[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF MINNESOTA]

THE USE OF PINACHROM AS A ONE COLOR INDICATOR

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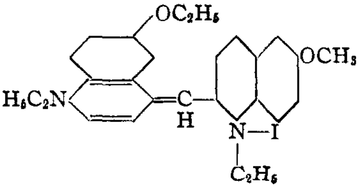
1. The one color indicators described by L. Michaelis and his collaborators are colorless at the acid side and yellow at the alkaline side of their color change interval. In many cases it would be more advantageous to have one color indicators with a more pronounced color change as, for example, phenolphthalein. In this relation, the properties of some methoxytriphenylcarbinols have been described.[1] Also the application of quinaldin red with an indicator constant of $2.70 - 0.007\ (t - 20°)$ may be mentioned.[2]

Besides *m*-nitrophenol we have no useful one color indicator for the *P*H determination in the neighborhood of the neutral point. As *m*-nitrophenol has some disadvantages, we made a special study of the behavior of cyanin and its derivative, pinachrom. The cyanin, which changes from colorless at the acid to blue at the alkaline side, has the disadvantage of being very unstable in solution, which is a serious objection against its general use.

The pinachrom, however, proved to be an excellent indicator and can be highly recommended for *P*H determinations near the neutral point. The pinachrom is used as a sensitizer in photography according to the tables of G. Schultz,[3] No. 611, where more literature about the preparation and properties of this compound can be found.

Pinachrom (M) is *p*-ethoxyquinaldin-*p*-ethoxyquinolin-ethylcyanin. It is very slightly soluble in water but is soluble in hydrochloric acid, forming a colorless liquid.

Probably the formula is



The substance is a weak base with a molecular weight of 518.

2. In my investigations, I used a pinachrom obtained from Farbwerke Meister Lucius and Bruning Hoechst a/M. A 0.1% solution in 70% alcohol was prepared. It has a dark violet color. Another solution was made in which one of the basic groups was neutralized by hydrochloric

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[1] Kolthoff, THIS JOURNAL, **49**, 1218 (1927); Lund, *ibid.*, **49**, 1346 (1927).

[2] See McClendon, *J. Biol. Chem.*, **59**, 437 (1927); Kolthoff, *Biochem. Z.*, will appear in 1928.

[3] G. Schultz, "Farbstoff-Tabellen," 6 Aufl., **1923**; Rowe, "Color Index," 1927, 1st ed., No. 807.

acid: 100 mg. of indicator is dissolved in 40 cc. of alcohol, 1.9 cc. of 0.1 *N* hydrochloric acid is added and the solution is made up to a volume of 100 cc. with water. This solution has a weak violet color. It was kept in a Pyrex bottle. In liquids with a very small or no buffer action—as in distilled water—the neutralized solution should be used; otherwise it is immaterial which solution is employed. The indicator has a color change interval between P_H 5.6 (faintly pink) and 8.0 (deep red-violet). When using 0.1 cc. of indicator in 10 cc. of buffer mixture, the solution is colorless at a P_H of 5.4 and has a very weak red-violet color at a P_H of 5.6. The reaction between the base and the hydrogen ions does not take place instantaneously. After the addition of the indicator, it is necessary to wait at least two minutes before the comparison is made.

When using the indicator without applying buffer mixtures its dissociation constant or hydrolysis constant should be known. From the equation $BOH + H^+ \rightleftarrows B^+ + H_2O$, the following expression is derived: $[(BOH)\ (H^+)]/(B^+) = K_h$, where K_h is the hydrolysis constant (or indicator constant), (BOH) is the concentration of the red-violet colored undissociated base and (B^+) the concentration of the colorless form.

Then $(H^+) = [(B^+)/(BOH)]\cdot K_h$.

In determining K_h, 0.4 cc. of 0.02% indicator solution in 40% alcohol was added to 10 cc. of freshly prepared phosphate buffer solution (according to W. M. Clark) and the color was compared with standards of known amounts of 0.002% indicator in 0.01 *N* sodium carbonate solutions. The 0.002% indicator solution was prepared in 0.001 *N* hydrochloric acid and is colorless.

The alkaline solutions of the indicator cannot be kept, as they are unstable. The free colored base itself is very slightly soluble in water and precipitates after standing for a short time. An interesting phenomenon was observed in preparing the alkaline solution used for comparison. If the tube is violently shaken after adding the indicator, a red froth is formed. This test gives a very simple demonstration of the adsorption of a substance in the interface water–air. After the froth has settled, the upper layer of the solution has a dark violet color, whereas the bulk of the liquid is almost colorless. If the violet layer is mixed with the rest of the solution, the resulting liquid has a much less deep color than it had originally after careful mixing of the indicator solution with the dilute sodium carbonate solution. This striking difference is caused by a precipitation of the indicator base by shaking. Almost all of the indicator is collected in the froth and, as the free base is slightly soluble, it forms a film on the water surface after the froth has settled and does not dissolve any more in the water.

Different tests have been made to prove that the decolorization was not due to an oxidation of the indicator. If the liquid after shaking is acidified and made alkaline, then the original color is restored. Moreover, it can be shown that during the shaking of the alkaline indicator solution, a small part of the base is adsorbed by the glass walls of the Pyrex glass tube. If the alkaline indicator solution is shaken until the solution is almost colorless and then alcohol added, the insoluble film of the free base can be observed. After mixing, the original color strength is restored (the comparison is made in a solution with the same amount of alcohol). If the experiments are repeated in an alkaline solution in 40% alcohol, a colorless froth is formed on shaking, and the color of the solution does not change. This test gives a simple demonstration of the replacing action of a capillary active substance on the adsorption of another capillary active substance.

It should be remarked here that a strongly alkaline solution of the indicator (in sodium carbonate or 0.05 *N* sodium hydroxide) is partly oxidized after a few days' standing.

The experiments described show that the alkaline solutions for comparison should be made by carefully mixing the indicator with the dilute sodium carbonate solution. If shaking is avoided, no troubles are encountered and reproducible results are obtained. The liquids for comparison, however, must not be kept longer than one hour. Addition of alcohol is not to be recommended, as it changes the shade of color.

In the buffer solutions with a *P*H between 5.6 and 8.0, the violet color is stable much longer so that it is often of advantage to perform the colorimetric determination of *P*H in the ordinary way with buffer mixtures.

In the following table are given the results of the determinations of K_h at different *P*H. It is evident that in respect to its properties as an indicator pinachrom behaves as a mono acid base, the monovalent cations being colorless.

TABLE I

HYDROLYSIS CONSTANT OF PINACHROM AT 22°

*P*H	$\frac{[BOH]}{[B^+]}$	$\frac{[BOH]}{[B^+]}[H^+] = K_h$	*P*H	$\frac{[BOH]}{[B^+]}$	$\frac{[BOH]}{[B^+]}[H^+] = K_h$
6.0	0.185/3.805	4.8×10^{-8}	7.0	1.32/2.68	4.9×10^{-8}
6.2	0.29/3.71	5.0×10^{-8}	7.2	1.85/2.15	5.0×10^{-8}
6.4	0.43/3.57	4.8×10^{-8}	7.4	2.25/1.75	4.9×10^{-8}
6.6	0.62/3.38	4.7×10^{-8}	7.6	2.65/1.35	4.9×10^{-8}
6.8	0.96/3.04	5.1×10^{-8}		Average K_h =	4.9×10^{-8}

The average value for K_h at 22° is equal to 4.9×10^{-8}, or $PK_h = 7.31$, corresponding to a dissociation constant of 2.04×10^{-7}, or $PK_b = 6.69$.

The temperature modulus of K_h is rather large, the color of the indicator increasing at higher temperatures. From measurements in phosphate buffers between 20 and 40° it was found that $PK_h = 7.34 + 0.013 (20° - t)$.

Pinachrom has the advantage of having a negligibly small salt error at very low salt concentrations. This may be derived from the results of Table II. A Clark buffer solution was diluted with carbon dioxide free water and the *P*H of the successive dilution was measured with the hydrogen electrode and in a colorimetric way with pinachrom as indicator. The comparison of the color was made with other freshly prepared Clark's buffers.

There was no difference between the behavior of the unneutralized and the neutralized indicator solution.

TABLE II

SALT ERROR OF PINACHROM AT SMALL SALT CONCENTRATIONS

Buffer soln.	*P*H with hydrogen electrode	*P*H with pinachrom
Original Clark buffer soln., salt content		
0.08 *N*	6.99	..
.04 *N*	7.06	7.05
.016 *N*	7.14	7.13
.008 *N*	7.17	7.18
.004 *N*	7.18	7.20
.0016 *N*	7.19	7.22

Practically there is no salt error at very small electrolyte content. The *P*H of the Minneapolis tap water was determined with the neutralized and unneutralized pinachrom solution. Both indicated the same *P*H of 7.48, whereas with neutralized solution a value between 7.45 and 7.50 was found. Hence the pinachrom is very useful for the determination of the *P*H of tap water and has distinct advantages over *m*-nitrophenol, which has a rather high "acid error."

The pinachrom can also be used for testing the quality of distilled water. As the latter has no buffer action at all, it makes some difference whether the unneutralized or neutralized indicator solution is used. The ordinary distilled water in this Laboratory gave with the unneutralized indicator solution a *P*H of 6.4, and with the neutralized solution a value of 5.95. With the sodium salt of methyl red a *P*H of 5.95 was found. Hence in this case the neutralized indicator should be used, as the solution of the base itself develops basic properties in the unbuffered water.

Probably the indicator will also be useful for the determination of the *P*H in blood serum and milk. So far, however, no measurements on the protein error have been made. In the presence of large amounts of salts pinachrom indicates a too acid reaction, in agreement with its basic character.

A Clark buffer solution with a *P*H of 6.8 was ten times diluted and different amounts of potassium chloride were added. The *P*H of the mixtures was measured with the hydrogen electrode and in a colorimetric way.

TABLE III

SALT ERROR AT LARGER SALT CONCENTRATION

Solutions	*P*H with hydrogen electrode	*P*H with pinachrom	Correction in *P*H for salt error
0.007 *N* buffer + 0.1 *N* KCl	6.71	6.58	+0.13
.007 *N* buffer + .25 *N* KCl	6.59	6.37	+ .22
.007 *N* buffer + .5 *N* KCl	6.48	6.18	+ .30

I also tried to make a mixed indicator[4] with some dyestuff in order to get a sharp color change at a special *P*H. The best results were obtained with

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[4] See Kolthoff, *Biochem. Z.*, **189,** 26 (1927), where other literature is discussed.

a mixture of 2 parts of 0.1% pinachrom and 1 part of 0.1% malachite green (or methylene green). At *P*H = 6.0 the color is green; at *P*H = 7.0 and 7.2, blue; at *P*H 7.4, violet; at 7.6 red-violet. The application of this mixed indicator does not give many advantages.

Summary

1. Pinachrom is an excellent one color indicator for the determination of *P*H between 5.8 and 7.8. $PK_h = 7.34 + 0.013\,(20° - t)$.

It is recommended for the determinations of *P*H in tap water and distilled water.

2. The salt error at low electrolyte content is negligibly small; at higher salt concentrations pinachrom indicates a too acid reaction.

3. A simple test is described by which the adsorption of a substance at the interface water–air, and the increased concentration of the capillary active substance in this interface can be shown.

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THE REDUCTION OF COLUMBIC ACID. I

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Introduction and History

The investigation of columbium and tantalum-bearing minerals began in 1801 when Hatchett,[1] in a black mineral from Massachusetts, afterwards named Columbite, found a new metallic oxide, "Columbium oxide." About the same time Ekeberg,[2] in a similar mineral from Kimit in Finland, called Tantalite, and in another from Ytterby in Sweden, named Yttrotantalite, detected a new oxide called "Tantalum oxide."

Due to the very close similarity in the chemical behavior of columbic and tantalic acids, and their frequent occurrence together in minerals, there existed up to the year 1865 considerable doubt as to the existence of more than one acid. Some believed them to be identical, while others attributed the observed differences in respective properties to the presence of two other elements, "Pelopium" and "Dianium."

It was not until 1865 that the compositions of columbite and tantalite were established. Marignac,[3] then, in his classic researches on columbium and its compounds, proved conclusively that all columbites and tantalites contained columbic and tantalic acids. Since that time, by far the greater part of the work done on columbium concerned either the prepara-

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[1] Hatchett, *Phil. Trans. Roy. Soc.*, **92**, 49 (1802).

[2] Ekeberg, *Ann. chim.*, **43**, 276 (1803).

[3] (a) Marignac, *Ann. chim. phys.*, [4] **8**, 1 (1865); (b) [4] **8**, 49 (1865).