	3.4	...	1.6	2.7	...	5.7	1.9	3.3	...
2.8	1.6	3.6	...	1.3	3.0	...	5.7	1.5	3.5	...

Standard for p_H 1.2-1.4 were too deep red to be matched.

TABLE IV.—METHYL ORANGE (SODIUM *p*-BENZENESULPHONATE-AZO-DIMETHYLANILINE).

Use 0.3-cc. indicator.

p_H .	Lovibond readings of buffer standards.			Matching blends (cc.).				Lovibond readings of blends.		
	R.	Y.	B.	Co.	Fe.	Cu.	H ₂ O.	R.	Y.	B.
3.0	8.4	2.5	...	8.1	0.3	...	1.6	8.4	2.4	...
3.2	8.0	3.2	...	7.5	0.6	...	1.9	8.0	3.2	...
3.4	6.6	4.4	...	6.5	1.0	...	2.5	6.6	4.3	...
3.6	5.8	5.4	...	5.8	1.9	...	2.3	5.7	5.3	...
3.8	5.0	6.8	...	4.8	2.9	...	2.3	5.0	6.8	...
4.0	4.4	7.2	...	4.0	4.0	...	2.0	4.5	7.2	...
4.2	4.1	7.8	...	3.4	5.0	...	1.6	4.1	7.9	...
4.4	3.4	8.2	...	2.8	5.8	...	1.4	3.3	8.2	...

Although not one of the Clark and Lubs' indicators, methyl orange was included, because of its freedom from dichromatism, in place of bromophenol blue. It is recommended by Kolthoff (15) as a useful indicator, having but a slight salt error.

TABLE V.—BROMCRESOL GREEN (TETRABROM-*m*-CRESOLSULPHONPHTHALEIN).

Use 0.3-cc. indicator.

p_H .	Lovibond readings of buffer standards.			Matching blends (cc.).				Lovibond readings of blends.		
	R.	Y.	B.	Co.	Fe.	Cu.	H ₂ O.	R.	Y.	B.
3.8	0.2	2.0	0.1	0.3	2.2	0.5	7.0	0.2	1.9	0.1
4.0	0.2	1.9	0.5	0.6	1.8	1.8	5.8	0.2	1.9	0.5

TABLE V.—Continued.

4.2	0.2	1.9	1.0	0.7	1.6	3.0	4.7	0.2	2.0	0.9
4.4	0.2	1.8	1.9	0.9	0.8	5.1	3.2	0.2	1.8	1.9
4.6	0.2	1.6	2.3	1.1	0.5	7.0	1.4	0.2	1.6	2.3
4.8	0.1	1.6	3.4	0.9	0.3	8.8	...	0.1	1.7	3.4
5.0	...	1.5	3.7	0.5	0.2	9.3	1.5	3.6

This is one of the comparatively recent dyes synthesized by Cohen (16); it is particularly free from dichromatism. Kolthoff (17) prefers to call it bromcresol blue since the name usually applied is that of the extreme alkaline color.

The standards for p_H 5.2–5.6 were too deep blue to be matched.

TABLE VI.—METHYL RED (*o*-CARBOXYBENZENE-AZO-DIMETHYLANILINE).

Use 0.2-cc. indicator.

p_H .	Lovibond readings of buffer standards.			Matching blends (cc.).				Lovibond readings of blends.		
	R.	Y.	B.	Co.	Fe.	Cu.	H ₂ O.	R.	Y.	B.
4.8	9.6	0.6	...	9.8	...	0.2	...	9.6	0.8	...
5.0	6.3	1.6	...	5.9	0.3	...	3.8	6.4	1.6	...
5.2	5.4	2.0	...	5.0	0.7	...	4.3	5.5	2.1	...
5.4	4.2	3.7	...	3.7	2.3	...	4.0	4.3	3.8	...
5.6	3.3	4.0	...	2.9	2.8	...	4.3	3.4	4.0	...
5.8	2.1	5.2	...	1.9	4.0	...	4.1	2.0	5.2	...
6.0	1.5	5.7	...	1.4	5.3	...	3.3	1.5	5.6	...

Although this indicator fades and is being replaced by chlorphenol red and bromcresol green, it was included since it is still used in many biological laboratories. Most workers prefer a straight alcohol solution of the dye because of the extreme difficulty of making an aqueous solution. By following the directions for preparing indicator solutions given above no such difficulty is encountered, and a neutralized indicator is obtained which gives more accurate results in non-buffered solutions.

Standards for p_H 4.4–4.6 were too deep red to be matched.

TABLE VII.—CHLORPHENOL RED (DICHLOROPHENOLSULPHONPHTHALEIN).

Use 0.5-cc. indicator.

p_H .	Lovibond readings of buffer standards.			Matching blends (cc.).				Lovibond readings of blends.		
	R.	Y.	B.	Co.	Fe.	Cu.	H ₂ O.	R.	Y.	B.
5.0	0.8	3.4	...	0.8	3.8	...	5.4	0.8	3.4	...
5.2	1.0	2.9	...	0.9	3.3	...	5.8	1.0	2.9	...
5.4	1.2	2.4	...	1.1	2.4	...	6.5	1.2	2.4	...
5.6	1.6	1.9	...	1.4	1.9	...	6.7	1.6	1.9	...
5.8	1.8	0.9	...	1.8	1.0	0.1	7.1	1.9	1.0	...
6.0	2.1	0.4	0.1	2.1	0.2	0.4	7.3	2.1	4.2	0.1
6.2	4.2	0.2	1.0	5.0	...	5.0	...	4.2	0.3	1.0

Standards for p_H 6.4–6.6 were too deep red to be matched.

TABLE VIII.—BROMTHYMOL BLUE (DIBROMTHYMOLSULPHONPHTHALEIN).

Use 0.3-cc. indicator.

p_H .	Lovibond readings of buffer standards.			Matching blends (cc.).				Lovibond readings of blends.		
	R.	Y.	B.	Co.	Fe.	Cu.	H ₂ O.	R.	Y.	B.
6.0	0.2	1.7	0.1	0.2	3.1	0.3	6.4	0.2	1.8	0.1
6.2	0.2	1.8	0.6	0.3	2.7	1.0	6.0	0.2	1.9	0.6
6.4	0.2	1.7	1.1	0.3	2.1	1.8	5.8	0.2	1.9	1.1
6.6	0.2	1.8	1.5	0.3	1.7	2.6	5.4	0.2	1.9	1.5
6.8	0.2	1.6	2.2	0.4	0.7	4.4	4.5	0.2	1.6	2.2
7.0	0.2	1.6	3.5	0.8	0.3	8.9	...	0.2	1.7	3.4
7.2	0.2	1.3	3.7	0.7	0.1	9.2	...	0.2	1.5	3.7

Standards for p_H 7.4–7.6 were too deep blue to be matched.

TABLE IX.—PHENOL RED (PHENOLSULPHONPHTHALEIN).

Use 0.3-cc. indicator.

p_{H} .	Lovibond readings of buffer standards.			Matching blends (cc.).				Lovibond readings of blends.		
	R.	Y.	B.	Co.	Fe.	Cu.	H ₂ O.	R.	Y.	B.
6.6	1.8	6.8	...	1.8	6.5	...	1.7	1.8	6.9	...
6.8	2.3	6.5	...	2.4	5.4	...	2.2	2.3	6.7	...
7.0	4.1	5.0	...	3.5	2.9	...	3.6	4.2	5.1	...
7.2	5.4	3.1	...	5.0	1.3	...	3.7	5.4	3.3	...
7.4	7.2	1.6	...	7.2	0.3	...	2.5	7.2	1.6	...

Standards for p_{H} 7.6–8.2 were too deep red to be matched.TABLE X.—CRESOL RED (*o*-CRESOLSULPHONPHTHALEIN).

Use 0.2-cc. indicator.

p_{H} .	Lovibond readings of buffer standards.			Matching blends (cc.).				Lovibond readings of blends.		
	R.	Y.	B.	Co.	Fe.	Cu.	H ₂ O.	R.	Y.	B.
7.2	1.3	2.2	...	1.0	2.8	...	6.2	1.2	2.2	...
7.4	1.7	2.0	...	1.4	2.2	...	6.4	1.7	2.1	...
7.6	2.0	1.3	0.2	2.1	1.2	0.7	6.0	2.0	1.5	0.2
7.8	3.1	0.5	0.4	3.0	0.1	1.7	5.2	3.1	0.5	0.4
8.0	4.0	0.4	0.8	4.6	...	3.7	1.7	4.0	0.5	0.8
8.2	5.0	0.2	1.6	5.6	...	4.4	...	4.9	0.4	1.5

Standards for p_{H} 8.4–8.8 were too deep red-violet to be matched.

TABLE XI.—METACRESOL PURPLE (ALKALINE RANGE).

Use 0.2-cc. indicator.

p_{H} .	Lovibond readings of buffer standards.			Matching blends (cc.).				Lovibond readings of blends.		
	R.	Y.	B.	Co.	Fe.	Cu.	H ₂ O.	R.	Y.	B.
7.6	1.2	1.8	0.4	1.3	1.5	1.2	6.0	1.2	1.8	0.4
7.8	1.1	1.3	0.4	1.2	1.0	1.1	6.7	1.2	1.3	0.6
8.0	1.3	0.8	0.7	1.5	0.4	2.0	6.1	1.3	0.8	0.6
8.2	1.5	0.4	0.8	1.8	0.1	2.4	5.7	1.5	0.4	0.8
8.4	1.9	0.2	1.2	2.5	...	4.0	3.5	1.9	0.2	1.2
8.6	3.0	0.2	1.9	3.5	...	6.5	...	3.0	0.4	1.9

Standards for p_{H} 8.8–9.2 were too deep purple to be matched.

TABLE XII.—THYMOL BLUE (ALKALINE RANGE).

Use 0.4-cc. indicator.

p_{H} .	Lovibond readings of buffer standards.			Matching blends (cc.).				Lovibond readings of blends.		
	R.	Y.	B.	Co.	Fe.	Cu.	H ₂ O.	R.	Y.	B.
8.2	0.2	1.5	0.4	0.6	1.8	1.2	6.4	0.2	1.5	0.4
8.4	0.2	1.2	0.7	0.8	1.2	2.3	5.7	0.2	1.2	0.7
8.6	0.2	0.8	1.4	1.0	0.4	4.8	3.8	0.2	0.9	1.5
8.8	0.2	0.4	2.2	1.4	0.1	7.0	1.5	0.2	0.5	2.2
9.0	0.2	0.2	3.2	1.5	...	8.5	...	0.2	0.4	3.0

Standards for p_{H} 9.2–9.6 were too deep blue to be matched.

All of these standards consume comparatively little time for preparation; they can easily be made in a day, after which they last indefinitely. They are not subject to the limitations of the usual buffer standards, such as fading and mold growth, and are not affected by light, temperature or CO₂ absorption.

Methods involving permanent standards are not intended to replace more accurate colorimetric or electrometric determinations. They are approximate methods, but of a sufficient degree of accuracy for usual routine work. The

standards in this paper have a limit of accuracy of about 0.1 p_H with the exception of a few cases where the error approaches 0.2 p_H .

The usual salt and protein errors should of course be taken into consideration; a full discussion of these can be found in the works of Clark, Kolthoff, Prideaux and Michaelis.

SUMMARY.

1. Seventy-three permanent standards, made by blending the "Co-Fe-Cu" inorganic salt solutions have been prepared, covering a p_H range of 1.2–9.0. Lovibond comparative readings have been made for these and the corresponding standard buffer solutions. These standards are notable for their stability and ease of preparation.

2. The indicators chosen were those found reliable and least subject to salt errors. By use of 20% neutralized alcoholic solutions, the indicators themselves are made to stand up for quite a period of time without decomposition, and may be used in unbuffered as well as buffered solutions.

3. Further studies are being made on other suitable indicators, especially in the p_H range 9.0–14.0.

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COLUMBIA UNIVERSITY,
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Dr. Edgar Fahs Smith has recently reprinted an article by him on "Fragments Relating to the History of Chemistry in America." In this he has a reproduction of the title page of "A New Nomenclature on Chemistry," prepared by Dr. Lyman Spalding, "Father of the U. S. P.," and also a cut of him taken from the "Life of Dr. Lyman Spalding," published by his grandson. The physicians and pharmacists, as well as chemists, should see that Dr. Lyman Spalding is honored with a place in the Hall of Fame at the next election.