_____*Review Article*____

Kinetic Methods of Analysis

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K INETIC CONSIDERATIONS always have been of prime importance to the analytical chemist in the development and use of his procedures. Because he can be satisfied with no less than quantitative recoveries, he must utilize optimum experimental conditions for his reactions. He uses these conditions not only to produce the desired stoichiometric reaction in the minimum time but also to obtain a specific reaction with the least interference from side or parallel reactions. Analytical chemists have gone a step further and are applying the kineticists' reaction rate techniques directly to the solution of analytical problems. This has yielded methods which give quantitative results based on reactions which are not necessarily complete. In fact, some methods require the occurrence of only a small fraction of the reaction. Also, procedures have been developed for the determination of traces of materials based on their catalytic effect on the rates of suitable reactions. Another result is the production of methods for the differential determination of two or more compounds in a mixture which are reacting concurrently but at different rates with a common reagent. These developments in the use of reaction rate approaches are considered in this review.

KINETIC METHODS FOR A SINGLE REACTIVE SPECIES

The application of kinetic principles to analysis renders possible the use of reactions which nor-

mally do not produce quantitative conversions because of the establishment of unfavorable equilibria or because of the necessity for impractically long reaction times. The concentration of a single reactive species can be determined in some cases based on an accurate knowledge of the rate constant and the measurement of the rate of reaction of the sample. A near quantitative conversion is not required because a measurement of the fraction of the material which has reacted during a specified time interval can be related directly to the original concentration. For example, if the reaction is first order, the integral form of the rate expression is

$$kt = 2.303 \log \frac{a}{(a - x)} \qquad (\text{Eq. 1})$$

Then, if the reaction is run under carefully controlled conditions, and if x, which represents the amount of the initial concentration, a, which has reacted in time, t, is measured, a can be calculated using Eq. 1. The same technique can be used for second-order reactions, in which case the integral form of the rate expression is

$$kt = \frac{2.303}{(b - a)} \log \frac{a(b - x)}{b(a - x)} \qquad (\text{Eq. 2})$$

Here b is the initial concentration of the reagent which must be known. If $a = b$, the integral form is then

$$kt = \frac{1}{(a - x)} - \frac{1}{a} \qquad (\text{Eq. 3})$$

In practice, it sometimes is more convenient to

refer the rate found for the sample to a calibration curve prepared from data obtained using known amounts of the pure compound.

Blaedel and Petitjean (3) investigated the possibility of developing kinetic methods based upon measurements of the rate of a reaction at several times during the early stages of the reaction. They carried out a preliminary study of the utility of the approach using the alkaline hydrolysis of ethyl acetate. The reaction vessel was part of a tuned circuit of a high frequency oscillator and conductance changes in the solution as the reaction proceeded caused changes in the frequency of the loaded oscillator. The amount of organic compound in the sample being analyzed was then determined by referring the measured rates of frequency change, f/t, to a working curve plotted from data obtained with known amounts of the substance being determined. The results obtained with ethyl acetate indicated that the method yielded highly precise determinations of single constituents in samples free of interferences. They then applied the developed method successfully to acetylacetone determinations by measurement of the reaction rate of the ketone with hydroxylamine hydrochloride. This particular oximation reaction does not reach substantial completion in a practical period of time and, therefore, presents problems in the usual straightforward procedures. These investigators concluded that data obtained during the first 5 or 10 min. of a reaction are capable of yielding results accurate to 0.3% for simple systems.

The same principle has been used to determine glucose in serum plasma or blood, based on the enzyme-catalyzed oxidation of the sugar to gluconic acid and hydrogen peroxide. Guilbault and co-workers (27) followed the rate of peroxide production by recording the potential difference between two platinum indicator electrodes polarized with constant current. The potential changes are proportional to the glucose concentration. Malmstadt and Piepmeier (47) used a pH stat to maintain the pH stationary at a preselected value with continuous neutralization of the gluconic acid formed during the reaction. The instrument which they described provides direct digital readout of initial rate data that are proportional to the concentration of glucose present. Results reproducible within 1–2% in the 50–250 p.p.m. range were obtained. Use also has been made of the color produced by the action of the peroxide formed on *o*-tolidine (2, 68) or on *o*-dianisidine (45). The elapsed time for the production of a definite absorbance change is proportional to the initial rate and to the concentration of glucose. The peroxide produced during the reaction has been determined also by its rapid molybdate-catalyzed oxidation of iodide and the glucose oxidation followed by measuring the iodine formed potentiometrically (46, 58), spectrophotometrically (2, 41), and amperometrically (56).

Pardue and Frings (60) used autometric amperometric control equipment to provide a direct readout of the time required for a definite amount of iodine to be produced from iodide as the result of the enzyme-catalyzed oxidation of galactose to peroxide. This amperometric method was further extended to furnish digital readout in concentration units (61). Frings and Pardue (23) also used an automatic spectrophotometric measurement of the peroxide produced by the enzymatic reaction by measuring the color produced by the oxidation of *o*-dianisidine. A similar arrangement was used to obtain automatic direct readout of p.p.m. cystine based on potentiometric measurement of the rate of an azide–iodine reaction (57, 59, 62).

Malmstadt and Hadjiioannou (42) reported a similar technique for the determination of alcohol in blood. The time required for a small fixed change in absorbance as a result of the selective enzyme-catalyzed oxidation of the alcohol was measured in the early stages of the reaction, and this value related directly to the alcohol concentration. The method was used to determine alcohol in the range 0.015–0.300 Gm./100 ml. of blood with a relative error of 2–3%. Sample sizes ranged from 0.1–0.25 ml. and the time of measurement ranged from a few seconds to 2 min.

Malmstadt and Hadjiioannou (43) determined some L-amino acids by an automatic spectrophotometric reaction rate method based on the coupled enzyme reaction in which oxidative deamination is specifically catalyzed by L-amino acid oxidase to form hydrogen peroxide. The time required, from a few seconds to about 2 min., for the reaction to produce a small fixed amount of colored product as a result of the reaction of the resultant peroxide with *o*-dianisidine was related to the initial amino acid concentration. The concentration range determined was 4–50 p.p.m. with a relative error of about 2%.

A pH stat with digital readout of data collected early in the hydrolysis of urea in the presence of urease was used by Malmstadt and Piepmeier (47). The pH was maintained constant by adding dilute hydrochloric acid to neutralize the ammonia formed. The increments of acid added during a preset time interval, about 2.5 min., was counted. The count was directly proportional to the urea concentration. The procedure was

tested in the 2- to 10-p.p.m. range, and the results were reproducible within 1–2%.

The relationship between the concentration of the catalyst and the rate of the catalyzed reaction often is used to quantitatively estimate the catalyst. The approach is not new, but new and revised procedures based on it continue to appear because, where applicable, it is one of the most sensitive techniques devised to determine minute traces of materials. Some of these methods are cited here as illustrations.

The catalytic effect of copper on the oxidation of cysteine to cystine and the subsequent measurement of oxygen evolved in a specified time has been used to determine copper in blood (82) and in milk (9). The reduction of ceric ion by arsenite in an acid medium is catalyzed by soluble iodides. Sandell and Kolthoff (71) measured the time required for this reaction to go to completion and found a linear relationship between the reciprocal of that time and the quantity of iodine present. The reduction of the yellow ceric to colorless cerous ion was followed colorimetrically. The change in absorbance was recorded continuously, and the slope of the recorded curve was related to the iodine concentration by Chaney (14). Lein and Schwartz (39) used the first-order velocity constant as a function of the iodine concentration. Malmstadt and Hadjiioannou (44) noted the time, 10–100 sec., required for the reaction to consume a small fixed amount of ceric ions, and, therefore, the absorbance to decrease by a preselected amount, measured automatically, and related this directly to the iodine concentration. The latter investigators reported the determination of iodine in the range of 0.015–0.45 mcg. with a relative error of about 1–2%. Hadjiioannou (28) used this procedure to determine iodine in common salt and in natural waters. This same reaction system was used for the determination of labile organic-bound iodine by Zak and Baginski (86). Yatsimirskii and co-workers (84) determined micro amounts of iodides by their catalytic action on the oxidation of thiocyanate with ferric iron and with nitrate. Changes in the extinction of the thiocyanate complex of iron were evaluated. The sensitivity of this method was reported to be 0.001 mcg./ml. and the error of a single determination was about 2%.

Underwood, Burrill, and Rogers (81) determined submicrogram quantities of silver using the catalytic action of silver on the persulfate oxidation of manganous ion to permanganate. Spectrophotometric measurement at 525 mμ after a specified time was related to a calibration curve prepared using standard silver solutions. The practical lower limit of the method was reported to be about a millimicrogram of silver.

Vanadium operates as a catalyst in the oxidation of p-phenetidine citrate by potassium chlorate in the presence of phenol as an activator yielding a colored product. Bontschev (10) determined 0.1 mcg. of vanadium by means of the linear relationship between the extinction at 510 mμ and the time of heating. Fishman and Skongstad (19) used the catalytic effect of vanadium on the rate of oxidation of gallic acid by persulfate in acid solution to determine vanadium in water. The absorbance at 415 mμ was determined and compared with standard solutions treated in an identical manner. The procedure is applicable for concentrations of vanadium in the range 0.1–8.0 mcg./L. and shows a standard deviation of 0.2 or less.

Alizarin is oxidized only slowly by hydrogen peroxide but in the presence of cobalt chloride in alkaline solution, the rate is increased in proportion to the concentration of alizarin and the cobalt. The color of the alizarin is destroyed by the oxidizing action of the peroxide, and then if the extinction of the solution is measured at intervals after the addition of the cobalt solution, the rate of reaction can be determined. A calibration curve was used (64) to determine cobalt in concentrations ranging from 0.001–0.0066 mcg./ml. Bognar and Jellinek (5) determined as little as 0.002 mcg. of cobalt with an accuracy of ±10% by measuring the rate of reaction of diphenylcarbazone and hydrogen peroxide in the presence of iron. They measured the time interval to the attainment of a specific extinction by the reaction mixture.

Yatsimirskii and Raizman (85) utilized the catalytic effect of zirconium and hafnium on the oxidation of iodide by hydrogen peroxide to determine the concentrations of these catalysts.

Bulgakova and Zalubovskaya (13) followed amperometrically the rate of iodide oxidation with hydrogen peroxide as a method to determine molybdenum in cadmium sulfide and in lithium fluoride crystals.

Pantaler (52) determined tungsten and molybdenum based on the catalytic oxidation of rubeanic acid by hydrogen peroxide in 0.1 N hydrochloric acid. He plotted absorbance against time and obtained the amount of tungsten or molybdenum by comparing the slope of the line with slopes given by standard solutions. He reported the method satisfactory for 0.1 mcg. of either metal in 25 ml. of reaction mixture.

Babkin (1) followed the rate of the manganese-catalyzed permanganate–oxalate reaction to determine concentrations of manganese in the

0.01–0.6 mg. range. The results were obtained from a calibration curve constructed for the relationship between reaction time and amount of manganese. Fernandez, Sobel, and Jacobs (18) used the oxidation of leucomalachite green with periodate in the presence of manganese for the determination of submicrogram amounts of manganese in human serum. The progress of the reaction was followed photometrically, and the slope of the rate curve was compared with the slope for a standard curve to calculate the manganese content.

Bognar and Sarosi (6) investigated the effect of osmium tetroxide on the kinetics of oxidation of hydroquinone, orcinol, and 1,3-dihydroxynaphthalene by hydrogen peroxide and found they could determine as little as 0.001 mcg. of osmium tetroxide in 5 ml. of solution. (Ethylenedinitrilo) tetraacetic acid (EDTA) does not interfere with the catalytic reaction so that this compound could be used to increase the selectivity of the method. The same workers (7) also determined osmium tetroxide based on its catalytic effect on the oxidation of 3,3′-dimethylnaphthidine by potassium chlorate. The formation of the violet-red colored product was followed spectrophotometrically.

Michalski and Wtorkowska (50) established the optimum conditions for the determination of sulfide and thiosulfate (51) from the catalytic effects on the iodine–azide reaction. The rate was determined from the changes in iodine concentration which was followed amperometrically with a rotating platinum indicator electrode. The value of the potential which should be applied to provide the limiting current, proportional to concentration, was found from polarographic measurements. Sulfide was determined in the range 10^{-15} to 10^{-13} Gm./ml. with a deviation from the mean of less than 10%.

The catalytic activities of acids and bases in selected reactions can be used for the determination of the concentrations of these species. For example, Clibbens and Francis (15) and Francis and Geake (22) found the decomposition of nitrosotriacetonamine into nitrogen and phorone to be a function of the catalytic activity of hydroxyl ion. The original concentration of nitrosotriacetonamine was known and the rate of decomposition found by measuring the nitrogen produced as a function of time. A relationship between the first-order velocity constant for the reaction and the hydroxyl ion was determined and used. Duboux (17) used a proportionality between the hydrogen ion concentration and the velocity constant for the acid-catalyzed inversion of sugar to determine acidity.

The general enzymatic reaction rate method can be used also to determine enzyme activity. Hadjiioannou and Santos (29) determined serum lactic dehydrogenase from the effect of this enzyme on the rate of oxidation of lactic acid in the presence of diphosphopyridine nucleotide to form a species absorbing in the ultraviolet. An automatic potentiometric method for the determination of glucose oxidase activity similar to the procedure used to determine glucose has been demonstrated (63). The direct electrochemical measurement of peroxide produced has been used also to determine glucose oxidase (27). The same electrochemical technique has been used to determine cholinesterase and acetylcholinesterase based on the rate of enzymatic hydrolysis of thiocholine ester by the enzyme sample (36). The method has been extended to the analysis of some highly toxic organophosphorous compounds which act as anticholinesterase compounds where the decrease in rate of the cholinesterase–butyrylthiocholine ester hydrolysis is linearly related to concentration of the organophosphorous compound (26).

Magerum and Steinhaus (40) introduced an interesting kinetic method for the determination of ultratrace concentrations of metals. They used a coordination chain reaction involving the exchange of triethylenetetramine–nickel II (trien –Ni II) and (ethylenedinitrilo)tetraacetocuprate II (EDTA–Cu II). The presence of free ligand, which can originate from the pure complexes or can be added separately, greatly accelerates the reaction. The bright blue color of trien–Cu II produced is used to follow the progress of the reaction. A metal at 5×10^{-8} *M* concentration can react with free ligand (EDTA) reducing the exchange rate. The metal ion detected in this manner need have no connection with the reactant metals, but only be capable of reacting with EDTA. The reaction is first order with respect to the EDTA concentration and was followed for at least half-life, normally 10–15 min. A calibration curve was prepared of the rate constant against concentration of EDTA used. Experimental results were given down to 10^{-8} *M*.

DIFFERENTIAL REACTION RATES TO ANALYZE MIXTURES

The differential reaction rate approach is effective for the analysis of mixtures of two or more compounds, each of which reacts at a different rate with a common reagent, resulting in products which are either identical or are indistinguishable by the analytical method used.

The rate of disappearance of the reagent or the appearance of a product is monitored to obtain data for the total reaction of the components. Usually a graphical analysis or an equivalent mathematical treatment of the data based on the appropriate rate law is then applied to quantitatively resolve the mixture. The process normally requires a prior knowledge of the reaction order. The applicable graphical plot for a mixture of two reactive components shows a linear portion arising from the reaction of the slower component after substantially complete consumption of the faster component has occurred. The extrapolation of this linear portion to zero time provides data which can be used to calculate the original composition of the mixture.

Thus, for a first-order reaction $\log (a - x)$ is plotted against t, based on Eq. 1. In this case, a represents the total concentration of the reactive components of the mixture and is determined by an independent method or by permitting the rate reaction to go to completion. For a two-component mixture, $a = a_1 + a_2$, if a_1 and a_2 are the concentrations of the faster and slower reacting constituents, respectively. When the final linear portion of the plot is extrapolated to $t = 0$, $x = a_1$ and $a - x = a - a_1 = a_2$, and sufficient data are then available to calculate the composition of the mixture. This process is illustrated in Fig. 1. If P is the value at the intercept $t = 0$, then from Eq. 1

$$\log a_2 = P \qquad \text{(Eq. 4)}$$

The concentration of the remaining component, a_1, is obtained by difference from a.

In some cases, it may be more convenient to transform a second-order reaction, one that is first order with respect to the reactant to be determined and first order with respect to the reagent, into one that is pseudo first order. To achieve this, the reagent is used in sufficient excess that its concentration can be considered constant. In this event, the data are treated according to the normal first-order rate laws. However, in most situations, if a large excess of reagent is
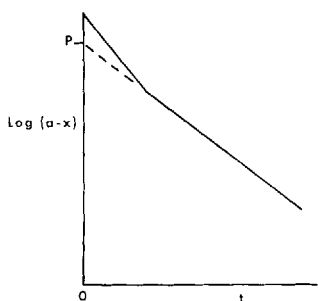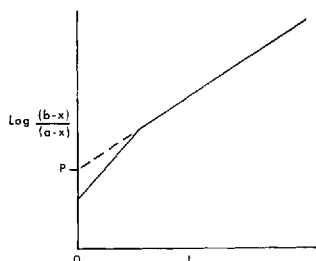
Fig. 2.—Graphical extrapolation procedure for a second-order reaction.

present, the reaction becomes so rapid that it is not manageable.

The procedure is based on Eq. 2 for a second-order reaction if the total concentration of the reactants and the concentration of the reagent are unequal. The initial concentration of the reagent, b, and the total initial concentration of reactants must be known and $\log [(b - x)/(a - x)]$ is plotted against time. Extrapolation of the final linear portion is performed as described for the first-order reactions and is illustrated in Fig. 2. For the second-order case, at the intercept, $t = 0$,

$$\log [(b - a_1)/(a - a_1)] = P \qquad \text{(Eq. 5)}$$

Eq. 5 is solved for the concentration of the faster reacting constituent, a_1, and a_2 is determined by difference from a.

A plot of $1/(a - x)$ against t is used for the special second-order rate situation where $a = b$ (Eq. 3). At the intercept of the extrapolated final portion of the plot with the zero time axis

$$1/a_2 = P \qquad \text{(Eq. 6)}$$

Eq. 6 can then be solved for a_2, and a_1 obtained by difference. This procedure based on the equivalency of the total concentration of the components of the mixture determined and the reagent concentration is more convenient in some cases and simplifies the computations. If there is not a precise match of a and b, however, a small error in the slope of the linear portion can result in a large error in the calculation of a_2, particularly if a_1 is relatively large. Also, if the imbalance is quite significant, the latter portion of the curve will not be linear. Reilley and Papa (66) rearranged Eq. 3 and obtained the form indicated in Eq. 7.

$$x = k_2 a_2 (a - x) t + a_1 \qquad \text{(Eq. 7)}$$

Here k_2 represents the rate constant for the slower reacting component. When x is plotted against $(a - x)t$, a straight line is obtained after substantially all a_1 has reacted. The slope of this straight line portion is then $k_2 a_2$, and a_1 is the intercept at $t = 0$.

Fig. 1.—Graphical extrapolation procedure for a first-order reaction.

A scheme has been presented for differential rate analysis for second-order reactions applicable to mixtures of organic compounds which react with differing stoichiometry (8). It involves the introduction into Eq. 2 of a term indicating the number of moles of a polyfunctional compound which reacts with the reagent. It is illustrated by application to the hydrolysis of mixtures of cyclotrimethylenenitramine and cyclotetramethylenenitramine by sodium hydroxide. However, such systems usually can be handled easily on the equivalency basis rather than on the mole basis without resorting to special equations.

ANALYSES BASED ON SINGLE AND DOUBLE POINTS

It is possible to calculate the concentration of each component in a binary mixture based on a single determination after a measured reaction time if the rate constant for the reaction of each component and the total original concentration of the components are known. If measurements are made at two separate times, it is also possible to calculate the original composition without the use of rate constants.

Lee and Kolthoff (38) expressed the first-order rate law as shown in Eq. 8.

$$a - x = a_1 e^{-k_1 t} + a_2 e^{-k_2 t} \qquad \text{(Eq. 8)}$$

The rate constants for the two components are k_1 and k_2. Therefore, if $(a - x)$ is measured and k_1, k_2, and t are known, then the values of a_1 and a_2 can be found from a calibration curve prepared from the analysis of known mixtures or from calculations based on Eq. 8. The calibration curve is the linear plot obtained from values of $(a - x)/a$ versus a_1/a. Garmon and Reilley (24) proposed a double point method also based on Eq. 8. They determined the amount of reaction at two different time intervals, substituted the known and measured values into Eq. 8, and solved the two equations simultaneously to obtain values for a_1 and a_2.

Reilley and Papa (66) based a single point method for second-order reactions on Eq. 3. To make this equation applicable they arranged the concentration of the reagent equal to the total concentration of the components in the mixture being determined, *i.e.*, they used the conditions that $a = b$. In addition to the value of x measured at a single time, the rate constant must be known. The rate constants need not be known if x is measured at two different times and the resulting two equations are solved simultaneously.

The single and double point methods have been extended so that it is not necessary to have the

condition of equivalency of the reagent concentration and the total sample concentrations (54). In this case, Eq. 2 is used with the requirement that the value for x is obtained after complete reaction of a_1, and the rate constant for the reaction of the slower component of the mixture is known. Again this process can be expanded to a double point method obviating the need for the rate constant if the process of solving simultaneous equations is used.

Roberts and Regan (67) proposed a somewhat different approach for the analysis of mixtures. They changed a normally second-order reaction to pseudo first order by having the concentration of the sample mixture in large excess relative to the reagent. Then they represented the first-order rate constant, k, by

$$k = k_1 a_1 + k_2 a_2 \qquad \text{(Eq. 9)}$$

Because $a_1 = a - a_2$, Eq. 9 can be rewritten to obtain

$$a_1 = \frac{k - k_2 a}{k_1 - k_2} \qquad \text{(Eq. 10)}$$

If k_1 and k_2 are separately determined for pure compounds, a is determined and k is measured for experimental mixture, then Eq. 10 can be solved for a_1, and a_2 can be obtained by difference from a.

Greinke and Mark (25) used the Roberts and Regan approach but followed the reaction in two different solvent mediums and obtained two different over-all pseudo first-order rate constants. Then by proper substitution in Eq. 9 and simultaneous solution of the two resulting equations, they were able to resolve binary mixtures.

APPLICATIONS OF DIFFERENTIAL KINETIC ANALYSIS

The differential kinetic approach has proven quite general and has been applied to the analyses of most of the organic functional groups. Siggia and Hanna (73) used the second-order graphical method involving extrapolation to the zero time intercept to analyze mixtures of hydroxyl-containing compounds. They monitored the reaction with acetic anhydride and determined primary alcohols in the presence of secondary alcohols, primary hydroxyl groups in polyhydric alcohols also containing secondary hydroxyl groups, and an alcohol in the presence of its next higher homolog as well as in the presence of one further separated in the series. Reilley and Papa (66) applied the graphical approach based on Eq. 7 and also the single and double point methods with the condition that $a = b$ to analyze mixtures of *n*-butanol and *sec*-butanol. Hanna and Siggia

(32) determined the primary hydroxyl group content of polyglycol ethers by graphical extrapolation of the kinetic data obtained by reaction of the hydroxyls with both acetic anhydride and phenyl isocyanate. Budd (12) substituted phthalic anhydride as the reagent for the same system when aldehydes or phenols were present. Hendrickson (33) used an infrared method to determine primary hydroxyl groups in polyglycol ethers. He used trityl chloride as the reagent, followed the disappearance of the hydroxyl band, and performed a graphical analysis of the kinetic data. Willeboordse and Critchfield (83) followed the disappearance of the NCO band in the infrared spectrum as the hydroxyl groups of mixtures of alcohols including polyglycol ethers reacted with phenyl isocyanate. They showed the applicability of the graphical approach and the single and double point methods to their process. They also analyzed ternary mixtures of alcohols based on the equations worked out by Schmalz and Geiseler (72). These equations require two data points and the rate constants, but become very involved and impractical for more than three component mixtures. Mark (48) published data showing that enzyme catalyzed reactions can be applied to the analyses of mixtures of ethanol and *n*-propanol by a kinetic method involving double points. He used alcohol dehydrogenase to catalyze the oxidation of the alcohols at different rates and followed the reactions spectrophotometrically.

Garmon and Reilley (24) analyzed mixtures of glycolic and diglycolic acids by measuring the color produced with 2,7-dihydroxynaphthalene at two different reaction times and used the double point technique to calculate the concentrations. Roberts and Regan (67) analyzed mixtures of carboxylic acids based on the rates of reaction with diphenyldiazomethane. They applied their technique of having the concentration of the carboxylic acid mixture in large excess relative to the concentration of the reagent and based the calculations on Eq. 10.

Lee and Kolthoff (38) analyzed mixtures of esters by comparing the amount of saponification after a selected time interval with a calibration curve prepared using known concentration mixtures.

Lee and Kolthoff (38) also analyzed mixtures of carbonyl compounds in the same manner based on the rates of decomposition of the bisulfite addition products. Only aldehydes and a few reactive ketones can be determined with this reaction. Fowler, Kline, and Mitchell (20, 21) determined aromatic aldehydes in mixtures with aromatic ketones by their rates of reaction with hydroxyl-amine hydrochloride. They used calibration curves to relate the amount of reaction after a specified time to the original concentration. Siggia and Hanna (73) applied the second-order graphical approach to the oximation reaction and analyzed mixtures of an aldehyde and a ketone, and mixtures of two aldehydes and mixtures of two ketones. They followed the reaction by maintaining the pH constant by titrating the hydrochloric acid as it was liberated from the reagent. Papa and co-workers (55) followed the course of the oximation reaction with a direct reading conductance meter. They made the reaction pseudo first order by adding the reagent in large excess and then compared the time interval for the reaction of a preselected fraction of the mixture and a predetermined calibration curve. Greinke and Mark (25) employed this same indicating technique but used two different alcohol–water concentration mediums to obtain data for their evaluation of mixtures of ketones based on Eq. 9. Sihtola, Neimo, and Sumiala (80) applied the second-order graphical approach to analyze the carbonyl groups in cellulose and were able to classify six different carbonyl groups present according to reactivity. They determined the slowest component first from the intersection of its extrapolated line with the zero time axis as usual. Then they subtracted the contribution of the slowest component from a and b, made a new plot, and performed the extrapolation again to obtain the value for the concentration of the next slowest component. They repeated this procedure until all components were evaluated. Papa, Mark, and Reilley (53) applied both single and double point methods to analyze fructose and glucose in aqueous media and in blood. They measured the rate of formation of molybdenum blue from Mo VI and also analyzed mixtures of sucrose and fructose and of sucrose and glucose. Mark and co-workers (49) used 2,3,5-triphenyl-2H-tetrazolium chloride as a reagent for the determination of mixtures of reducing sugars. A red formazan was formed which was measured spectrophotometrically to follow the reaction. The double point method and the solution of simultaneous equations were used in the calculations. Krivis and Supp (37) demonstrated the use of polarography combined with a graphical handling of the data to evaluate mixtures of Δ^4-3-ketosteroids and $\Delta^{1,4}$-3-keto-steroids. The mixtures were reacted with semicarbazide and hydrocortisone–prednisolone and cortisone–prednisone mixtures were differentiated.

Hanna and Siggia (30) adapted the reaction of amines with phenyl isothiocyanate to the differential kinetic analysis of mixtures of primary and

secondary amines, mixtures of homologous primary and of secondary amines, and mixtures of aliphatic and aromatic amines. The reaction to form thiourea is a second-order process and the graphical extrapolation technique was applied.

Siggia, Hanna, and Serencha (78) measured the rate of evolution of ammonia as a result of alkaline hydrolysis to analyze mixtures containing both a nitrile and an amide as well as binary mixtures of each of these compounds. Standard first-order rate plots were made from the data obtained and the linear portion extrapolated to zero time to calculate the composition of the mixture.

Siggia, Hanna, and Serencha (75) used both bromination with free bromine and catalytic hydrogenation with hydrogen gas followed by graphical extrapolation of the rate curves to resolve qualitatively mixtures of unsaturated compounds. The decrease in bromine content of the bromination reaction mixture was followed colorimetrically with time and showed a second-order rate process. In the hydrogenation process, the hydrogen was present in a large excess causing the reaction to proceed in a pseudo first-order pattern. Polgár and Jungnickel (65) described a method to measure the amount of substitution realized when bromine was added to unsaturated linkages. Several additional determinations were made at times in excess of that needed for complete saturation of the compound. They plotted the results against time and extrapolated the final linear portion to zero time to obtain the correct value for the addition reaction. Kolthoff and Lee (35) and Saffer and Johnson (69) applied the rates of reaction of perbenzoic acid with olefinic double bonds as a basis for differential analysis. They found the reaction valuable to distinguish between the amounts of internal and external double bonds in polymers. Reference of the amount of reaction after a specified time was related to calibration curves to determine the compositions of mixtures. Schmalz and Geiseler (72) also used the perbenzoic acid reaction to analyze mixtures of unsaturated compounds. They determined the rate constants for the components based on a single reaction of a mixture. Their calculations were based either on the differential equations, using graphically determined values of the derivatives, or on the integrated rate laws, using the area under the experimentally determined reaction rate curves. The derivated rate constants were then used to calculate the composition of the sample mixtures. Connor and Wright (16) used the difference in rates of the addition of mercuric acetate to double bonds to determine the *cis–trans* concentration ratio in isomer mixtures, specifically for the

analysis of mixtures of ethyl oleate and ethyl elaidinate.

Salzman and Gilbert (70) differentiated natural smog oxidants into components by following the rate of oxidation of potassium iodide colorimetrically. The reaction showed a pseudo first-order rate process because the iodide was present in a large excess. They applied the graphical method to resolve the mixtures.

Hass and Weber (34) found a linear relationship between the second-order rate constants for the reaction of mixtures of the monochlorides of *n*-pentane and isopentane and the composition. Brown and Fletcher (11) used the slope and zero time intercept of the first-order rate plot of the hydrolysis of mixtures of isomers of diethyl-*tert*-butylcarbinyl chloride for analysis.

Siggia, Hanna, and Serencha (77) measured the rate of liberation of nitrogen from diazonium compounds in the presence of cuprous chloride to analyze mixtures of these compounds. They found the decomposition followed a first-order pattern and applied the graphical extrapolation procedure.

Block, Morgan, and Siggia (4) combined the graphical differential reaction rate technique with the micro-Dumas method to analyze mixtures of nitrogen-containing compounds. The reactions studied were first order with respect to the nitrogen-containing compound.

Special approaches available to monitor rapid reactions should provide the mechanics for the performance of the differential rate analyses of mixtures of inorganic compounds. Hanna and Siggia (31) demonstrated that continuous flow techniques can be applied to the analysis of mixtures of iodate and bromate, periodate and bromate, and dichromate and bromate. These analyses involved the observance of the point of appearance of the starch–iodide color in the flow tube as a result of the reaction with potassium iodide. Various measurement techniques can probably be applied for other reactions; therefore, the scope of the flow systems for differential analysis is potentially wide.

Siggia, Hanna, and Serencha (76, 79) demonstrated that for analytical purposes, data obtained from the rates of distillation of mixtures, the rates of effusion of mixtures of gases through a small orifice, and the dialysis of mixtures under specific conditions could be treated in a manner similar to the treatment of data from chemical reactions. The distillation procedure was applied to mixtures of organic acids, mixtures of alcohols, mixtures of carbonyl compounds, and mixtures of aromatic compounds. The effusion method was applied to mixtures of both inorganic and organic gases. The dialysis method was found

applicable to mixtures of inorganic salts, of salts of organic acids, of inorganic acids, of organic acids, of amines, of sugars, and of amino acids.

Siggia and Hanna (74) have pointed out that in the case of some binary mixtures the effect of the presence of the faster reacting component is to decrease the rate of reaction of the slower component. When the graphical approach is used, this effect works to an advantage because the experimental differences between the slopes are more pronounced than are expected from theoretical plots. Methods that rely on rate constants obtained using pure materials for the final calculations, however, can give significant errors. The effect was noted particularly for mixtures of some ketones determined by the oximation procedure and mixtures of oleates determined both by bromination and hydrogenation.

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