and to the quinonoid interpretation of the cause of color in the triphenylmethane series in general. This applies in no lesser degree to the free triarylmethyl radicals themselves. In the solid state they are nearly all colorless or only pale yellow, but when they are dissolved in some solvent, like benzene, intensely colored solutions result. We must assume from the evidence presented in this paper that the radicals dissolve with a simultaneous partial transformation to the quinonoid state.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF COLUMBIA UNIVERSITY, No. 267.]

RESEARCHES ON QUINAZOLINES. XXXIII. A NEW AND SENSITIVE INDICATOR FOR ACIDIMETRY AND ALKALIMETRY, AND FOR THE DETERMINATION OF HYDROGEN-ION CONCENTRATIONS BETWEEN THE LIMITS OF 6 AND 8 ON THE SORENSEN SCALE.[1]

BY MARSTON TAYLOR BOGERT AND GEORGE SCATCHARD.[2]

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Introductory.

As a means of measuring hydrogen-ion concentration, the use of an indicator possesses the obvious advantage of convenience of operation, practically instantaneous readings and inexpensive apparatus.

Ostwald's explanation[3] of indicator action as due to an equilibrium between an ion of one color and an un-ionized molecule of another color (or colorless), has been quite generally abandoned in favor of the theory that an equilibrium exists between the two forms in both states.[4]

$$\begin{array}{ccc} H^+ + In^- & \rightleftharpoons & HIn \\ \downarrow\uparrow & & \downarrow\uparrow \\ H^+ + \mathit{In}^- & & \mathit{HIn} \end{array}$$

[1] Sörensen's system of expressing hydrogen-ion concentration has been used throughout, the "Index" (P_H) being the negative common logarithm of the concentration of hydrogen ion, *i. e.*, the negative exponent of the power to which 10 must be raised to express this concentration (Sörensen, *Compt. rend. Laboratoire de Carlsberg*, **8** (1909); Fales and Nelson, THIS JOURNAL, **37**, 2771 (1915)). Thus, the index 6 represents a hydrogen-ion concentration of 10^{-6}, and 8 of 10^{-8}. This provides a single unit for expressing acidity and alkalinity, and one which can be measured directly by the use of the hydrogen electrode. Further, it is the relative and not the absolute change in hydrogen-ion concentration which affects both the electromotive force of the hydrogen electrode and the color of the indicator.

[2] The experimental work upon which this paper is based was carried out by Mr. George Scatchard in partial fulfilment of the requirements for the degree of Doctor of Philosophy under the Faculty of Pure Science of Columbia University.

Acknowledgments are also due to Dr. H. A. Fales, of this University, for much valuable assistance and advice.

[3] *Wissenschaftliche Grundlagen der analytischen Chemie*, 1901, 117.

[4] Stieglitz, THIS JOURNAL, **25**, 1112 (1903); A. A. Noyes, *Ibid.*, **32**, 816 (1910).

Yet, for a satisfactory indicator, just those conditions are necessary which approximate Ostwald's simple hypothesis so closely as to permit the application of the dilution law to them as to a simple dissociation. That is, for a two-color indicator, one tautomer must predominate in the ion and the other in the un-ionized molecule: while, for a one-color indicator, the colored form must be ausent in one state. In either case, the equilibria between tautomers must be unaffected by external factors. With these conditions fulfilled, we may then ignore all but the predominating forms.

For a monobasic acid indicator (In.H), the following relation will therefore hold:

$$(H^+) = K(In.H)/In^-;$$

and for a monacid basic indicator (In.OH),

$$(H^+) = K_W/(OH^-) = K_W(In^+)/K_B(In.OH)$$

An indicator base may therefore be regarded as an acid with $K_A = K_W/K_B$.

We have omitted the term γ for the degree of ionization of the salt which Noyes uses in his equations, because its use assumes that the equilibrium between the tautomers in the un-ionized salt is the same as in the ion, and that the other ion which goes to make up the salt exists in the solution in the same concentration as the indicator ion. The second condition is seldom even approximately realized in practice, the first we do not know about. Since the error introduced by assuming $\gamma = 1$ is small compared to that made by the other approximations, it does not seem worth while to use the very much more complicated form demanded by an exact expression.

For polybasic acids, or polyacid bases, the relations are more complex, but probably even then a single color change is affected by only one ionization, and the above relation holds at least approximately, which is all that is required for qualitative purposes.

By the depth of color of a one-color indicator, or the tint of a two-color one, the ratio of ionized to un-ionized indicator is measured, and thus the hydrogen-ion concentration. Although theoretically this ratio should vary with the change of hydrogen-ion concentration throughout the whole possible range, there is for every indicator a definite limited region within which this change of concentration is determinable by the color change. Outside this region either the small amount of one color is masked by the large amount of the other, in the case of two-color indicators; or, for one-color indicators, the amount of color is too minute to be visible, or too deep for the recognition of a slight change. The range of usefulness hence varies with the different indicators, but is usually one or two units of index. The location of this particular region of serviceability, depend-

ing as it does upon the ionization constants, naturally differs for different indicators. The location and extent of the serviceable range, and the accuracy of measurement, depend upon the amount of indicator required to give visible color. In general, one-color indicators have wider ranges than two-color ones, because of the masking of one color by preponderating amounts of the other, as already pointed out; and they give more accurate readings because it is easier to estimate depth of color than change of tint.

In titrations where the purpose in using an indicator is merely to determine when a certain definite concentration of hydrogen ion has been reached, the great advantage of a one-color indicator arises from the fact that the easiest of all color changes to detect is the first appearance of color in a colorless solution.

The accuracy of an indicator is impaired by any factors which cause a variation in the equilibrium between the tautomers. Certain substances affect the color change by disturbing the equilibrium without changing the concentration of hydrogen ion. Neutral salts affect most indicators and are particularly troublesome with those of the rosaniline series. Certain organic solvents, like alcohol, likewise often exert a disturbing influence.

Since the use of indicators for determining hydrogen-ion concentration is frequently resorted to in the case of biological liquids, the interfering factors in such solutions are of especial interest, notably the proteins and the commonly used preservatives. The effect of proteins is quite likely due to their colloidal nature, and becomes more serious as the molecular weight and complexity of the indicator increase, being most noticeable with azo indicators of Congo Red type. On the other hand, the disturbing action of the ordinary preservatives, such as chloroform and toluene, is usually due to extraction of the indicator, and those most seriously affected are the basic azo indicators.

Another factor to be reckoned with, is the color of the solution to be titrated. This difficulty may be partially remedied by giving the standard the same color, but it cannot be wholly overcome. For example, a yellow solution would impair but little the usefulness of phenolphthalein, but it would make trouble with methyl orange, and might preclude entirely the use of *p*-nitrophenol; the disturbance caused by a pink solution, on the other hand, would increase in just the opposite direction.

Still another matter of importance, is the permanence of an indicator color, particularly on standing exposed to ordinary daylight. Some indicators, like hematoxylin, run through a rapid series of color changes which make them unsuitable for measuring hydrogen-ion concentration; others fade on account of the precipitation of the indicator; practically all lose their color on long standing in strong daylight. This fading

may be so slow that the standards can be used for several days after they have been made up, or it may be so rapid that the indicator must be added at practically the same instant to the solutions which are to be compared.

For many titrations, an indicator must be used changing near a fixed point, because of the effect of the hydrolysis of the salt formed upon the hydrogen-ion concentration, and the common ion effect of the salt upon the acid or base. Except near this point, the change in hydrogen-ion concentration for a given amount of acid or base added is very slow; as is well illustrated by Hildebrandt's measurements, with a hydrogen electrode, of the hydrogen-ion concentrations at different points in certain titrations.[1] But, for the titration of a strong acid with a strong base, these influences are so slight that, with a proper correction for a blank on the water, the indicator may be selected throughout a wide range. In such cases, the delicacy is greatly increased when the end point is near the neutral point, because the color change depends upon the relative change in concentration, while the volume added affects the absolute change.

Assuming complete ionization, it requires 0.009 cc. of 0.01 molar monobasic acid to change the concentration of 100 cc. from an index of 7 to one of 6, while it requires ten times this amount to change it from 6 to 5, and 100 times to change it from 5 to 4. As the range of dinitrobenzoylene urea is from 6 to 8, it is appropriate to consider here other indicators available for this same change.

Azolitmin changes from red to blue with a change of hydrogen-ion concentration from 4.5 to 8.3; but the color change is too gradual for accurate work, and the indicator is useless in presence of protein.

p-Nitrophenol changes from colorless to greenish yellow from 5 to 7, and is but little affected by outside disturbing factors. Its chief objection is its color, which renders it unsuitable for use in artificial light or in yellow solutions.

Alizarin changes from yellow to red from 5.5 to 6.8. It is affected by protein and cannot be used in yellow solutions.

Neutral red changes from red to yellow from 6.8 to 8. It is affected by protein, chloroform or toluene.

Rosolic acid (commercial Coralline) changes from yellow to red from 6.8 to 8. The color change is poor and the indicator is affected by protein.

Cyanine changes from colorless to blue at 7 to 8, but is useless in presence of protein.

Hematoxylin and hematein give too rapid color changes.

Phenolsulfonephthalein changes from yellow to reddish violet from

[1] THIS JOURNAL, **35**, 847 (1913).

6.50 to 8.50. The effect of salts and protein upon it is now being studied by Lubs and Clark.[1]

o-Cresolsulfonephthalein shows the same range and color change as the last, but its color change is less satisfactory. Lubs and Clark are studying the action of protein and salts upon it.

Bromothymolsulfonephthalein is also being studied by Lubs and Clark in the same way as to the effect of protein and salts. It shows a color change from 6 to 7.25 from yellow to blue.

So far as we are informed, there is no satisfactory indicator now known covering this range of 6 to 8. Sörensen says of this whole region, "il serait certes désirable de trouver des indicateurs supplémentaires."

The color change in the case of dinitrobenzoylene urea is from colorless to greenish yellow, the color developing regularly with the change in hydrogen-ion concentration from 6 to 8. This color change closely resembles that of *p*-nitrophenol, possesses the same advantage as a one-color indicator, and the same disadvantage due to its color being yellow. This is of interest, as the compound is structurally more closely allied to *p*-nitrophenol than to any of the other indicators cited, since it may also be regarded as of nitrophenol type. It is but slightly affected by neutral salts and not at all by chloroform or toluene.

Sörensen found that egg albumen was the most troublesome of all proteins with indicators, but that it did not affect *p*-nitrophenol. Comparative tests with egg albumen showed that dinitrobenzoylene urea is no more disturbed by it than is *p*-nitrophenol. The color of this new indicator fades but slowly. In phosphate solutions[2] it did not fade appreciably in two days, and but very slightly in a week.

Thus, it would appear preferable to rosolic acid (Coralline) for the preparation of neutral ammonium citrate solutions for fertilizer or soil analyses, since the hydrogen ion concentration of such solutions lies near the middle of its range, and it is possible to make much more accurate readings with it than with the older indicator.

In titrations, the acid side of the end point gave an index of 5.93 and the alkaline side 6.09, as measured by electromotive-force determinations with the apparatus described by Fales and Nelson.[3] These tests were made on phosphate solutions, as the reduction of the indicator by the hydrogen made it impossible to carry out these e. m. f. measurements in its presence, and because a buffer was necessary to keep the hydrogen-ion concentration constant. The figures recorded correspond to a change in hydrogen-ion concentration of 3.6×10^{-7}, or less than 0.004 cc. of

[1] *J. Wash. Acad. Sci.*, **5**, 609 (1915).

[2] The primary and secondary alkaline phosphate solutions used throughout were prepared as described by Sörensen, *i. e.*, were $^1/_{15}$ molar.

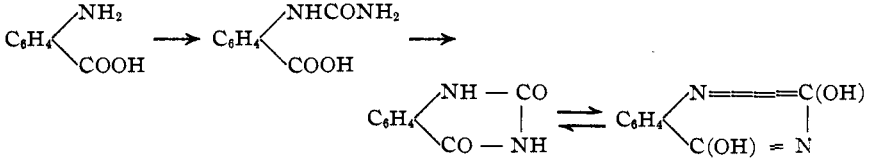
[3] THIS JOURNAL, **37**, 2781 (1915).

0.01 molar monoacid alkali to 100 cc. of solution (assuming ionization to be complete). In practice, the change was obtained with one drop of 0.01 molar NaOH. To give an equally distinct change under similar conditions requires in the case of *p*-nitrophenol 5–6 drops, and for methyl orange 10–12. Titrations of 0.01 molar HCl checked to within 0.1%.

This indicator can be used in hot or cold solutions, and its color is unaffected by nitrous acid. It gives a sharp end point with ammonium hydroxide solutions and hydrochloric acid. It can be used with NaOH and acetic acid, but it is impossible to titrate to the first yellow color. It cannot be employed to titrate carbonates, but the error due to small amounts of CO_2 is negligible.

It gives a second color change at 11 to 13, when the yellow color becomes much deeper, but this is too alkaline for most practical purposes. An attempt was made to use this color change for titrations of boric acid with NaOH, but it proved abortive, partly because the indicator gave a pale yellow color on the acid side of the end point, but principally because in such a strongly akaline solution, a small change in index requires a large amount of alkali.

Dinitrobenzoyleneurea is readily prepared from anthranilic acid, by converting the latter first into the uramino (ureido) acid, which then easily condenses to benzoylene urea,



The benzoylene urea is nitrated with sulfuric and nitric acids, yielding a dinitro derivative, whose monosodium salt is used as the indicator. The location of the two nitro groups has not been definitely determined as yet, although one is quite certainly in position 6. The indicator action may be due solely to keto-enolic tautomerism, or the nitro groups also may play a part by pseudo-acid tautomerism themselves. In this connection, the second color change already noted at 11 to 13 is quite possibly due to the ionization of the second hydrogen.

The study of benzoylene urea and related compounds is being continued, and it is hoped that other serviceable indicators will come to light in the prosecution of the work.

Experimental.

Dinitrobenzoylene Urea, $C_8H_4O_6N_4$.—20 g. anthranilic acid were dissolved in 700 cc. water and 15 cc. concentrated hydrochloric acid (calculated, 12 cc.) by warming. The solution was filtered, cooled, a solution of 15 g. KNCO (calculated, 12 g.) in 50 cc. water added slowly with mechanical stirring, this stirring being continued for 20 minutes after

all the KNCO solution had been added. The uraminobenzoic acid precipitated as a pasty white mass of microscopic needles. 300 g. NaOH were added with cooling. This dissolved the uramino acid and the sodium salt of benzoylene urea soon separated in crystalline form. After standing for four hours, the sodium salt was filtered out, dissolved in a liter of boiling water, precipitated with acetic acid, the free benzoylene urea filtered out, washed with water, and dried at 120°. Yield, 21.8 g., or 92%. The product formed colorless needles, m. 353–4° (corr.), which m. could not be raised by further crystallization.

10 g. of this benzoylene urea were heated on the water bath with 100 cc. concentrated sulfuric acid (which did not quite dissolve it all), and 12 cc. (calculated, 8 cc.) concentrated nitric acid (gr., 1.42) added. Heat was evolved, and the mixture turned bright red, but soon changed to bright yellow. After heating for an hour at 100°, the mixture was cooled and poured into a liter of ice and water, the precipitate filtered out, washed with water and recrystallized from a liter of 50% acetic acid. The crystals were removed, washed and dried at 120°. Yield, 14.4 g., or 92%.

Subs., 0.2026; 0.2786; H_2O, 0.0290, 0.0419; CO_2, 0.2846, 0.3895.

Calc. for $C_8H_4O_6N_4$: C, 38.08; H, 1.60. Found: C, 38.31, 38.13; H, 1.60, 1.68.

Subs., 0.1858, 0.1655; 36.60 cc. N at 17° and 765.4 mm.; 32.75 cc. N at 20° and 765.3 mm.

Calc. for $C_8H_4O_6N_4$: N, 22.19. Found: N, 22.28, 22.30.

As thus prepared, the compound formed pale greenish yellow prisms, decomposing at 274–5° (corr.), which decomposition point could not be altered by further recrystallization.

100 cc. of its aqueous solution saturated at 23° gave 0.0164 g. residue at 110° and 100 cc. of the water used gave 0.0007 g. residue. This solubility of 0.0157 g. per 100 cc. is equivalent to 0.00062 mols per liter, and corresponds to 14–15 drops of 0.01 molar indicator solution to 10 cc. of liquid to be tested.

The substance is very difficultly soluble in cold alcohol, or in ether, benzene, toluene, ligroin, chloroform, carbon tetrachloride or carbon disulfide; slightly soluble in acetone, ethyl acetate, cold acetic acid or boiling alcohol; moderately soluble in boiling water; readily soluble in boiling glacial acetic acid. It can be recrystallized from water or acetic acid. In solution of sodium hydroxide or carbonate, it dissolves with a yellow color, but is reprecipitated from such solutions by saturation with CO_2.

For the preparation of the sodium salt, 25 g. of the dinitrobenzoylene urea were dissolved in 115 cc. molar NaOH and 500 cc. boiling water, the solution filtered and cooled. A mass of long, bright yellow needles crystallized out. These were removed, pressed as dry as possible, and then left over concentrated sulfuric acid in an evacuated desiccator.

The rest of the dinitrobenzoylene urea was recovered by acidifying the filtrate. The sodium salt thus dried *in vacuo* was then heated to constant weight at 140–150°:

0.3195 g. subs. lost 0.0194 g. H_2O. Calc. for $C_8H_3O_6N_4Na.H_2O$: H_2O, 6.17. Found: H_2O, 6.07.

Subs. (dried at 140–50°), 0.3001; Na_2SO_4, 0.0777. Calc. for $C_8H_3O_6N_4Na$: Na, 8.39. Found: Na, 8.38.

The solubility of the salt at 20° and at 2° was determined by taking a measured volume saturated at the desired temperature, heating to boiling, acidifying with hydrochloric acid, cooling, filtering out the precipitated dinitrobenzoylene urea, and drying to constant weight at 120° in a Gooch crucible. This gives the difference in solubility between the sodium salt and the free dinitrobenzoylene urea.

(1) 50 cc. solution saturated at 20° gave 0.4898 g. dinitrobenzoylene urea, indicating a solubility of 0.0389 mols per liter, or 1.1359 g. $C_8H_3O_6N_4Na.H_2O$ per 100 cc.

(2) 50 cc. solution saturated at 2° gave 0.1294 g. dinitrobenzoylene urea, indicating a solubility for the sodium salt of 0.0103 mols per liter, or 0.3008 g. per 100 cc.

In both the above calculations, the solubility of free dinitrobenzoylene urea at the temperature used has been assumed as zero. This, of course, is not strictly accurate, as the free dinitrobenzoylene urea is itself slightly soluble; but it is sufficient for all practical purposes, and those who wish closer figures can readily obtain them from the solubility results recorded above for the free dinitrobenzoylene urea.

The indicator solution was prepared by dissolving 0.292 g. of the salt in 100 cc. water, and was therefore 0.01 molar. The dropper used delivered 22–23 drops per cc. Fifteen drops of this indicator solution in 10 cc. 0.01 molar HCl gave no precipitate; 20 drops gave considerable. The solubility is thus about four times the amount ordinarily used in practice.

Determination of Hydrogen-Ion Concentration.—Phosphate and borate solutions were prepared as described by Sörensen. To 10 cc. of each, four drops of indicator solution were added. That with an index of 6 gave a colorless solution, while that with an index of 8 gave a distinct greenish yellow, the color developing evenly in the intermediate solutions.

Borate solutions with an index of 9 and 10 gave the same color as those with 8; 11 gave a slightly deeper color. 0.1 molar NaOH (index 13) gave a much deeper greenish yellow, one drop of the indicator giving as much color as four drops in a solution with index of 8.

Effect of Toluene and of Chloroform.—Phosphate solutions were prepared of index 6.4, 7.0 and 7.6. 30 cc. of each solution were made up, and each of these solutions then divided into three lots of 10 cc. each. These separate lots were then grouped into three sets of three lots each,

one of each index. One set was shaken with excess of toluene and filtered. Another set was given a similar treatment with chloroform. The third set was kept as a blank. Four drops of indicator were then added to each solution. No difference could be detected between the solutions of the same concentration in the three sets.

Effect of Protein.—25 cc. of egg white were diluted to 100 cc. with water, filtered, and 2 cc. of this solution added to a mixture of 14 cc. primary and 6 cc. secondary phosphate solution. This was then divided into two 10 cc. portions, to one of which *p*-nitrophenol was added and to the other the new indicator. Both showed an index of 6.60.

Salt Effect.—160 cc. secondary phosphate solution and 40 cc. primary phosphate solution were mixed and 12 g. NaCl (approximately molar) added. Electromotive-force measurements of this solution showed an index of 6.74, while the new indicator gave 6.90. Such a solution is more concentrated than any generally measured. The effect is apparently due to the large concentration of Na^+ ion, which is responsible for the presence of un-ionized salt molecules which are colored like the ion.

Fading Effect.—Three sets each were made up of phosphate solutions of index 6.6 and of 7.6. Four drops of indicator were added to one set upon a certain day, to the second set on the second day, and to the third set on the third day. On this third day, no difference could be detected between the three sets of the same concentration. After standing for a week, the first set was compared with freshly prepared standards, with the following results: that with index of 7.6 had faded to 7.45–7.50, and that with index of 6.6 to 6.55–6.60.

Titrations.—Titrations of 0.01 molar NaOH and HCl, using 10 drops indicator, gave 1.0011, 1.0004, 1.0009 and 1.0016, for the ratio of base to acid.

Titrations of 0.1 molar NH_4OH and HCl, using 10 drops indicator, gave 1.1257, 1.1262 and 1.1261, for the ration of acid to base.

Titrations of 0.1 molar acetic acid and NaOH, using five drops of indicator, gave 1.0129 and 1.0138, for the ratio of base to acid; but it was necessary to titrate by comparison with a color standard, instead of to the first appearance of a yellow color.

Effect of Temperature on the End Point.—Two 100 cc. samples of 0.005 molar NaCl (the approximate concentration at end point of titrations of 0.01 molar solutions) were heated to boiling and 10 drops of indicator added. One was titrated to one side of the end point, and the other to the other side. On cooling, no change was observed in the color in either case.

Effect of Nitrous Acid.—30 cc. molar HCl were diluted to 200 cc. (making it 0.15 molar), 1.5 g. $NaNO_2$ (0.1 molar) added, and 40 drops of indicator (10 drops per 50 cc.). After standing for an hour at room

temperature, the solution was heated to 65° for a few minutes, cooled, and then titrated twice with 0.1 molar NaOH. A sharp end point was obtained in both cases, and the results checked closely, *viz.*, 1.0716 and 1.0715.

Summary.

A dinitrobenzoylene urea has been discovered whose monosodium salt is a very sensitive indicator for hydrogen-ion concentrations between the limits of 6 and 8 on the Sörensen scale, changing from colorless to greenish yellow.

Structurally, and in its behavior as an indicator, it resembles *p*-nitrophenol more closely than any of the other well-known indicators. Like the latter, its chief disadvantage is its yellow color, which renders it unsuitable for work in artificial light.

It is but slightly affected by neutral salts, not at all by chloroform or toluene, proteins (egg albumen) have no more influence upon it than upon *p*-nitrophenol; its color fades very slightly in a week, and is unchanged by nitrous acid. It can be used in cold or in boiling (100°) solutions. It gives a sharp end point with NH_4OH and HCl, but cannot be used to titrate carbonates.

For the preparation of neutral ammonium citrate solutions, for fertilizer or soil analysis, it should prove superior to rosolic acid (commercial Coralline).

It can be prepared easily from anthranilic acid by the method described.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY OF THE UNIVERSITY OF ILLINOIS.]

A COMPARATIVE STUDY OF THE DISTRIBUTION OF UREA IN THE BLOOD AND TISSUES OF CERTAIN VERTEBRATES WITH ESPECIAL REFERENCE TO THE HEN.

BY WALTER G. KARR AND HOWARD B. LEWIS.

Received June 5, 1916.

The problems of protein metabolism and protein requirements of the organism are today among the most absorbing and fundamental in the whole realm of physiological chemistry. Closely associated with these problems is the problem of the formation and distribution of urea, since this has long been recognized as the chief end-product of protein metabolism in the higher vertebrates, with the exception of birds and reptiles, in which its place as the chief end-product of nitrogenous metabolism is taken by the more complex uric acid. Although much work has been done on the relation of urea to the intermediary metabolism of protein and amino acids, many problems remain to be solved, the solution of which is now a possibility with the more suitable and accurate