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Review Article

Photometric Titrations

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INTRODUCTION

Scope of This Review

An increased interest in the photometric titration technique has resulted in the publication of several reviews in recent years. Among these are articles by Underwood (1), Goddu and Hume (2), and Headridge (3), and a book by Headridge (4). These authors have given adequate attention to the history of photometric titration methods—the summary by Goddu and Hume (2) is particularly recommended—and this material will not be repeated here. Our treatment will emphasize the principles upon which photometric titrations are based, with special attention given to techniques developed in the past few years. Examples illustrating each type of titration have been drawn from the literature or from our unpublished results.

Experimental Operations[1]

One advantage of end-point detection by photometric means is simplicity of the experimental operation, which merely requires the evaluation of the suitable optical property—which is accomplished with a spectrophotometer, a fluorimeter, or a polarimeter—as a function of titrant volume. Any commercially available instrument may be employed. Two types of titration cells have been used.

The first and most widely used arrangement places the titration cell directly in the light path of the instrument; it thus takes the place of the usual sample cell. Titration is carried out directly in the cell. Some means of stirring the solution must be provided; this may be a mechanical or a magnetic stirrer. The presence of a magnet in the vicinity of a photoelectric cell may affect the response of the cell, and this possible source of error must be kept in mind. The titration cell may be an ordinary glass beaker for titrations using the visible region of the spectrum. Ultraviolet radiation requires special cell walls of silica. Provision must be made for the buret tip and, where necessary, a source of nitrogen for flushing the solution and cell compartment.

Many modifications of this basic assembly have been reported. The examples given by Goddu and Hume (2), Headridge (4), and Bobtelsky (5) illustrate the types of arrangement possible.

The second kind of titration system consists of a titration cell external to the instrument and connected to a flow-through photometer cell with some kind of pump to circulate the solution. Both mechanical and magnetic circulation have been used. An advantage of this method is that the magnet can be removed from the vicinity of the photocell. Moreover, it is very easy to carry out a simultaneous photometric and potentiometric titration by inserting electrodes in the titration flask. Further advantages are the ease with which the solution may be thermostated and protected from the atmosphere. The photometer does not require any modification other than a small hole in the cell compart-

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[1] Throughout this review the spectrometric nomenclature preferred by *Analytical Chemistry* will be used (6).

ment cover to permit passage of the tubing connecting the flow-through cell and the titration flask. A convenient assembly has been described by Rehm, *et al.* (7).

Several commercial photometric titrators are available. Headridge (4) has discussed several instruments designed primarily for use in absorbance titrations, while Howerton and Wasilewski (8) have described one for use in studying either absorbance or fluorescent end points. Some of these provide a means for recording, manually or automatically, the photocell response as a function of titrant volume. Other more sophisticated instruments can terminate the titration at the end point. Although these titrators can save much time and effort in routine analyses, for developmental and investigative work we recommend use of an ordinary spectrophotometer or fluorimeter and one of the titration cell assemblies discussed above.

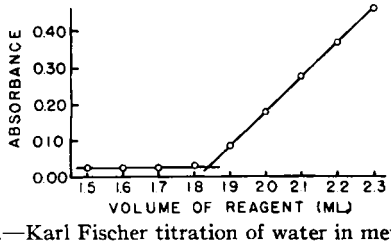

Fig. 1.—Karl Fischer titration of water in methanol solution; absorbance measured at 525 mμ (9).

ABSORBANCE MEASUREMENTS IN HOMOGENEOUS SYSTEMS

In this section we consider titrations in homogeneous solutions which are accompanied by changes in the light absorbing properties of the solution. All such photometric titrations are based upon Beer's law, $A = abc$, which states that the absorbance, A, of the solution is directly proportional to the concentration, c, of the absorbing substance; b is the path length, and a is the absorptivity. Absorptivity is a function of wavelength, solvent, temperature, and molecular structure. If the solution contains more than one absorbing component, the observed absorbance is the sum of the absorbances of the individual components. These general statements are of course subject to exceptions, but unless otherwise stated we shall assume adherence to Beer's law in these systems.

Self-Indicator (Direct) Titrations

Single Samples.—The simplest systems are those in which the sample, the product of the titration reaction, the titrant, or a combination of these, absorbs light of the selected wavelength,

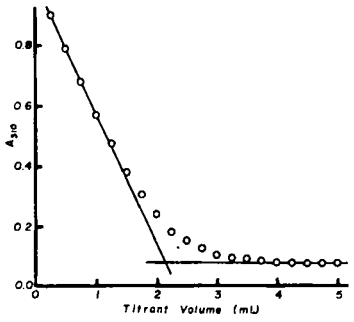

Fig. 2.—Titration of aniline with acetic anhydride (10).

such that a change in absorbance occurs during the titration. Since absorbance is directly proportional to concentration, while electromotive force is logarithmically related to concentration, photometric titration may be expected to be more sensitive than potentiometric titration (2) (assuming suitable absorption characteristics of the components of the titration solution). If the concentrations and absorptivities of all species are known, the titration plot may be quantitatively predicted. It will consist of straight-line segments. The end point is marked by the intersection of two such lines. For the titration of a single substance the titration may take one of several forms, depending upon the absorption characteristics of the solution components.

If the titrant, but neither the sample nor the product, absorbs at the selected wavelength, the plot will look like Fig. 1, which shows the Karl Fischer titration of water followed at 525 mμ (9). By extrapolation of the two line segments, the end point is located. A classic example of this type of titration is the titration of ferrous ion with potassium permanganate.

If the sample absorbs, but the product and titrant do not, as the titration proceeds the absorbance will fall to zero. Figure 2 shows an acylation reaction—the titration of aniline with acetic anhydride (10). At the wavelength selected, only the aniline absorbs (actually the

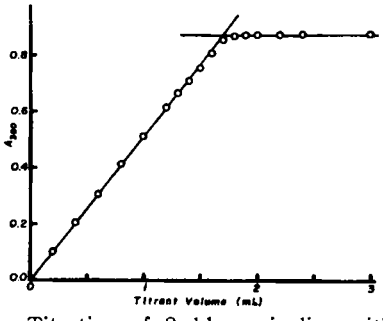

Fig. 3.—Titration of 8-chloroquinoline with perchloric acid in acetic acid (11).

product does absorb slightly) so the absorbance falls. (Sources of curvature will be discussed later.)

If the sample and the titrant do not absorb, but the product of the titration reaction does, a plot will be obtained like that shown in Fig. 3. The acid form of 8-chloroquinoline absorbs strongly at 380 mμ, so the absorbance rises linearly with titrant volume until the titration is complete, when it levels off (11). Another example would be the titration of *p*-nitrophenol with a strong base, the absorbance being followed near 400 mμ.

If the sample does not absorb, but both titrant and product do (but to different extents), then a break will again be observed. If the titrant is a much stronger absorber than the product, a graph like that shown in Fig. 4 will be obtained. In this titration the product absorbed strongly

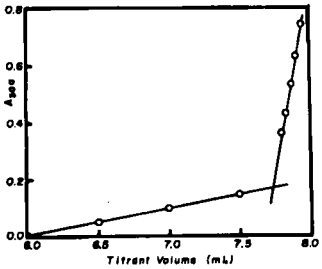

Fig. 4.—Titration of *o*-cresol with bromate-bromide solution (12).

enough that for convenience the absorbance was arbitrarily set at zero about 2 ml. prior to the end point (12). In general, these direct titration plots require only relative absorbance values because the end point is detected through the change in absorbance.

If the product is a stronger absorber than the titrant, the slope of the line following the end point will be less than that before it. Of course, other possible combinations of absorbers exist but examples of these are quite rare.

Mixtures.—One of the advantages of self-indicator photometric titrations appears to be their ability to distinguish between closely related components in a mixture. Hume and his co-workers have explored the applicability of the method (11, 13, 14). Restricting attention to acid-base titrations (though similar conditions should be applicable to other systems), two kinds of binary mixtures described below may be encountered.

First, the weaker compound (acid or base) exhibits absorption at longer wavelengths. Then the wavelength can be selected such that the weaker compound absorbs while the stronger one does not. The resulting titration curve will look

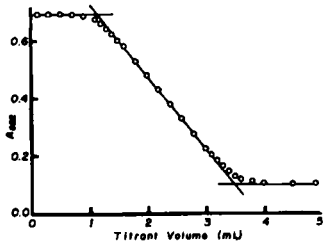

Fig. 5.—Titration of 2-methyl-5-nitroaniline and 4-methyl-2-nitroaniline with perchloric acid in acetic acid (11).

like Fig. 5 (11), which shows the nonaqueous titration of 2-methyl-5-nitroaniline [pKb (H_2O) 12.0] and 4-methyl-2-nitroaniline [pKb (H_2O) 13.5]. The *ortho*-nitroaniline, which is the weaker base, is the only compound which absorbs at around 520 mμ. Therefore, no absorbance change is seen in the initial stage of the titration, for the stronger component is being titrated. At the conclusion of this stage, the weaker base begins to consume titrant, and the titration is accompanied by a decrease in absorption. Finally, when both components have been titrated, the absorbance drops to a low constant value.

The second situation is when the stronger compound absorbs at the longer wavelengths; this is less favorable. The titration of the stronger component is accompanied by a fall in absorbance if its spectrum is shifted appropriately on titration. However, in order to follow the titration of the weaker component it is now necessary to shift to a lower wavelength. Since the stronger compound probably absorbs intensely here, and traces of it remain untitrated, curvature of the titration plot may result.

In favorable instances multicomponent mixtures may be susceptible to analysis, particularly if the wavelength shift technique is applied. Thus, Hummelstedt and Hume (14) titrated the four component mixture of (in descending order

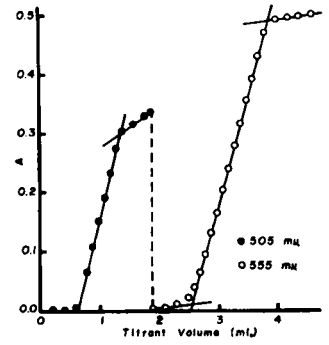

Fig. 6.—Titration of diphenyl phosphate, 2,4-dinitrophenol, *p*-nitrophenol, and *m*-nitrophenol with tetrabutylammonium hydroxide in isopropyl alcohol (14).

of acid strength) diphenyl phosphate, 2,4-dinitrophenol, *p*-nitrophenol, and *m*-nitrophenol (Fig. 6). At 505 mμ, diphenyl phosphate does not absorb. This strong acid is titrated first. Then the 2,4-dinitrophenol is titrated, the anion absorbing strongly. This accounts for the second segment of the curve, and allows extrapolation back to yield the end point for the diphenyl phosphate titration. (In a sense, therefore, 2,4-dinitrophenol may be considered an indicator for the photometric titration of the colorless diphenyl phosphate.) The next component to be titrated is the *p*-nitrophenol, the anion of which also absorbs at 505 mμ, but with sufficiently different intensity from the dinitrophenolate to permit location of the second end point.

Since *m*-nitrophenolate absorbs very strongly at 505 mμ, and its titration would give rise to extremely high absorbances, the wavelength is now shifted to 555 mμ, and the titration is continued to yield the third and fourth end points.

Sources of Error.—In the most satisfactory instances the direct photometric titration plot consists of straight-line segments intersecting at the end point. Many titrations, however, yield lines which exhibit more or less curvature, and which do not permit thoroughly reliable extrapolation to the end point. One source of this curvature is the dilution effect. If the volume of the titration solution is significantly

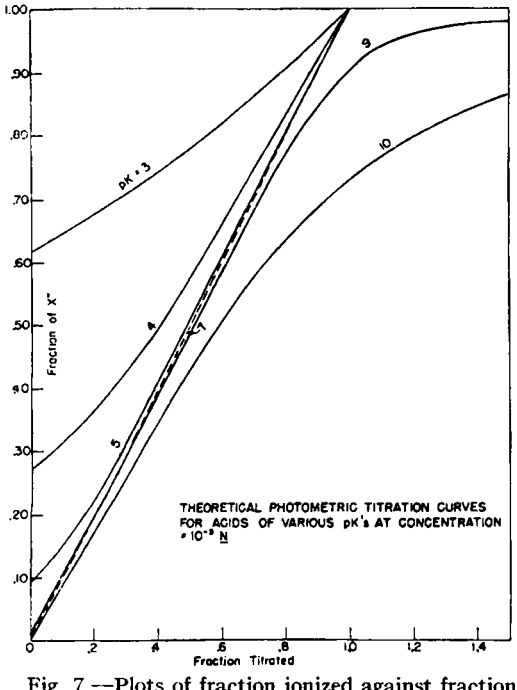

Fig. 7.—Plots of fraction ionized against fraction titrated for acids of various pKa's at 10^{-3} *M* concentration (13). [Courtesy of *Analytical Chemistry*.]

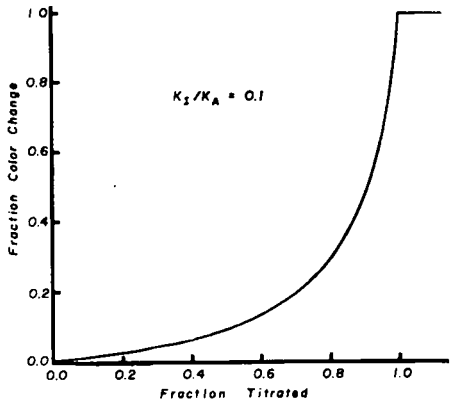

Fig. 8.—Theoretical color change curve for titration of weak acid with strong base in presence of indicator (16).

increased by the addition of titrant, evidently the concentration of each absorbing species will decrease due solely to this volume change. The result is an apparent failure of Beer's law, with negative curvature over the entire length of the line segment. Large errors may result. This dilution effect may be entirely compensated for by multiplying each observed absorbance value by the correction factor $(V + v)/V$, where V is the initial volume of the sample solution and v is the volume of titrant solution added. To render the dilution effect negligible, it is common practice to employ highly concentrated titrants.

A second effect which leads to apparent deviations from Beer's law is more specific in nature. If one or more of the absorbing species is involved in a molecular interaction with another component of the solution so that a change in its absorption spectrum results, a curvature may be observed in the titration plot. Thus, Hummelstedt and Hume (14) observed curvature in the titration of some phenols and ascribed it to phenol-phenolate complex formation. Such effects are sensitive to the nature of the solvent, which may be changed to eliminate them.

The most common cause of curvature is incompleteness of the titration reaction. This is manifested by curvature in the vicinity of the end point, and it obviously makes an accurate extrapolation more difficult. For example, considering acid-base reactions, it is possible to construct a graph of the fraction of the acid in the ionized state as a function of the fraction of the acid titrated, as done by Goddu and Hume (13), Fig. 7. This plot is made for acids of various pKa values, all at $10^{-3}M$ concentration in aqueous solution. In the case of acids whose absorption is due solely to the anion form, these graphs are equivalent to the absorbance-volume plots.

Of particular interest here are the curves for the weaker acids. An acid of pKa 7 gives the expected straight line. As the acid becomes weaker, say pKa 9, a pronounced curvature is observed in the region of the end point. Evidently, extrapolation in such a case is less certain. This source of curvature—namely, incomplete reaction (that is, "hydrolysis of the salt" in the present case)—is responsible for most of the curvature observed in this type of photometric titration plot. Goddu and Hume (13) estimate that satisfactory titrations can be made in aqueous solution if the product $C_{HA}Ka$ is equal to or greater than 10^{-12}. Grunwald (15) has presented a mathematical correction procedure which employs the data taken in the curved portion of the plot to yield the correct end point.

Indicator Titrations

A major limitation to the application of the direct or self-indicator photometric titrations just discussed is of course the requirement on the absorption spectra of the solution components. By incorporating an indicator in the solution, the titration becomes nearly general in its applicability and in this section we consider the types of photometric indicator titration plots which have been developed.

Absorbance-Volume Plots.—Visual indicator titrations are the most convenient of all, but sometimes the color change is too gradual to permit accurate location of the end point. It seems reasonable to expect that photometric observation of the color change might lead to more accurate and precise titrations. It is possible to predict the curve to be found. Figure 8 shows the curve relating the fraction of indicator color change to the fraction of weak acid titrated by a strong base for the case in which $K_I/Ka = 0.1$; in other words, the indicator is a weaker acid than is the sample. It is assumed that the titration reaction is complete. If the base form of the indicator were colored, Fig. 8 would be equivalent to the absorbance-volume plot (16). The end point is marked by the sharp change in direction. Note that the approach to the end point is characterized by a line which approximates to a straight line, but is really a curve. In any actual case we may expect that this will be a possible source of error. It is not possible to extrapolate from a great distance prior to the end point because the line is curved.

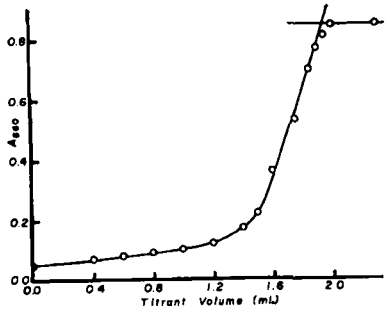

Fig. 9.—Titration of magnesium with EDTA, using Eriochrome black T indicator (18).

Though Fig. 8 has been calculated for an acid-base reaction, the same considerations will hold for complexometric titrations, though in this case conditional stability constants would apply. Fortuin, *et al.* (17), have presented a graph for a complexometric indicator titration which is similar to Fig. 8, and these authors have examined some of the factors which influence the accuracy of the end-point determination. Complexometric titration has in fact been the area of greatest use of absorbance-volume plots in indicator titrations. Figure 9 shows a typical example—the EDTA titration of magnesium with Eriochrome black T indicator—obtained by Zak, *et al.* (18). The experimental curve approximates to the theoretical shape, but some curvature is noted near the end point. Ringbom, *et al.* (19), have carried out similar calculations and experiments for the complexometric titration of very dilute solutions.

Such plots have not been widely used in acid-base titrations. An ingenious application of indicators to these reactions has been made by Goddu and Hume (13), who carried out matched-strength indicator titrations. The colorless acid sample was titrated in the presence of an indicator of the same acid strength and of known concentration. The titration plot resembles that for the colored acid alone, being a direct type of plot, but the apparent intensity of absorption is diminished because the colorless acid is titrated simultaneously. The end point is for the sum of the two acids, and the colorless acid is found by difference. Another kind of absorbance-volume indicator titration plot has already been encountered in the discussion of Fig. 6, where it was pointed out that the weaker acid 2,4-dinitrophenol served as an indicator for the titration of diphenyl phosphate. Goddu and Hume (13) and Reilley and Schweizer (20) have employed such systems in acid-base titrations. The two types of titration discussed in this paragraph differ from the kind represented by Figs. 8 and 9 in that the indicator concentration in the solutions considered here is comparable to the concentration of the sample substance, while for titrations of the kind in Figs. 8 and 9 the indicator

concentration is generally negligible in terms of titrant consumption.

A variation of the absorbance-volume plot has been introduced by Bruckenstein and Gracias (21), who evaluate (for the case of aqueous acid-base titrations) the point at which the second derivative $\Delta^2 pH/\Delta V^2$ is zero; this is taken as the end point. The method employs differential spectrophotometry of solutions containing an indicator, the data being taken in the region of the end point. The original paper should be consulted for details.

Indicator Ratio Methods.—The absorbance-volume plot is limited primarily by its dependence upon data obtained in the vicinity of the end point, and these are often the poorest data for extrapolation purposes. Moreover, it is necessary to collect data on both sides of the end point for such extrapolation. Recently, some related methods have been developed by Higuchi and his co-workers which utilize data further from the end point for linear extrapolation; in these methods data are necessary on only one side of (that is, before or after) the end point. The treatment given here will consider the titration of a base with an acid. The titration system is pictured as a competition between the sample base B and indicator base I for the titrant acid HA (Eqs. 1 and 2).

$$BHA + I \rightleftharpoons IHA + B \qquad \text{(Eq. 1)}$$

$$K_{ex} = \frac{[IHA][B]}{[I][BHA]} \qquad \text{(Eq. 2)}$$

The reaction between B and HA (the titration reaction) is considered to proceed to completion, and the amount of titrant consumed by the indicator is assumed negligible. Now let X' be the total concentration of acid added to the system at any time prior to the end point, and let S' be the total concentration of base initially present.

$$X' = [BHA] \qquad \text{(Eq. 3)}$$

$$S' = [B] + [BHA] \qquad \text{(Eq. 4)}$$

The concentrations X' and S' can be converted to the corresponding volumes X and S with the relationships $X' = XN/V$ and $S' = SN/V$, where N is the titrant normality, V is the total volume of the titration solution, X is the volume of titrant added at any point, and S is the volume of titrant required to react stoichiometrically with the total base present. Combination of these relations with Eqs. 2, 3, and 4 gives the fundamental titration equation

$$K_{ex} = \frac{I_a}{I_b} \cdot \frac{S - X}{X} \qquad \text{(Eq. 5)}$$

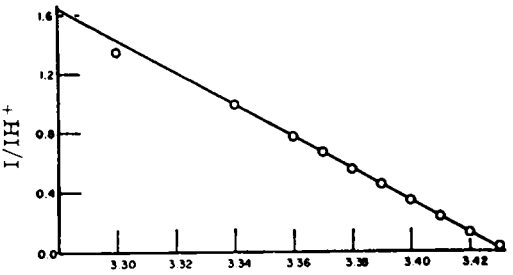

Fig. 10.—Type I plot for titration of triphenylguanidine with perchloric acid in acetic acid, using quinaldine red indicator (16). [Courtesy of *Analytical Chemistry*.]

where I_a/I_b, the indicator ratio, is written for the quantity [IHA]/[I]. K_{ex} is called the exchange constant, and evidently it is a measure of the relative basicity of I and B.

The indicator ratio is calculated from the spectrophotometric data by

$$I_a/I_b = \frac{A_b - A}{A - A_a} = \frac{A - A_b}{A_a - A} \qquad \text{(Eq. 6)}$$

where A_b is the absorbance of the solution when the indicator is totally in the base form, A_a is the absorbance when the indicator has been completely converted to the acid form, and A is the absorbance when it is in any mixture of the two forms; all solutions must contain the same concentration of indicator. Usually the wavelength is chosen so that either A_a or A_b is zero. To render negligible the effect of dilution by the titrant, it is common practice to incorporate the indicator in the titrant in the same concentration it has in the sample solution; the dilution effect is thus eliminated. Several uses of Eq. 5 will be considered below.

Type I Systems.—The titration Eq. 5 can be rearranged to $XK_{ex}(I_b/I_a) = S - X$. Now consider a system in which K_{ex} is very small, of the order 10^{-2}. This means that B is a much stronger base than is I. The indicator color change will all occur in the final few per cent of the titration. In such a case, therefore, it is permissible to make the substitution $S = X$

$$SK_{ex}(I_b/I_a) = S - X \qquad \text{(Eq. 7)}$$

This is a linear equation in I_b/I_a and X; the intercept on the X axis is S, the end-point volume. An example of this method of titration, which is termed the Type I plot, is shown in Fig. 10 (16). The Type I method is capable of great precision, and it should find use in the analysis of high-purity chemicals and the development of primary standards. Because of the nature of the assumption made in the derivation of Eq. 7 data can only be taken close to the end point. It should

be noted that with this plot all data are collected before the end point is reached.

TYPE II SYSTEMS.—In the more general case the basic titration Eq. 5 must be employed (16). This equation can be placed in three forms which are linear in the titration variables (22). (For convenience K is written for K_{ex}.)

$$1/X = (K/S)(I_b/I_a) + 1/S \qquad \text{(Eq. 8)}$$

In this form the indicator ratio I_b/I_a is linear with $1/X$, the reciprocal of the titration volume (16). An example is shown in Fig. 11 (22). From the slope and either intercept the end-point volume, S, and the exchange constant, K,

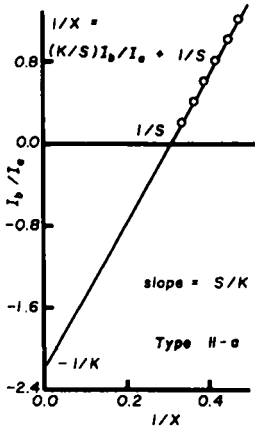

Fig. 11.—Type II-a plot for titration of urea with perchloric acid in acetic acid, using Nile blue A indicator (22).

can be determined.[2] This photometric titration plot, called the Type II-a plot, has been applied to acid-base titrations in aqueous and nonaqueous systems (16, 23–26). It has enabled successful titration to be made of very weakly acidic and basic substances not otherwise accessible to acid-base titration.

Equation 9 gives another form of Eq. 5. The

$$X(I_b/I_a) = S/K - X/K \qquad \text{(Eq. 9)}$$

corresponding plot, known as the Type II-b plot, is shown in Fig. 12 for the same data graphed in Fig. 11 (22). Again both S and K are readily determined from any two of the three features of the line (two intercepts and one slope). The third form (Eq. 10) is graphed in Fig. 13; this is the Type II-c plot.

$$I_a/I_b = (I_a/I_b)S/X - K \qquad \text{(Eq. 10)}$$

All Type II plots employ data taken before the end point, and in fact can utilize information taken much earlier in the titration than is possible with absorbance-volume plots. Besides permitting the use of much more data, some special advantage may result. For example, precipitation of the titration product, which would vitiate the absorbance-volume plot, may not prevent accurate location of the end point by the Type II plot.

In addition to providing a value of S, the end-point volume, these methods give a value for the exchange constant. In the case of acid-base reactions the exchange constant is a measure of acidity or basicity, and many such constants have been evaluated by this means (16, 27–29). The same equations will apply to 1:1 complexation reactions, and it seems that one of the most promising uses of the Type II plots may be in the evaluation of complex stability constants.

TYPE III SYSTEMS.—If the indicator base is so weak that it does not begin to change color until essentially all of the sample base has been titrated, then the color change is governed not by the competition (1) but by

$$I + HA \rightleftharpoons IHA \qquad \text{(Eq. 11)}$$

The free acid concentration [HA] is given by

$$[HA] = (I_a/I_b)(1/K') \qquad \text{(Eq. 12)}$$

where the significance of K' depends upon the nature of the reaction and the titration conditions; thus in an aqueous acid-base reaction K' is the reciprocal of the dissociation constant of the conjugate acid form of the indicator. Equation 12 can be transformed into

$$X - S = (I_a/I_b)(V/K'N) \qquad \text{(Eq. 13)}$$

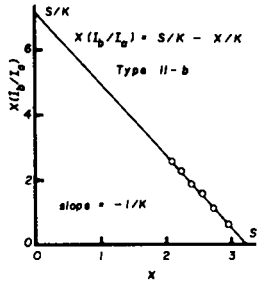

Fig. 12.—Type II-b plot; see Fig. 11 for details (22).

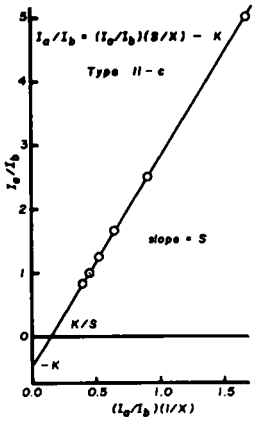

Fig. 13.—Type II-c plot; see Fig. 11 for details (22).

[2] The plot as shown places the independent variable on the horizontal axis, as is customary. This is why the slope is S/K rather than K/S, indicated by Eq. 8.

where the quantity $(X - S)N/V$ has been substituted for [HA]; in this case X is greater than S. This equation is linear in X and I_a/I_b, and the X axis intercept is equal to the end-point volume, S. From the slope, the equilibrium constant can be evaluated. Figure 14 shows an example of this kind of titration, which Rehm and Higuchi called the Type III plot (30). Note that with the Type III plot all data are taken after the end point.

Sources of Error.—In many systems application of the indicator ratio methods just discussed reveals curvature in the titration plots. This deviation from the behavior predicted on the basis of Eq. 5 can be accounted for by a more complete treatment of the system. Suppose that the equality expressed by Eq. 3 fails to hold; in other words, some of the acid added to the system is present in a form other than BHA. (The reasons why this may be so will be discussed shortly.) Let the concentration equivalent to this acid be denoted by R'. Then Eqs. 3 and 4 may be more accurately given as

$$X' = [BHA] + R' \qquad \text{(Eq. 14)}$$

$$S' = [B] + [BHA] \qquad \text{(Eq. 15)}$$

These equations can be combined with Eq. 2 as before to give the general titration equation

$$K_{ex} = \frac{I_a}{I_b} \cdot \frac{S - (X - R)}{(X - R)} \qquad \text{(Eq. 16)}$$

where R is the volume of titrant acid equivalent to that added but not consumed in the actual titration reaction. In the limiting case in which R is negligible Eq. 16 becomes equivalent to Eq. 5. When R becomes significant compared

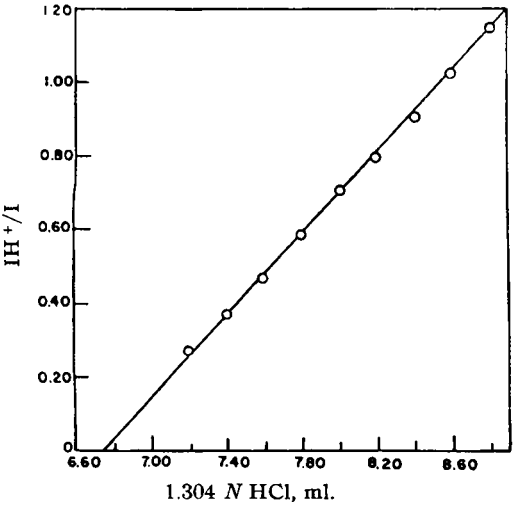

Fig. 14.—Type III plot for titration of aniline in water with hydrochloric acid, using Metanil yellow indicator (29). [Courtesy of *Analytical Chemistry*.]

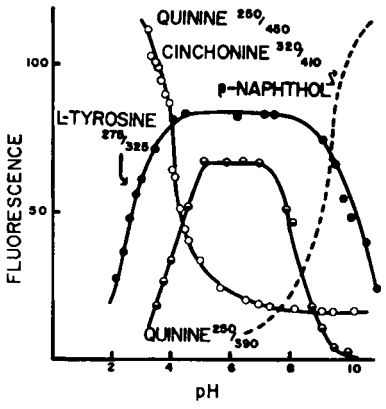

Fig. 15.—Representative pH-fluorescence curves for the three most common types of fluorescent substances. Key: ---, acid type (β-naphthol); —○—, basic type (quinine, activation/fluorescent wavelength 250/450 mμ); —◉—, bifunctional type (quinine 250/390 mμ).

with X, then curvature is observed in the conventional Type II plots as described. There may be several sources of this deviation:

(*i*) The indicator is present in sufficient concentration that it accounts for the consumption of a significant volume of titrant acid; then $R' = [IHA]$.

(*ii*) The solvent contains a basic impurity C which consumes titrant; then $R' = [CHA]$.

(*iii*) Most important, the titration reaction does not go to completion (that is, the product of the reaction undergoes solvolysis).[3] Then $R' = [HA]$.

A practical experimental technique has been developed which eliminates all three sources of error (27). The procedure involves the titration of two samples identical except for the amount of sample base. Let sample 1 be the smaller of the two. Then Eq. 16 represents the titration of this sample, the volumes being denoted X_1 and S_1. A similar equation describes the titration of sample 2. The quantity R is identical (or practically so) in the two titrations at a given value of the indicator ratio. The two equations are now subtracted, at constant I_a/I_b, thus eliminating R. The resulting equation can be placed in the usual Type II forms; for example, the Type II-b equation becomes $(X_2 - X_1)(I_b/I_a) = (S_2 - S_1)/K - (X_2 - X_1)/K$ which may be compared with Eq. 9. The end point represents the titration of the difference between samples 1 and 2. This technique is called the modified Type II titration (27). It is effective in eliminating the sources of error listed above. Some special cases of mixture analysis have also

[3] The limitations thus imposed on the use of Eq. 5 have been analyzed in detail by Bruckenstein and Nelson (24).

been treated (27). Details for its application will be found in the original papers (22, 27).

The general application of indicator ratio titration plots to the analysis of mixtures has not yet been attempted. It must be kept in mind that a second basic substance will cause an interference which cannot be circumvented by the modified Type II technique unless this impurity is introduced in the solvent. If the impurity is present in the sample its amount cannot be varied independently of the sample base and its influence cannot therefore be subtracted out.

Although the discussion throughout has treated the titration of a base with an acid, the reverse case is analogous, the ratio I_a/I_b appearing as its reciprocal, I_b/I_a.

FLUORESCENCE MEASUREMENTS IN HOMOGENEOUS SYSTEMS

Fluorescence is a function of the molecular species present in solution and may often therefore be used as a means of determining the change in ionic form occurring during a titration. The fluorescence of a dissolved substance is very nearly a linear function of the concentration of the fluorescing ion so that it may be used in a manner quite analogous to absorbance. Fluorescence varies with concentration according to

$$F = PC \qquad \text{(Eq. 17)}$$

where F is fluorescence (in relative photometer units), C is the concentration of the fluorescing species, and P is a constant relating the two which is a function of the structure of the molecule, the incident light power, and the Beer's law constants for the system.[4]

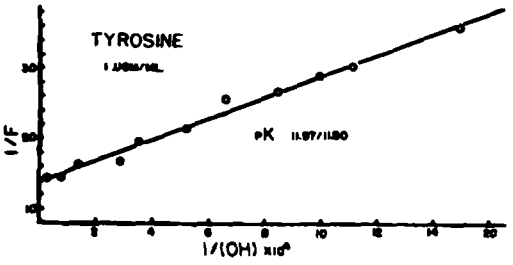

Fig. 16.—Titration of 1 mcg. tyrosine (1 mcg./ml.), pK (found/lit.) 11.97/11.80.

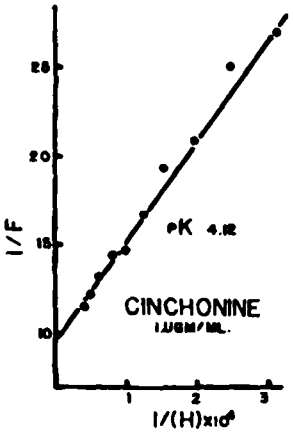

Fig. 17.—Titration of 1 mcg. cinchonine (1 mcg./ml.), pKfd 4.12.

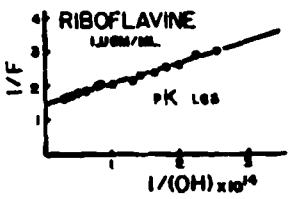

Fig. 18.—Titration of 1 mcg. riboflavin (1 mcg./ml.), pK fd 1.63.

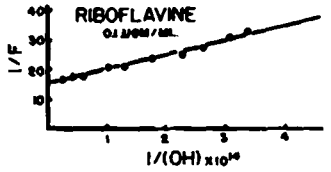

Fig. 19.—Titration of 0.1 mcg. riboflavin (0.1 mcg./ml.).

Just as with absorbance, titrations involving fluorescence may be performed using either the activity of the compound itself as a measure of ion concentration (self-indicator or direct titration) or the change of the fluorescence of an indicator to determine the effect of added titrant (indicator titration).

Self-Indicator Direct Titrations

Fluorescence-pH curves may assume three general shapes (see Fig. 15). Those molecules with two functional groups generally show a humplike curve as shown for tyrosine and one wavelength combination for quinine. The acid type is represented by naphthol and the basic type by quinine and cinchonine. As may be expected, any of these fluorescence-pH slopes may be used for titration.

Plots to determine the end points in direct titrations using fluorescence suffer from the same problems that are observed in absorbance "self-indicating" titrations, in that there is a lower concentration limit at which the effect of

[4] The exact expression is $F = ZI_0(1 - e^{-abc})$, where the term F is the observed fluorescence, Z is a constant relating the efficiency of the conversion of absorbed light to that fluoresced, both terms being measured in relative photometer units, and I_0 incident light power.

The complete expansion of the above is

$$F = ZI_0\left(abc - \frac{(abc)^2}{2!} + \frac{(abc)^3}{3!} - \ldots\right)$$

so that the assumption of linearity, i.e., $F = (ZI_0ab)\,C = PC$, involves an error of about 1 per cent or less for molecular weights of about 300, 1-cm. cells, absorptivities of about 1000, and concentrations of about 10^{-6} M.

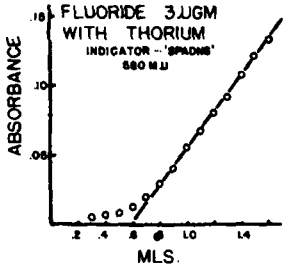

Fig. 20.—A fluorescent indicator titration of fluoride with thorium (35).

the solvent swamps that of the sample (11). In the extremely dilute solutions measurable by fluorescence, data may easily be obtained well beyond the limiting product $C_{HA}Ka = 10^{-12}$, but the end point is not readily observed. Data from titrations of these dilute solutions can, however, be used to obtain pKa values for fluorescent compounds.

Rearrangement of the general equations for the dissociation constants for acids and bases, using the approximation in Eq. 17 to estimate the concentration of the fluorescing species, yields equations suggesting linear plots for easily obtaining Ka values for fluorescing substances

$$1/F = Ka/PC(1/H^+) + 1/PC \quad \text{(Eq. 18)}$$

$$1/F = K_w/KaPC(1/H^+) + 1/PC \quad \text{(Eq. 19)}$$

for either the case where the conjugate acid (Eq. 18) or the conjugate base (Eq. 19) is fluorescent. By the simultaneous measurement of pH and fluorescence, the quotient of slope and intercept of the appropriate graph will yield values for the Ka (31) even in very dilute solution; see Figs. 16–19. Quantities of material as small as 0.1 mcg. and probably lower can be determined reproducibly as only 1 ml. of solution is required for titration.

Indicator Titrations

Fluorescence titrations with indicators are much more commonly used than are direct titrations; many examples are available, particularly those in which metal complexation or chelation is involved, *e.g.*, see (4). Their prime use has been for metal titrations, but they need not be so limited. Fluorescent compounds can be quite effective as pH indicators, for instance, although they would seem to offer little advantage over standard indicators except for their intense color. Their ability to fluoresce brightly in very thin layers, however, has suggested their use in titrations involving very dark or opaque solutions and suspensions (32). Many fluorescent pH indicators are available (33, 34), and the entire pH range is accessible.

Despite the considerable application of fluorescent indicator titration, it appears that investigators using this technique have not made the most of the recent advances in the field of data handling. A scan of the likely literature indicates that, although they are amenable to such processing, fluorescent indicator titrations are not being evaluated through the use of the "indicator ratio" equations, despite the strong evidence (from absorbance titration results) of their superiority over direct fluorescence *versus* volume plots. Figure 20 (35) shows the results of a thorium titration of fluoride, using SPADNS (a phenylazonaphtholene sulfonate) as an indicator, plotted as a direct titration. After total precipitation, the color of the thorium-SPADNS complex develops. While this is a satisfactory end point, the data prior to the end point could have been used to get the same end point value using a Type I plot (Fig. 21). If the addition of thorium had been carried on far enough to permit the top of the complex-color curve to be observed, this system probably would be better graphed as a Type III plot.

The sharpness of break at the end point in some of the direct titration plots in the literature hides the fact that the end points are not always so clearcut. Considerable rounding often occurs near the end point, making extrapolation dif-

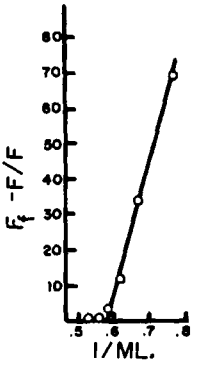

Fig. 22.—A replot of the data of Howerton (8) for the 80 μl. Labtrol 4 × 10^{-4} EDTA titration of zinc (calcein), according to Type II mathematics.

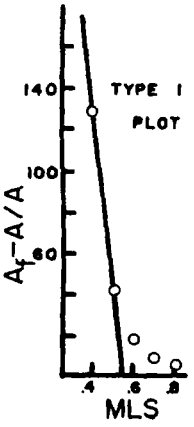

Fig. 21.—A replot of the data of Fig. 20 according to Type I mathematics.

ficult. The back-titration of EDTA with zinc in a calcium determination with calcein indicator, by Howerton and Wasilewski (8), was such a situation. The data obtained by these authors, replotted with Type II mathematics, yielded Fig. 22, showing clearly the applicability of such end-point extrapolations.

The sensitivity of fluorometric titrations is great. Titrations of very fluorescent substances (using Eqs. 18 or 19) in the 0.1 to 1 mcg. range can easily be duplicated to within 2 to 5 per cent without special precautions. The indicator method without plotting improvements yields 1 to 5 per cent accuracy on samples of about 1 mcg./ml. The more sophisticated data handling methods should afford better results.

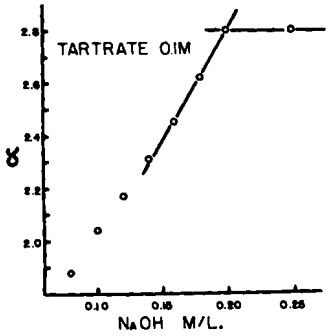

Fig. 23.—The titration of optically active tartrate with NaOH using optical rotation to locate the end point (37).

METHODS BASED ON OPTICAL ROTATION

End points depending upon changes in optical rotation can be used only for compounds having rather specific structural features. They must be optically active, and their activity must change, preferably by a great deal, during the course of the titration.

Self-Indicator (Direct) Titrations

While a considerable amount of work has been published on the effects of pH on optical rotation, especially by Britton and his co-workers (36, 37), these were not actually titrations. These workers were mainly interested in using optical rotation to detect the ionic species present during complexing. Their data were obtained as in a titration, however, and can be used to demonstrate the methods. Examples of some of their original data, replotted as for a titration, indicate that (although these workers apparently did not see the value of it at the time) polarimetric titration end points can be obtained (Figs. 23 and 24). Britton and co-workers do not appear to have been the first to use polarimetric-pH data, although they seem to have been the most prolific (38). These figures show the titration of tartrate and lactate, their own rotation being used as an indication of the degree of titration (self-indicator). The change in the case of tartrate is quite spectacular, while the lactate case leaves something to be desired; but both are usable.

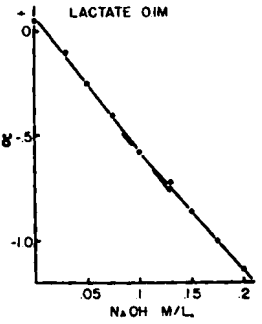

Fig. 24.—The titration of optically active lactate with NaOH using optical rotation to locate the end point (36).

The recent availability of spectropolarimeters has made it convenient to select the wavelengths most suitable for titration studies, creating possibilities for selective titrations of mixtures, and for maximizing end-point breaks by selecting appropriate wavelengths.

Indicator Titrations

Recently, Kirschner and Bhatnagar (39) have described work done with a Beckman DU modified by the Rouy method (40) and enlarged to take a 10-cm. cell. They report sample curves for an acid-base titration (HCl with NaOH) using about 0.04 *M* D-tartaric acid as the indicator. The sharpness of the end point is notable, but is largely due to the very high indicator concentration (see Fig. 25). The reactant concentrations were 0.1 *N* HCl and 5 *N* NaOH, so that one might expect sharp end points. The authors assert that the second break is due to the second ionization constant of tartaric acid; *i. e.* this is a "self-indicator" titration that they suggest can be used to find the concentration of the indicator present. While the end point shown on the figure and in their paper is clear enough, a con-

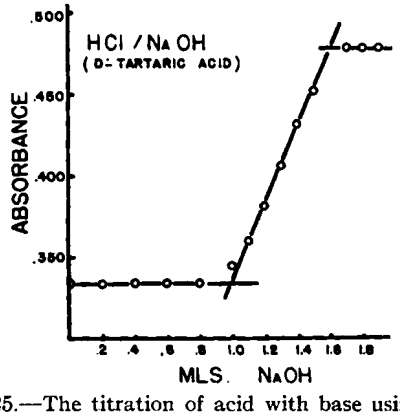

Fig. 25.—The titration of acid with base using an optically active indicator (39).

siderable improvement in the data treatment for this type of titration, which should be helpful when less definite end points are obtained, can be achieved by using the Type III "linear plot"—the one designed for indicators that change after the end point. The results of this type of plotting are shown in Fig. 26. The authors have also shown this procedure to be useful in the study of the interaction of metals and optically active complexers, *l*-histidine in the case shown in their paper, although this use has been developed to a much greater degree by Britton and his co-workers (37) and others, *e.g.*, see (41) and (42).

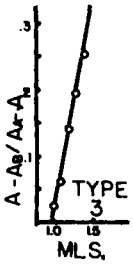

Fig. 26.—A replot of the data of Fig. 25, according to Type III mathematics.

It would not seem that this would be a particularly sensitive method, although it is certainly specific, and very likely would yield good pK values for specific groups even when associated with other groups with similar pK's. The large volume and high indicator concentration that seem to be required would appear to limit its usefulness. All that is required for improvement is the discovery of some really active pH-sensitive compounds.

One important point that should be recognized is the rather large temperature effect on optical rotation of some optically active molecules—up to 10 per cent /° C. for tartaric acid, for instance (43)—and the relative ease with which the racemization of isomers can occur, particularly in strong acid or base. Thus, if one proposes to develop a very accurate and sensitive method using this technique, temperature control is necessary.

ABSORBANCE MEASUREMENTS IN HETEROGENEOUS SYSTEMS

Self-Indicator (Direct) Titration

Titrations using the separation of another phase to signal the end point have been grouped into three categories: turbidimetry, nephelometry, and heterometry. These are all characterized by measurements of the effects of a dispersed phase on the passage of light through the system, and the first two (turbidimetry and nephelometry) while requiring different equipment are, from the standpoint of visually detected end points, the same. The end points for heterogeneous titrations are always made at the point of complete precipitation and visual detection of this point is some small problem, as one must determine the titrant volume that causes the last bit of precipitation in a cloudy solution.

Several very clever methods have been devised to overcome this limitation, GayLussac's "equal turbidity" method being one (44). All of the other schemes devised to sharpen the observation of the faint opalescence produced in the supernatant by "one more drop of titrant" have encountered the problem of the common ion effect. As a point of interest, it was to determine this "amount still in solution" in the AgCl titration that led Richards and Wells (45) to develop the nephelometer during their early studies on the atomic weight of silver.

Instrumentally, turbidimetry and nephelometry differ; transmitted light is measured in turbidimetry, scattered light in nephelometry. While both are instrumentally similar save for geometry, nephelometry (in which one measures a small change against a dark background) should be fundamentally more sensitive than turbidimetry, which, like colorimetry, involves measuring a small change against a bright background.

Heterometry is a field in itself. Largely pioneered by Bobtelsky and co-workers (46), it is not actually a titration tool at all, but rather an investigational tool for studying multiphase relationships and phase changes. It would seem from Bobtelsky's earlier work that quantitative titration was a valuable side product rather than an aim of the development of heterometry.

Heterometry differs from turbidimetry more in point of view than in any profound manner. Its development stemmed, it seems, from the desire to study multiphase systems without additives (such as polymers to retard crystal growth and influence particle size) and it produced a simplified turbidimetric titration procedure by ignoring "principles" considered to be absolute in turbidimetry and nephelometry. Thus, in many cases, Bobtelsky has found that differing rates of nucleation and growth, admittedly problems, affect the absolute values of the absorbance or light transmission but not the critical or "break" points which are of interest in titration.

For the moment we shall ignore the theoretical difference between scattered light measurements and transmitted light measurements (we will merely assume that one need only change ordinates to plot one or the other). Figure 27 shows the eight types of precipitation titrations. Graph 1 depicts the classical one obtained from

the ideal case where the precipitate is virtually insoluble. Reagent A plus reagent B creates a nearly insoluble precipitate AB. Additional A has no effect on the state or amount of precipitate.

Graph 2 describes the case in which the precipitate forms but, at the equilibrium point B, a second 1:1 solubilizing reaction begins which proceeds to completion at about A_2B. Graphs 4 and 7 show similar situations where the dissolution is either nonstoichiometric or involves other ratios of titrant to titrand than that in Graph 2. Point B in Graphs 1 and 2 represent stoichiometric formation of a product and thus are usable end points.

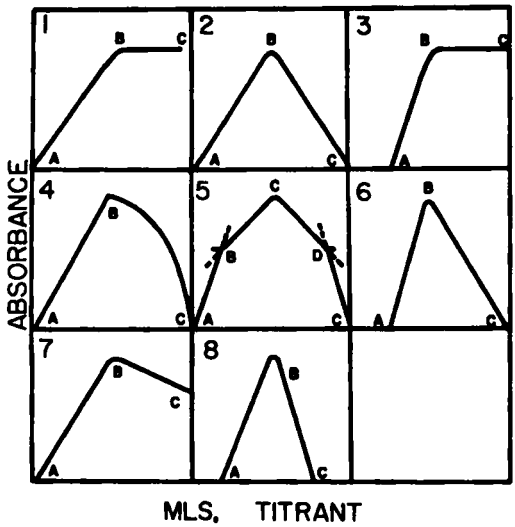

Fig. 27.—The eight general types of multiphase titrations, after Bobtelsky.

Graph 3 shows the formation of either a partially soluble product or a soluble interaction product (complete at point A) which secondarily interacts with another molecule of titrant to produce complete precipitation at point B. This is much like the Ag(AgCN) reaction. Graphs 6 and 8 show the same situation, but include quantitative precipitation followed by further solubilization of the precipitate; different ratios of titrant-titrand are required to achieve the same result in the two cases shown. The initial point A then represents either the solubility of the product or the stoichiometric formation of the soluble interaction product.

Graph 5 shows the formation of insoluble intermediates which rearrange to more stable forms upon addition of more titrant.

There is a great number of possible combinations of these basic systems, and in addition no limit to the possible number of intermediates. The detection of a quantitative end point depends upon a knowledge of the reaction in order to know which portion or portions of the curve can be used, although in theory any "critical point" should be usable. Complicating the analytical problem are practical limitations such as partial solubility of the precipitate, rate of precipitation at the end point, and coagulation caused by excess titrant which produces nonlinear portions that defy extrapolation.

The problems of speed of nucleation and whether crystal growth is occurring rather than new nucleation have been theoretically discussed by Fischer and co-workers (47). Slow crystallization is a prime concern because it is just at the end point, where the degree of supersaturation is lowest, that the rate of precipitation is also the slowest, making equilibration at this point of some concern. In this connection Bobtelsky asserts that equilibration is usually reached "after a few seconds to one minute" as evidenced by stable photometer readings on his "heterometer." Because light scattering and turbidity should both be functions of particulate surface area, and the mass of precipitate a function of titrant volume, calculated data for surface area as a function of added titrant should closely approximate absorbance-volume data from titrations. Fischer (47) was able to perform this calculation for the two extremes to be expected for absorbance *versus* titrant volume plots (Fig. 28). The upper (straight) line in Fig. 28 represents the situation where only nucleation occurs without crystal growth. The surface area increase is roughly linear with the mass of preicpitate (light absorbance thus should be a linear function of the added titrant volume). When the worst possible situation occurs, an initial seeding followed only by crystal growth and no further nucleation, the parabolic lower curve of Fig. 28 results. All possible variations

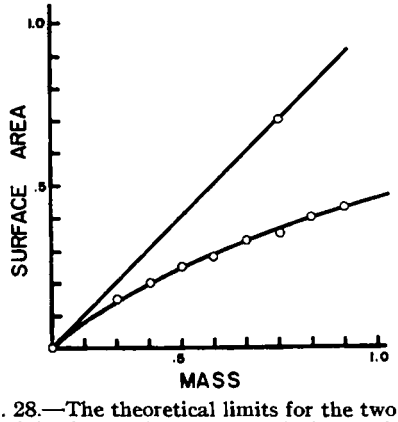

Fig. 28.—The theoretical limits for the two types of precipitation to be expected during a titration (47). The upper line, nucleation without crystal growth, lower line, growth only without nucleation. All combinations should lie between these two extremes.

and degrees of these two should lie between these two extremes. The more curved (crystal growth) cases will produce titration end points which are hard to detect, being marked by the intersection of a nearly horizontal curved line with the horizontal one occurring after the end point.

The solution to this problem often simply requires the addition of a protective colloid. While this is usually effective, as one might expect, crystal-face poisoning is a highly selective phenomenon and no universal nonionic protective colloid has been found. Protective colloids have one other beneficial effect: they inhibit the adsorption and premature precipitation of the ions being titrated onto the surface of the precipitate, a troublesome problem that can produce early end points.

Despite the problems outlined, turbidometric end points can in many cases be quite straightforward. Consider the titration of 0.585 mg. of sulfate with $1.6 \times 10^{-3}M$ barium ion [Fig. 29 (48)] or the titrations of calcium with phthalate by Bobtelsky [Fig. 30 (49)].

An interesting nonmetallic, organic titration was published by Lambert (50). An anionic surfactant was titrated with a cationic agent to produce an almost perfect reproduction of one of Bobtelsky's theoretical curves (Fig. 25) involving a slightly soluble interaction product suddenly achieving saturation, then redissolving (or in this case reacting further to produce a soluble compound); see Fig. 31. These curves are endlessly repeated in the polymer literature, but this is an interesting pharmaceutical example.

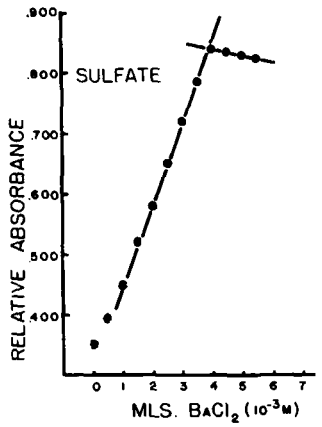

Fig. 29.—The nephelometric titration of sulfate (0.585 mg.) with barium ion (48).

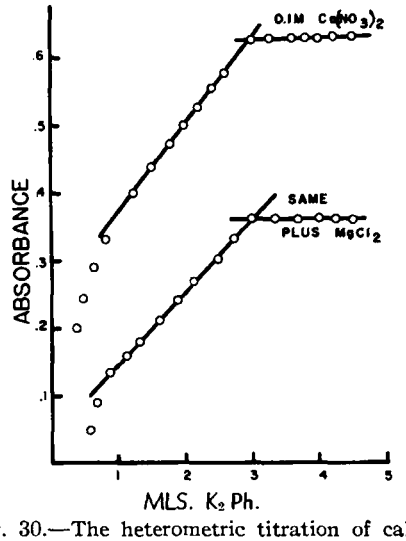

Fig. 30.—The heterometric titration of calcium ion with phthalate in the presence and absence of magnesium (49).

Indicator Titrations

There was a flurry of research done several decades ago on added indicators whose function was to precipitate at the end point of a titration (51). This "new principle," as it was termed by its inventor in 1925, seems to have died a very undeserved death. Several series of potentially useful indicators were tested, of which the most promising seemed to be the rhodamines and the *p*-aminoazobenzenes. Many of the derivatives of these parent compounds were quite effective and were able, according to the author, to precipitate in an extremely narrow pH range. These indicators were effectively used for the titration of several common acids (along with barbital, boric, and arsenous acids) with excellent results. The sharp change in the pH-solubility profile of these indicator compounds makes them somewhat more difficult to use than the usual indicator that telegraphs its end point by the color changes produced as the titrant is added. Fairly dilute solutions must be used as concentrated titrant produces an immediate precipitate which is only very slowly redissolved; thus high local concentrations should be avoided. The true end points for these titrations should be determinable photometrically using a Type III plot as discussed before.

Photoelectric titration using nephelometry has several pitfalls—the effects of stirring in particular—that are not present in other types of titration. In Fig. 32 the results of a fluorometric precipitation titration are plotted both with and without stirring during photoelectric readings (52). The stirred titration solution always results in higher photometer readings (fluorescence or absorbance) than the unstirred. Because the line after the end point is the same for both and the line before the end point is much steeper and thus less sensitive to suspended particles, stirring results in a poorer end point.

Nephelometric titration methods can be quite sensitive. Bobtelsky feels that his heterometric

method is capable of using solutions of $10^{-6}M$ concentration. In absolute amounts, 1–3 mcg. seems to be the current extreme, with the precision ranging from 0.1 to 5 per cent. Naegli and co-workers obtained a precision of about 0.2 per cent using isonitroso-acetyl-paraminazobenzene as a precipitation indicator (51). This was without instrumental aid; with the improved plotting procedures now available for indicator titrations, an instrumental titration using his indicators should be capable of much greater sensitivity and at least equal precision.

CONCLUSION

The literature of the past few years has recorded a great increase in both developmental work and applications of photometric titrations (though the same may be said of almost every analytical technique). Some of the advantages peculiar to these methods may be expected to lead to their continued wide use. The simplest types of photometric titrations are those utilizing direct plots of photometer readings against titrant volume to locate the end point, and when such plots yield sufficiently accurate results they should certainly be employed. The limitations of such procedures have been outlined, and the alternate procedures, involving indicators and the evaluation of an indicator ratio function, have been presented in sufficient detail to permit their use. The application of these more general methods to complexometric and precipitation indicator titrations should be especially fruitful.

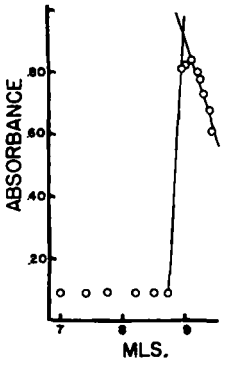

Fig. 31.—The nephelometric titration of an anionic surfactant (Igepon, 10^{-3}) with a cationic agent (Ceepryn, 10^{-3}) (50).

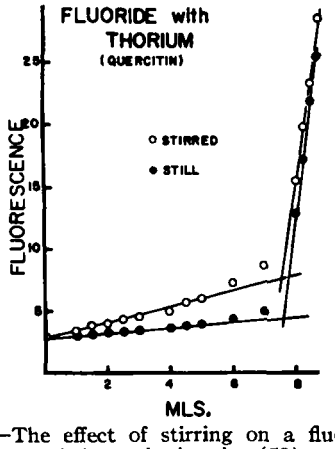

Fig. 32.—The effect of stirring on a fluorescent-nephelometric titration (52).

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