

INVESTIGATIONS IN THE FIELD  
OF TRACE ANALYSIS

A THESIS

Presented to

The Faculty of the Division of Graduate  
Studies and Research

by

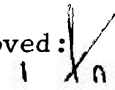
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INVESTIGATIONS IN THE FIELD  
OF TRACE ANALYSIS

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## SUMMARY

The studies reported in this thesis involve: the investigation into dissolving and fusing of samples, the theoretical study of fluorometric titration curves, the evaluation of a new fluorotitrator employing the fluorometric titration of lead, and a brief study on the extraction behavior of the lead-PAN complex.

At first glance the areas of research may seem somewhat unrelated, but each resulted from a definite need for improvement in these areas when considering trace analysis.

Two problems that can cause significant errors in the field of trace analysis are contamination and loss. A particular procedure that suffers from these problems is the decomposition of silicates. Thus, methods have been developed in order to minimize contamination and loss when fusing and dissolving such samples. After an alkaline fusion of a silicate, the flux cake is placed together with a beaker containing hydrochloric acid in a vacuum desiccator and after partial evacuation the acid is allowed to isothermally diffuse onto the cake. Dissolution takes place without danger of loss by splattering and at the same time the acid is highly purified.

Also, Teflon crucibles and dishes have been investigated in conjunction with fusions employing overhead radiation from a torch lamp. For the analysis of most materials, Teflon offers the possibility of carrying out an opening free from almost all contamination problems normally encountered with other crucible materials.

With the development of new instruments specifically designed to make use of the potentially more sensitive technique of fluorometric titration, a need was seen to initiate a general study and to calculate fluorometric titration curves for systems under a variety of assumed conditions. Equations have been derived and curves computed and characterized for self-indicating fluorometric titrations based upon complex formation. Computer programs are given to perform the appropriate calculations and to print out the results.

In order to meet the needs to effectively perform titrations with fluorogenic agents, White and Flaschka have built a fluorotitrator that allows such titrations at high sensitivity and without interferences from ambient light. An evaluation of this instrument to test its versatility and to establish its limitations was conducted. Overall the instrument has proven to be very reliable and valuable as a research tool. The stability of the instrument was checked and a typical result showed a drift of 2 divisions (on a 100 division scale) over a two hour period. Based on an initial setting of 80, this corresponds to a drift of only 1 division per hour.

The major part of the evaluation was accomplished by investigating the self-indicating fluorometric titration of lead with EDTA using Calcein Blue as the fluorogenic agent. The fluorescence was measured at 445 nm employing a secondary filter having a peak transmittance at 440 nm. No primary filter was used. The results were within the bounds of acceptability and indicative that good results can be obtained at various concentration levels using the new titrimetric method. The results could

provide a good basis for the development of a new method for the determination of lead.

Unlike the majority of metal complexes of PAN (1-(2-pyridylazo-2-naphthol), the lead complex could not be extracted. Based upon this fact, attempts were made to determine zinc (which can be extracted) in the presence of large amounts of lead. This approach showed only limited success.

PART ONE

INVESTIGATIONS INTO DISSOLVING AND FUSING OF SAMPLES

## CHAPTER I

## INTRODUCTION

The quality of an analytical result is described by two parameters, accuracy and precision. The types of errors that affect these two parameters are listed in any elementary textbook of analytical chemistry and need no further discussion here, except for those two errors that are of significance in the field of micro-, submicro- and trace analysis; namely, contamination and loss.

Contamination and Loss

Contamination is defined as the introduction of contaminants, that is, of extraneous material into the analytical system. The contaminants can be classified as follows:

- 1) entities identical to those to be determined
- 2) entities that yield analytical responses identical or similar to those caused by the substances to be determined
- 3) entities that change the intensity of an analytical signal

To exemplify the above situations the following respective cases may be cited: In a determination of silica, contamination is caused by silica released from the vessel material. In an EDTA titration of magnesium, any calcium introduced is cotitrated. In a fluorometric analysis, a quenching contaminant may severely decrease the intensity of fluores-

cence.

It is important to point out here that the above definition of contamination puts the emphasis on the term "introduction". Entities behaving in a fashion similar to those mentioned above may be present in the original material as legitimate constituents and would, therefore, be classified as interferences, but not as contaminants.

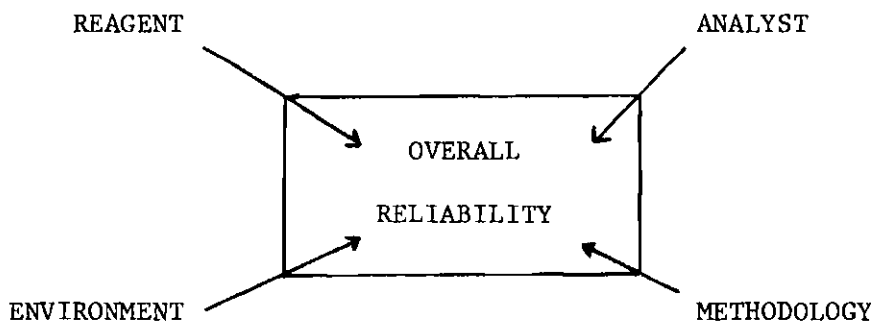
Losses result from removal of substance to be determined by volatilization or by adsorption on beaker walls, filter paper, etc.

Contamination and loss occur, to various degrees, during any analysis regardless of scale. However, when dealing with micro or trace determinations the influence is especially noticeable, as can readily be appreciated from the following example. In an EDTA titration of 100 ml of a  $10^{-1}$  M  $Pb^{2+}$  solution obtained from an ore extraction, 0.001 millimole of  $Pb^{2+}$  is lost by adsorption; the resulting error is -0.01%. In a d.c. polarographic determination for  $Pb^{2+}$  in gasoline, 100 ml of a  $10^{-4}$  M solution obtained after proper pretreatment is analyzed. If 0.001 millimole of  $Pb^{2+}$  is lost, the resulting error is -10%. Finally, in a pulse polarographic determination of atmospheric  $Pb^{2+}$  after proper sampling, 100 ml of a  $10^{-7}$  M solution is analyzed. If it is assumed that 0.001 millimole of  $Pb^{2+}$  is again lost, the resulting error is -100%. Analogous examples could be cited to show the effect of contamination, where the resulting error would be positive.

In determining major or even minor constituents in macro analysis, the amount involved in contamination and loss can often be neglected, because it is such a small fraction of the total amount of substance to

be determined. On the other hand, for trace or even micro determinations the amount of contaminants may be greater than that of sought-for substance present. With regard to losses, the concentration of sought-for substance may be significantly reduced due to adsorption and losses may even be total.

The influence on the overall reliability of a result stems from many sources. It is convenient to arrange these into four main groups. The situation can be represented as follows:



Each of these parameters will affect both accuracy and precision but to varying degrees. In an actual analytical case it is difficult to assess the extent of individual influence of each parameter. However, a theoretical discussion of each parameter separately will aid in pinpointing its influence on either precision or accuracy.

Such a theoretical discussion will be given below with the intent to derive from it ways and means to exclude or at least minimize the adverse effects.

#### Methodology

Any step in a method, such as dissolution, precipitation, filtration opens the door for contamination and loss. Consequently, one way of

improvement is to combine, if possible, several manipulations into one; thus reducing the number of steps and thereby the chances for error.

#### Analyst

"Good Housekeeping" in trace analysis is extremely important. Careless manipulations, of course, will cause serious loss and/or contamination. Practical preventative considerations go without saying and therefore additional comment here is unnecessary. However, when attempting trace analysis, there are other points the analyst must consider, and correct judgement concerning facts discussed in the section on environment are extremely important.

#### Environment

In trace analysis the appropriate design of laboratories, selection of surfaces, materials and ventilation systems are most important. It may even be necessary to remove from the laboratory and store separately substances that could possibly cause contamination. As an example, aqueous ammonia can be cited as a possible threat in the micro Kejehdahl determination of nitrogen.

Utensils, such as stirring bars, spatulas, vessels and other devices that come into direct contact with samples are often serious sources of contamination.

On the other hand, various elements may be adsorbed on the surface of vessels, and thus be lost since it is often very difficult to desorb them completely. Much attention must be paid to selection of the vessel materials and to their cleaning and storage. Main considerations in the selection of the materials are: chemical composition and resistivity

and thermal resistivity. Price may also be of interest.

### Reagents

Impurities present in reagents and the water used in trace analysis become serious sources of contamination. Reagents can be purified in the laboratory or obtained commercially in very pure form. When stored in solid form relatively few problems result. The difficulties arise when the reagent is used in dissolved form. Frequent preparation of the reagent solution is quite advantageous because aside from reagent decomposition, prolonged storage can result in contaminants from the container.

Even though these precautions are taken, some reagents still contain impurities at ppm level and below. Since it is almost impossible to remove all impurities from a reagent, special steps must be taken to compensate for them, and such a method is the "running of a blank". The blank solution is prepared in the identical way as the sample solution, but without addition of the sample. In the preparation of the blank, an equal volume of the solvent is added in place of the sample if it is a liquid.

The use of a blank is effective in as far as the compensation goes of the impurities originally present in the reagent or its solution which are there in a constant ratio. The blank does not compensate for impurities from environment and vessels walls, etc., because they are spurious and consequently unpredictable and variable. These are the ones that cause the fluctuation in the blank value. What is acceptable for avoiding contamination of the sample solution also holds in this respect for the

blank preparation.

It should also be pointed out that for very dilute reagent solutions, losses of reagent by adsorption of the solute on the wall of the container can cause severe problems because the actual concentration of the reagent may be severely reduced.

## CHAPTER II

## DECOMPOSITION OF SAMPLE BY FUSION

Most analytical procedures require the sample to be brought into solution. Various reagents and techniques exist for decomposing and dissolving materials for analysis (I-1). The most common reagents for attacking the sample besides water are the mineral acids. Solutions of potassium and sodium hydroxide also find occasional application.

There are, however, materials which are attacked slowly, if at all, by the usual liquid reagents and, therefore, more rigorous means are required. Commonly this involves fusion with a flux (I-2). The term flux here is used for the reagent that during fusion attacks the material to be opened.

In trace analysis fusion methods present many problems. In the first place, normally the weight of flux required to decompose the material is up to ten times the sample weight and the possibility of significant contamination of the sample by impurities present in the flux material thus becomes very real. Since such a large amount of flux is required, much acid or base, depending on the type flux employed, is necessary for the dissolution and neutralization of the fusion cake, possibly causing additional contamination. Furthermore, the aqueous solution of the fusion cake will have a high salt content that may lead to difficulties in the subsequent steps of the analysis. The high temperatures required for a fusion are also, although to a lesser extent, a problem because of

the danger of loss by volatilization. Finally, the container in which the fusion is performed in most cases is attacked resulting in introduction of additional impurities.

A particular procedure that suffers from many of the problems thus far discussed is the decomposition of silicates. The majority of procedures were developed at a time when openings were accomplished by fluxing with sodium carbonate preferably in a platinum crucible. More aggressive reagents were known but unless exact temperature limitation was applied, corrosion of the crucible went beyond tolerable limits. Progress has changed the situation; new crucible materials and fluxes have become available and thermostatic control is no longer a problem. Still, the decomposition of silicates necessitates improvement because of the importance of the material and the frequency with which such analyses are performed. The present investigation is directed toward possible areas of improvement.

#### Types of Fluxes

With few exceptions the common fluxes used in the decomposition of silicates are compounds of the alkali metals. The basic fluxes employed in the present investigation for attack of silicates include sodium carbonate, sodium hydroxide, and potassium hydroxide. In the following sections the properties of these fluxes are described.

#### Sodium Carbonate

Sodium carbonate, with a melting point of  $851^{\circ}\text{C}$ , is used extensively to decompose silicates. When heated with sodium carbonate the majority of metallic constituents present are converted to the corresponding

carbonates or oxides. The melt is treated with acid resulting in dissolution of the metallic ions, with partial separation of the silicon as hydrated silica. Platinum or silver crucibles are commonly used for carbonate fusions. Reasons for this selection will be explained later when discussing crucible materials. Some old procedures prescribe a mixture of sodium and potassium carbonate because the melting temperature is significantly lower than that of either compound alone. However, an appropriately longer time has to be employed for completion of the attack. With reaching high temperatures no longer being a problem, the carbonate mixtures are now seldomly used.

#### Sodium and Potassium Hydroxides

Due to the similarities in the properties of the hydroxides, they will be discussed jointly. The melting points of anhydrous potassium and sodium hydroxides are  $360^{\circ}$  and  $328^{\circ}\text{C}$ , respectively. The actual melting points are usually somewhat lower due to the presence of water and carbon dioxide. Fusion with these hydroxides is used to decompose quartz, silicates, silicides and carbides of various metals, and certain minerals. Silver crucibles are usually used when doing fusions with these hydroxides.

#### Processing the Fusion Cake

The fusion of the silicate with an alkali results in an increase in the proportion of bases in the silicate, thereby rendering it acid-decomposable. This preferred "acid decomposable form" present as a fusion cake is transferred (often together with the crucible) to another vessel and dissolution effected by adding hydrochloric acid at once or after leaching with water. In the process of dissolving the fusion cake,

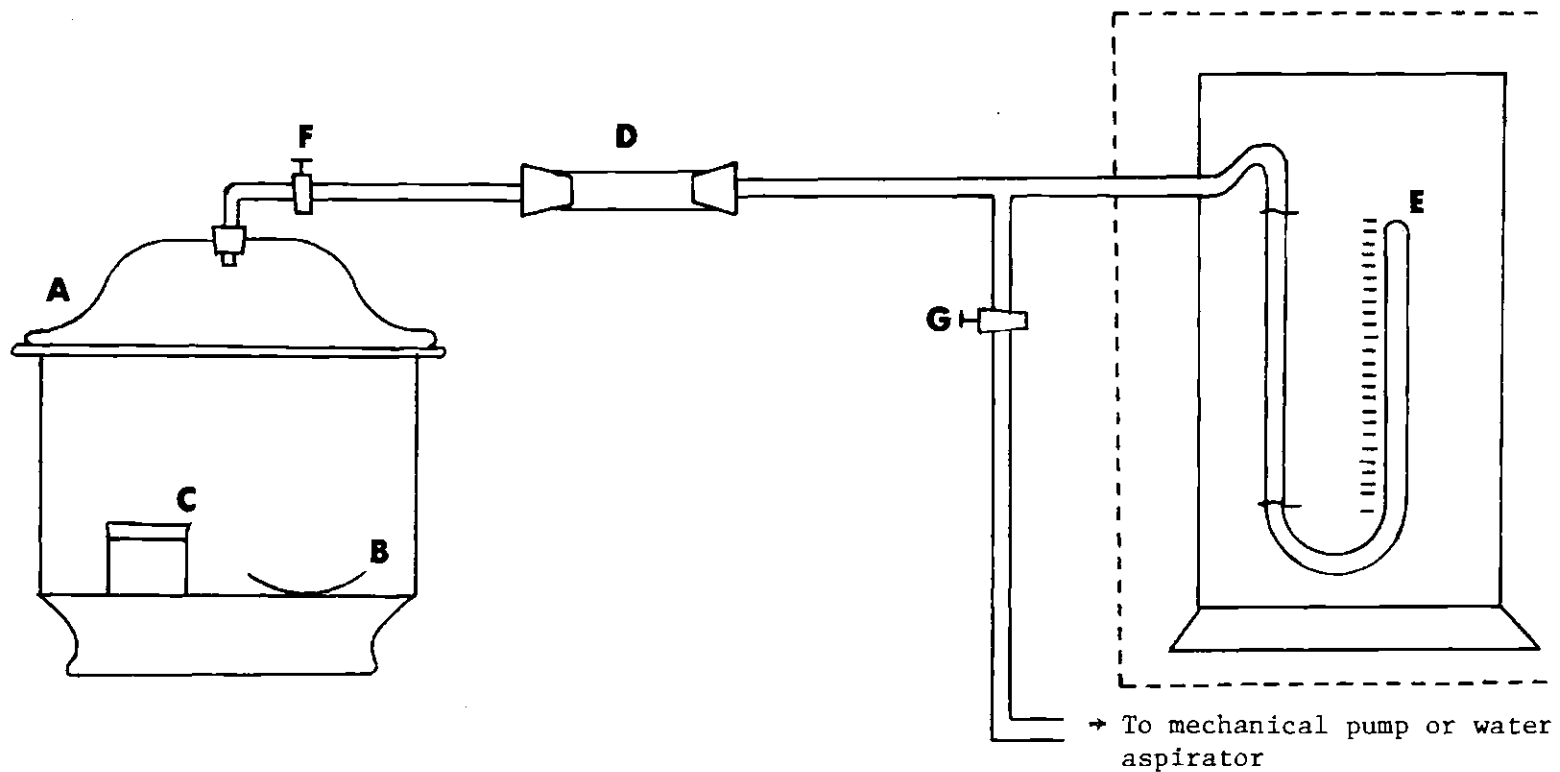
several transfer steps are required. Contamination can result, therefore, not only from the acid used, but also from the exposure of the sample and reagents to the environment. From the guidelines discussed earlier, contamination from the acid can be kept low by employing very high purity acids either purchased or prepared in the laboratory. However, this still does not eliminate the problems encountered in the transfer steps. The situation can be greatly improved by combining the purification with the transfer.

Hydrochloric acid is one of the volatile acids lending itself to the purification by isothermal diffusion as proposed many years ago by Abrahamczik (I-3). Instead of purifying the hydrogen chloride by diffusion into high purity water and then transferring the pure acid to the cake, the diffusion of the hydrogen chloride can proceed directly onto the cake.

#### Isothermal Attack on Cakes Solely Containing Flux Material

In the preliminary investigations only the flux itself was used to test the method of isothermal attack.

The arrangement shown in Figure 1 was used. The isothermal attack is carried out in the vacuum desiccator, A. Inside the desiccator are a beaker, C, containing concentrated hydrochloric acid, and a watchglass, B. The pressure inside the desiccator can be reduced via either a mechanical pump or a water aspirator. When using the mechanical pump, a trap, D, is employed to prevent hydrogen chloride from reaching the pump. The trap consisted of a glass tube, 8 cm long by 2 cm in diameter, filled with sodium hydroxide pellets and plugged with one-hole stoppers on both



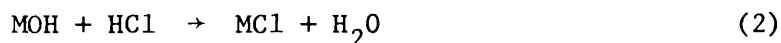
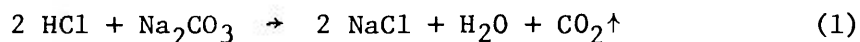
A-Vacuum Desiccator; B-Watchglass; C-Beaker containing acid; D-Trap; E-Mercury Manometer; F-G-Stopcocks.

Figure 1. Apparatus for Isothermal Attack  
(not drawn to scale)

ends. A mercury manometer, E, is incorporated to check the degree of evacuation. Two stopcocks, F and G, located as shown, are used to close-off the system. Stopcock G is used only when the mercury manometer is to be operative.

#### Procedure

A weighed amount of the flux is transferred to the watchglass and placed in the vacuum desiccator. The pressure is then reduced to the appearance of gas bubbles in the acid at which point stopcock F is closed. As the hydrogen chloride vapor diffuses onto the cake, attack occurs. Depending on which flux material is used, the reaction proceeds according to either of the following equations



where M in equation (2) represents sodium and potassium, respectively. In the case of sodium carbonate, carbon dioxide is evolved during attack, and, depending on the size of the desiccator, if a large amount of carbonate is present, the pressure rises to an extent that reevacuation is required in order to keep diffusion at a practical rate. After a specified time the flux material is removed and titrated to the methyl orange end point with standardized hydrochloric acid.

The subsequent calculations are based on the acid-base titration of a known amount of unreacted flux material. The results are expressed as percent sample converted, where 100% represents complete attack of the

sample by the acid vapor. Table 1 shows the relation between time and percent conversion. As can be seen from these data, the sodium carbonate required a much longer time for complete reaction than did the sodium and potassium hydroxide.

The above experiments were repeated using a fused sample of sodium carbonate. The sodium carbonate was heated in a platinum crucible until it melted at which point the crucible was dipped into water causing rapid solidification of the melt. The crucible was then heated slightly and inverted. By tapping the crucible bottom, the cake could be readily removed completely. The cake was then processed following the previously discussed procedure. The results are summarized in Table 2. As can be seen, the time required for satisfactory reaction is much longer in comparison to the non-fused material. It was observed that, as the reaction proceeded a crust of sodium chloride formed on the surface of the fusion cake. It is the opinion here that this crust prevented proper penetration of the acid, hampering complete attack.

Realizing that an increase in surface area should shorten the time required for complete attack of the cake, several methods of accomplishing this were investigated.

For sodium and potassium hydroxides the temperature required for fusion is low enough so that the liquid melt can be poured onto a large Teflon watchglass without fear of corrosion of the Teflon. After the hydroxide melt was poured onto the watchglass, another smaller Teflon watchglass was pressed down on top of the melt. As the melt solidified, a thin sheet, approximately 5-7 cm in diameter and 1 mm in thickness,

Table 1. Relation Between Time and Percent Conversion for the Isothermal Attack on Unfused Flux Material

Sodium Carbonate			Sodium Hydroxide			Potassium Hydroxide		
Weight mg	Time min	% Conversion	Weight mg	Time min	% Conversion	Weight mg	Time min	% Conversion
196	15	59	400	15	65	404	15	65
197	30	74	402	30	75	404	30	78
193	45	86	401	45	95	403	45	94
195	60	91	406	60	100	401	60	100
197	75	97						
196	90	99						

Table 2. Relation Between Time and Percent Conversion for the Isothermal Attack of Fused Sodium Carbonate

Weight gm	Time hr	% Conversion
4.74	10	21
4.54	17	41
4.65	24	56
4.76	41	78
4.46	60	90

was produced. This sheet was then placed slightly inclined, supported at one end by a small glass rod, on a watchglass; transferred to the desiccator and attack allowed to proceed as before.

Teflon cannot be used with sodium carbonate because the temperature required for fusion is far above the limits the Teflon will stand. When using the sodium carbonate, the best method is to break the fusion cake into smaller pieces before isothermal attack.

It must immediately be realized that these modifications, especially breaking the fusion cake into pieces, have already deviated from the guidelines mentioned earlier in that this adds another step to the procedure and thus allows the introduction of impurities. However, in situations where the introduction of impurities is of only minor concern this method can be applied without causing difficulties.

In many instances the analyst can schedule his workday in such a manner that the cakes can be placed in the desiccator near the end of the day and allowed to react overnight. In this way, the analyst can make use of the "dead time" sometimes encountered with almost all methods used in silicate analysis.

Having established empirical conditions for the attack on the flux material alone, the next step was to test the method using an actual silicate sample.

#### Isothermal Attack on Cakes Containing Flux Plus Silica

Approximately half a gram of silicon dioxide was opened in the established manner with a 10- to 20-fold excess of sodium carbonate. The cake was then removed from the platinum crucible as before. Initially

the cake was placed unbroken in the desiccator. By mere visual observation it was apparent that no attack was occurring. A second sample of silicon dioxide was opened, but this time the cake was broken into small pieces. Again, no attack took place. The acid seemed to be prevented from attacking the melt due to the incorporation of the silicate in the melt's structure.

Several experiments were conducted in which the cake was placed into the desiccator and after evacuation dry hydrogen chloride gas from a small lecture tank was introduced. In all cases the cake showed no signs of being attacked. Obviously, the reaction between gas and solid is not favored.

A possible solution was discovered somewhat by accident. A cake from the fusion of a silicate with sodium hydroxide in a nickel crucible was allowed to remain in contact with the air for an unusually long time. The hydroxide cake, being very hygroscopic, absorbed enough moisture from the air to form a layer of concentrated solution on its surface. When isothermal attack was effected, reaction proceeded as normal. This observation allows some insight into the possible mechanism of attack. When water is present originally, the chlorides formed are dissolved and new surface of the cake is exposed; therefore, the acid need only diffuse through the solution, a process that is much faster. In addition, transport through the liquid phase takes place by convection. Therefore, the water content of the cake plays an extremely important role in effecting attack, and it is thus advantageous to moisten the cake before exposure to the hydrogen chloride vapor.

Since sodium carbonate is not hygroscopic, the moistening of the cake is accomplished in another way. Here a few drops of ultra pure water were added, which proved sufficient to allow isothermal attack. In cases where the fluxes used are very hygroscopic, such as sodium and potassium hydroxides, the cake is moistened by allowing it to remain in contact with the air for a few minutes. Caution must be exercised so that no contamination results from the atmosphere.

In addition to the water content and surface area of the cake, many other parameters affect the time required for the cake to react completely. These are briefly summarized below.

The sample-to-flux ratio is of importance, especially when materials with high silica content are processed. The higher the concentration of silica the slower the attack on the cake. The amount of cake is, of course, an important parameter.

The effect of heating was also investigated. The temperature inside the desiccator could be varied within small limits, but not much influence on the rate of attack was noticed. When heating only the acid by directing the beam of a heat lamp on the beaker a small increase in diffusion rate can be achieved. However, this slight advantage has to be compared with the great disadvantage of much unwanted condensation of acid on the desiccator walls.

## CHAPTER III

## SURVEY OF CRUCIBLE MATERIALS

Although many of the findings thus far discussed allow improvements of fusion methods used in trace analysis, this successful application to a large extent depends upon the crucible material employed. If a crucible material were available that is perfectly inert chemically, price would be of lesser concern, since, with proper care, such a crucible could be used indefinitely. However, presently such a material is not available, and price becomes very important, depending upon the conditions under which the crucible is to be used.

In light of this, a brief discussion of the more commonly used crucible materials should suffice in order to make the proper choice concerning the combination of flux and crucible. The limitations of each crucible material when used in conjunction with any of the three fluxes presently under investigation; namely, sodium carbonate, sodium hydroxide and potassium hydroxide, will be discussed in the subsequent sections.

Platinum

Platinum (m.p. 1770°C) by tradition is used with solids melting at high temperatures. It is one of the most widely used crucible materials and is employed most frequently in conjunction with sodium carbonate as the flux. It can also be used when openings are performed with sodium and potassium hydroxides; however, unless very strict temperature limitations are applied, much corrosion occurs. In the opening of a sample with sodium

hydroxide at 500°C, some 100 to 200 milligrams of platinum are lost from the crucible (I-4). This not only presents a contamination problem, but also is financially unfeasible because of the limited use obtained from each crucible.

Platinum is wetted by the melt, and therefore, its removal by pouring is not quantitative. This poses problems when attempting to form the melt into a thin sheet (the importance of such sheets has been discussed in a previous section, see page 14).

#### Gold

Gold is less suitable as a crucible material because it is corroded to a much greater extent than are platinum and silver. Because of the relatively low melting point of 1036°C, gold is not suitable in case of high melting fluxes. For example, sodium carbonate has a melting point of 851°C, but the temperature used to melt it and keep it melted usually approach 1000-1100°C; thus, a gold crucible cannot be used.

In addition, gold is also unpopular because of its price. At the time of this investigation, gold was the most expensive crucible material.

#### Silver

Since silver melts at 960°C, it is completely excluded from use with sodium carbonate. Silver crucibles are usually suggested in procedures when using hydroxides for fluxing. Silver is only slightly corroded and is, therefore, much more suitable for such openings. The contamination by silver ions, although noticeable, commonly poses no serious problem in the subsequent analysis since the metal in the majority of analyses does not interfere and, if necessary, can be easily removed as silver

chloride.

Like platinum and gold, silver is also wetted by the melt preventing its quantitative removal. Silver is also preferable, since it is much less expensive than either platinum or gold.

#### Carbon (Graphite)

In comparison to the already mentioned materials, carbon is unusually inexpensive except when of high purity. Owing to its great absorptive properties, removal of trace elements, and therefore, manufacture of high purity carbon is difficult. Also, carbon poses a combustion problem and thus it is limited to use at high temperatures in inert atmospheres. Carbon is not satisfactory for the present needs because the melt enters into the pores of the material and upon solidification total removal of the cake is impossible.

#### Nickel

In some conventional methods nickel crucibles are used extensively. Naturally, nickel cannot be used when this metal is to be determined or when it poses problems as an interference. Nickel crucibles are attacked heavily by most fluxes; however, due to its relative low cost this is no problem since ruined crucibles can be discarded in favor of new ones with little expense.

When trace analysis is of prime concern, ordinary nickel crucibles are unsuitable. In this particular case, the trace impurities present in the nickel must be considered and thus high purity nickel is required and then the price rises accordingly. Nickel is also wetted by the melt.

### Iron

Iron as a crucible material is very limited, even though it is comparatively inexpensive. It is corroded very heavily and poses serious problems as an interference in subsequent steps of an analysis. Because of this it is almost always unsuitable for use in trace analysis.

### Zirconium

More recently Dobson (I-5) has made a study of problems surrounding contamination and loss caused by crucibles while investigating the determination of trace amounts of chromium, iron, and cobalt in synthetic rubies and sapphires. He found zirconium to be the most useful crucible material. With zirconium no exchange of trace metals took place between crucible and melt. The only disadvantage encountered when using zirconium is that surface oxidation occurs to various extents when the crucible is heated on a gas burner.

The superior resistance of zirconium to weight loss during most fusions is unique. Thus, the usual contamination problems encountered with the previously discussed crucible materials are virtually eliminated. Although, sodium peroxide is not a flux presently under investigation, Figure 2 shows graphically the average weight loss in a peroxide fusion for zirconium as compared to several other crucible materials. The superior resistance of zirconium is clearly demonstrated. It can be appreciated that an extension of such studies to the much less corrosive sodium carbonate should give similar results. In the present investigation it was found that zirconium is not corroded by hydrochloric acid in the isothermal attack method and thus can be employed without hesitation.

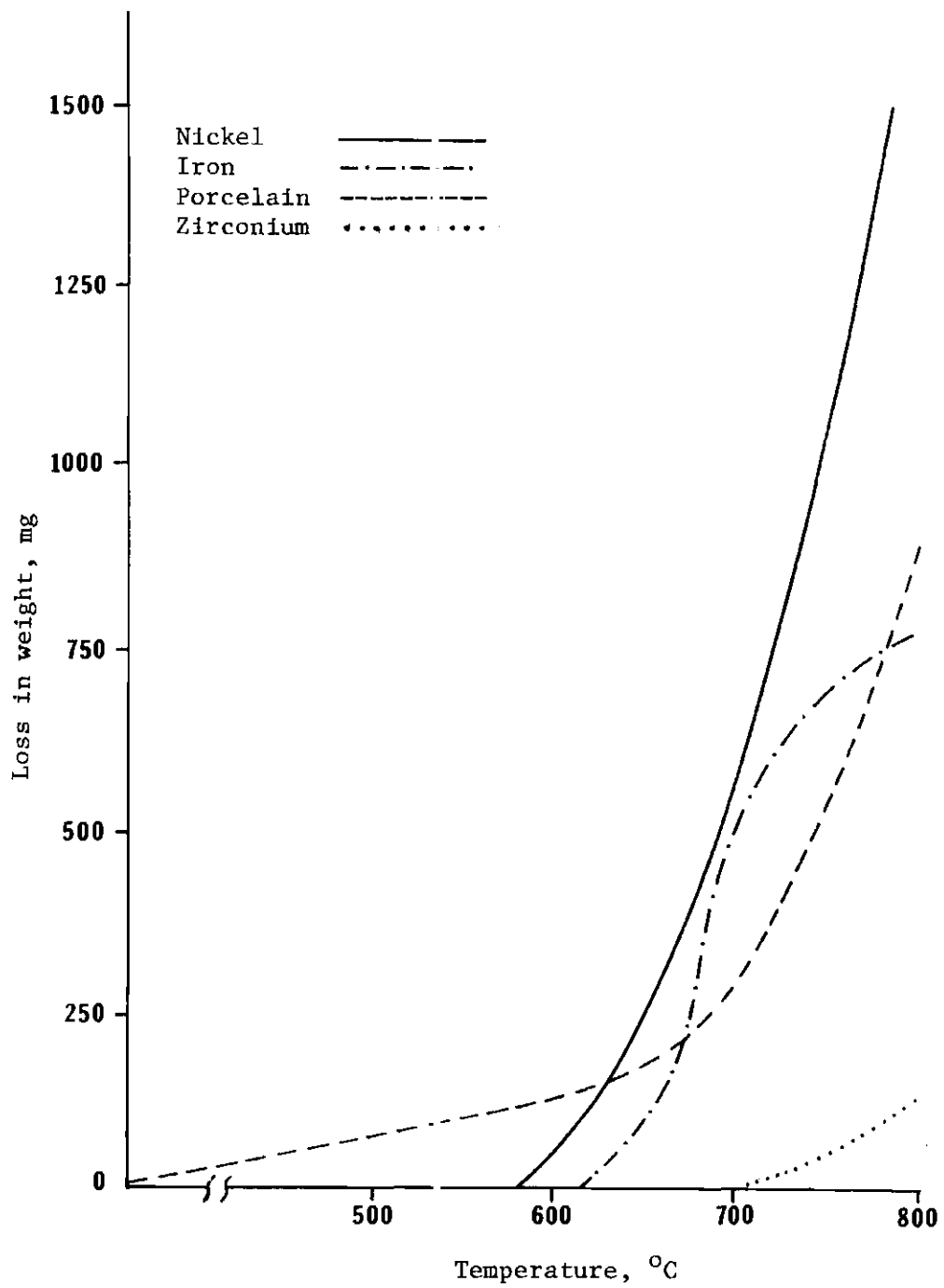


Figure 2. Average Weight Loss in a Peroxide Fusion For Various Crucibles (I-7)

### Teflon

As far as chemical inertness, price, and the presence of metallic impurities are concerned, Teflon is an ideal material; however, it has a major disadvantage in that it cannot be used at temperatures higher than about 300°C (I-6). Although, thermal decomposition takes place somewhat above this temperature, 300°C is normally the limit because the material becomes pliable and the vessels lose their shape. Thus, Teflon in conjunction with sodium carbonate fusions (>850°C) is totally unsuitable.

With regard to sodium and potassium hydroxide, Teflon offers several advantages over other crucible materials. Unlike the majority of materials, Teflon is not wetted by molten sodium or potassium hydroxides and thus removal of the melt by pouring is quantitative. For the analysis of most materials, Teflon offers the possibility of carrying out an opening free from almost all contamination problems normally encountered with other crucible materials. These possibilities warranted further investigation.

## CHAPTER IV

## FUSIONS IN A TEFLON CRUCIBLE

The Teflon "crucibles" used in the present investigation are Teflon beakers of various sizes. The fusions were performed by heating the Teflon crucible containing the flux material on a thermostated hotplate. Attempts to melt either sodium or potassium hydroxide (m.p.  $318^{\circ}\text{C}$  and  $360^{\circ}\text{C}$ , respectively) resulted in the deformation of the Teflon crucible. The situation was improved by providing the Teflon crucible with a solid support (see Figure 3). An aluminum block of appropriate size was fashioned and a hole bored at the center. The diameter of the hole was such that the crucible had to be inserted with some force. In this way, it was fixed permanently in the hole; thus, the still-liquid melt could readily be poured from the crucible by merely inclining the block assembly. To aid its manipulation, the block was fitted with a handle at the side. A second hole was bored close to the crucible hole and of the same depth but with a diameter adequate to house a thermometer.

Because of the slow heat transfer through Teflon, it was necessary to establish a relation between the temperature inside the crucible and that of the block itself. This was done by measuring the temperature, with a second thermometer, of an oil heated in the crucible and relating this temperature reading to that of the block thermometer. These measurements were begun immediately when the block assembly was placed on the hotplate. A "calibration curve" (see Figure 4) of block temperature versus

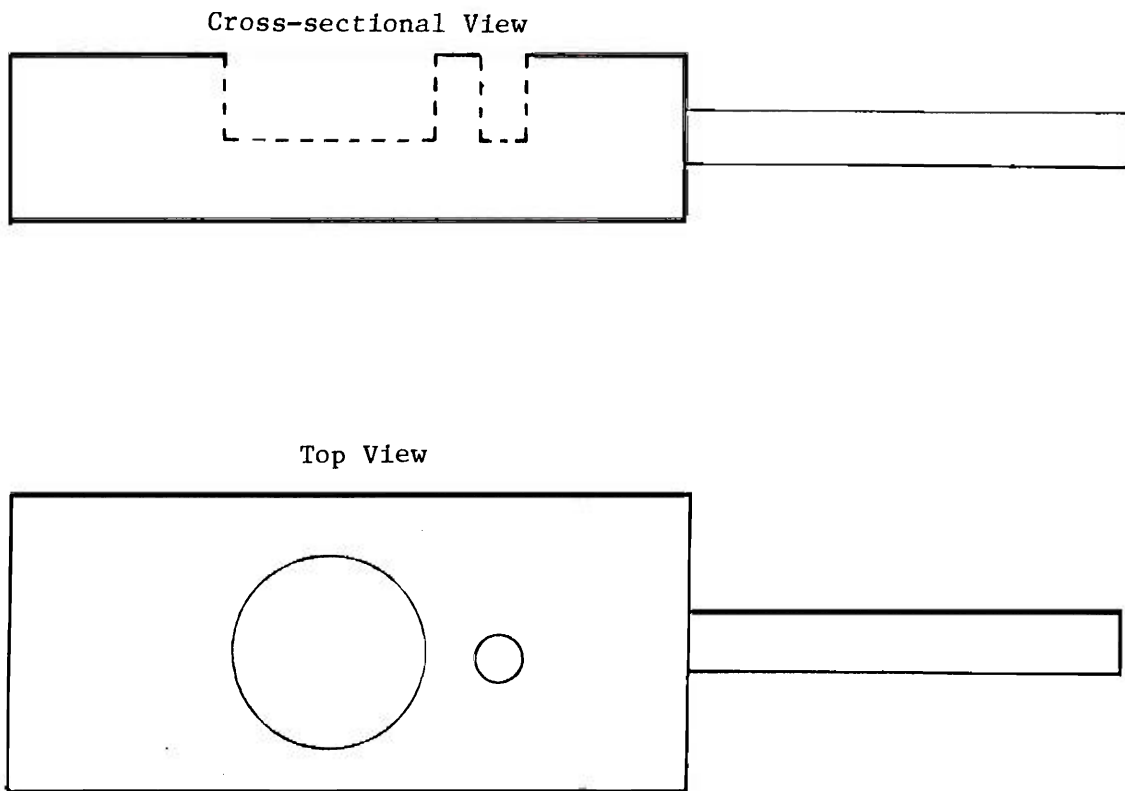


Figure 3. Aluminum Block Support for Teflon Crucibles

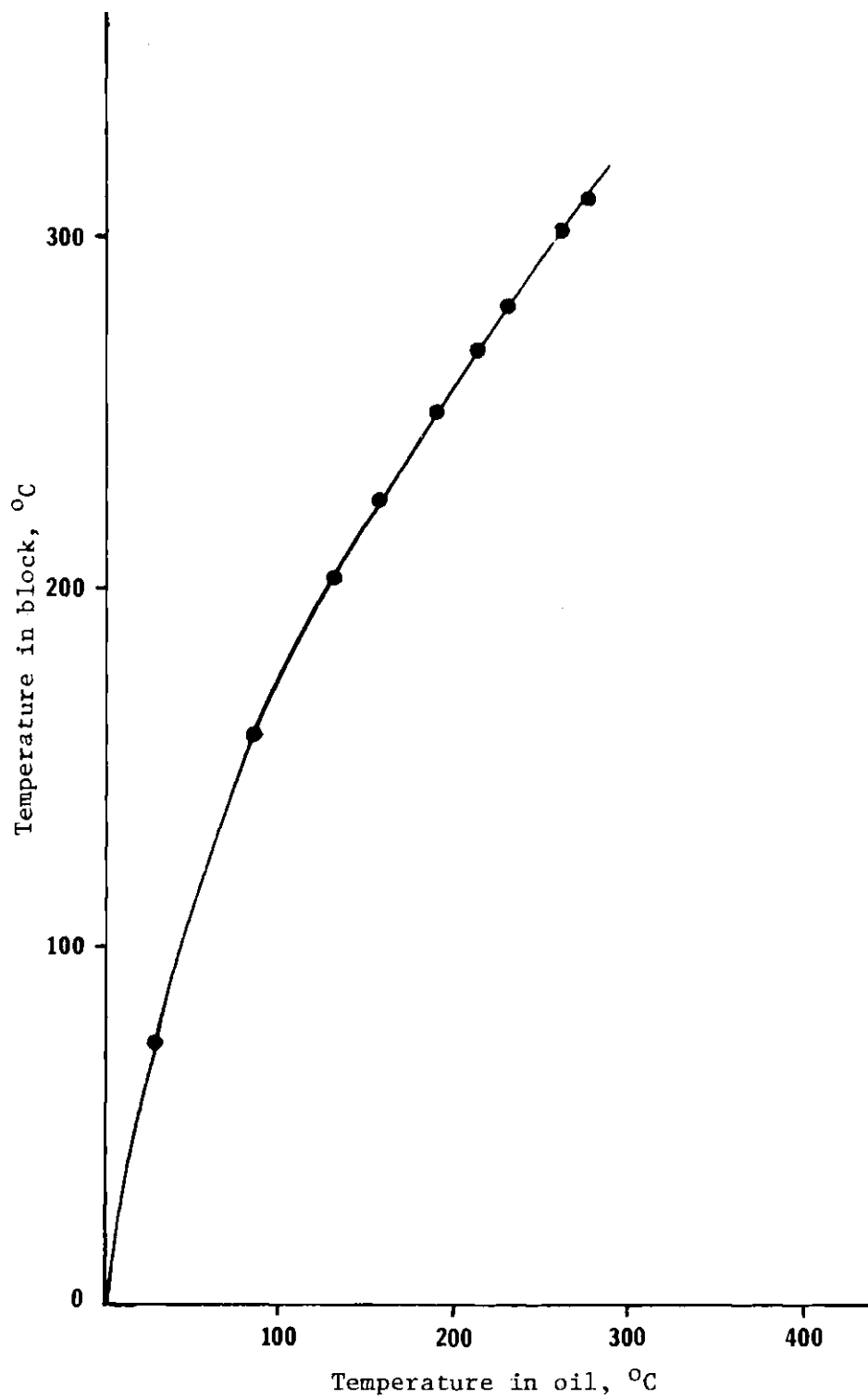


Figure 4. Calibration Curve for Aluminum Block Assembly

temperature in the crucible was then plotted. It should be noted that such a "calibration curve" holds only for the particular situation and that a curve must be established for each individual assembly.

Although the melting point of potassium hydroxide is listed as 360°C, with the commercial product containing about 15% water, liquification takes place at a much lower temperature and fluxing with this compound is no problem. Sodium hydroxide, which melts at 318°C, can be melted in a supported Teflon crucible, but normal operation requires that the block temperature be raised dangerously close to the critical limit of the Teflon. A thermostated electric furnace would probably be better in some respects but would pose other problems, e.g., trace impurities loosened from the oven material and heating wire.

Since the presence of water proved to facilitate the melting of potassium hydroxide, it was thought that adding some water to the sodium hydroxide would achieve an equally favorable situation but experiments quickly showed the fallacy of this assumption. Initially the wet pellets liquified quite readily, but then the water started to evaporate and as this proceeded, progressively more heat is required to keep the melt liquid. In addition, the water usually escapes with much undesirable splattering.

Sodium hydroxide would also be the preferred flux from another point of view. If potassium hydroxide is used and a perchloric acid treatment is intended in a determination of silica the formation of sparingly soluble potassium perchlorate would occur. Mixtures of sodium and potassium hydroxides that melt at lower temperatures could be used; for example, according to Rechetnikov and Unzhakov (I-8), the sodium-

potassium hydroxide system has a eutectic point at  $170^{\circ}\text{C}$  at a 50 mole percent composition. However, the formation of potassium perchlorate occurs with almost any mixture of potassium and sodium hydroxides; and, therefore, the use of such mixtures present no real advantage over the use of pure potassium hydroxide.

The heat needed to perform the openings in the present method was obtained by heating the Teflon crucible directly. The thermal problems encountered with Teflon could be greatly improved by applying heat directly to the flux material. One possibility is to use a small heating coil in direct contact with the flux; however, contamination from the coil material would then become a problem. A method, which appears more suitable to the present needs, is the application of heat by overhead radiation.

## CHAPTER V

## INVESTIGATIONS INTO OVERHEAD RADIATION

Overhead radiation by a 250 or 500 watt projection lamp with built-in condensing reflector or a model TL-2 Smith-Victor torchlamp was found to be quite adequate for liquifying the flux. As can be seen from Figure 5, a wide range of temperatures can be accommodated by varying the distance between lamp and reaction vessel.

It is advisable to place a glass partition between the crucible and lamp. This prevents impurities from falling into the melt and protects the lamp from damage in the event of splattering. An inverted beaker will do, but the best method is to have glass cylinders cut of different diameters and lengths to fit over the various crucibles and then to cover the cylinders with a thin plate glass sheet of appropriate size. This assembly allows easy cleaning, can be made exactly to size, and does not impair light focusing and viewing the crucible's contents.

Both sodium and potassium hydroxide can be brought to melt with overhead radiation; however, the sodium compound does not lend itself well to the operation. With the sodium hydroxide, melting takes place where the light beam is focused while the surrounding areas remain solid. When the beam is moved the material then in focus melts while the material previously irradiated solidifies. This at least is the situation with lamps up to 500 watts. Higher energies, of course, would bring the whole flux to melt, but temperatures then created locally would by far exceed

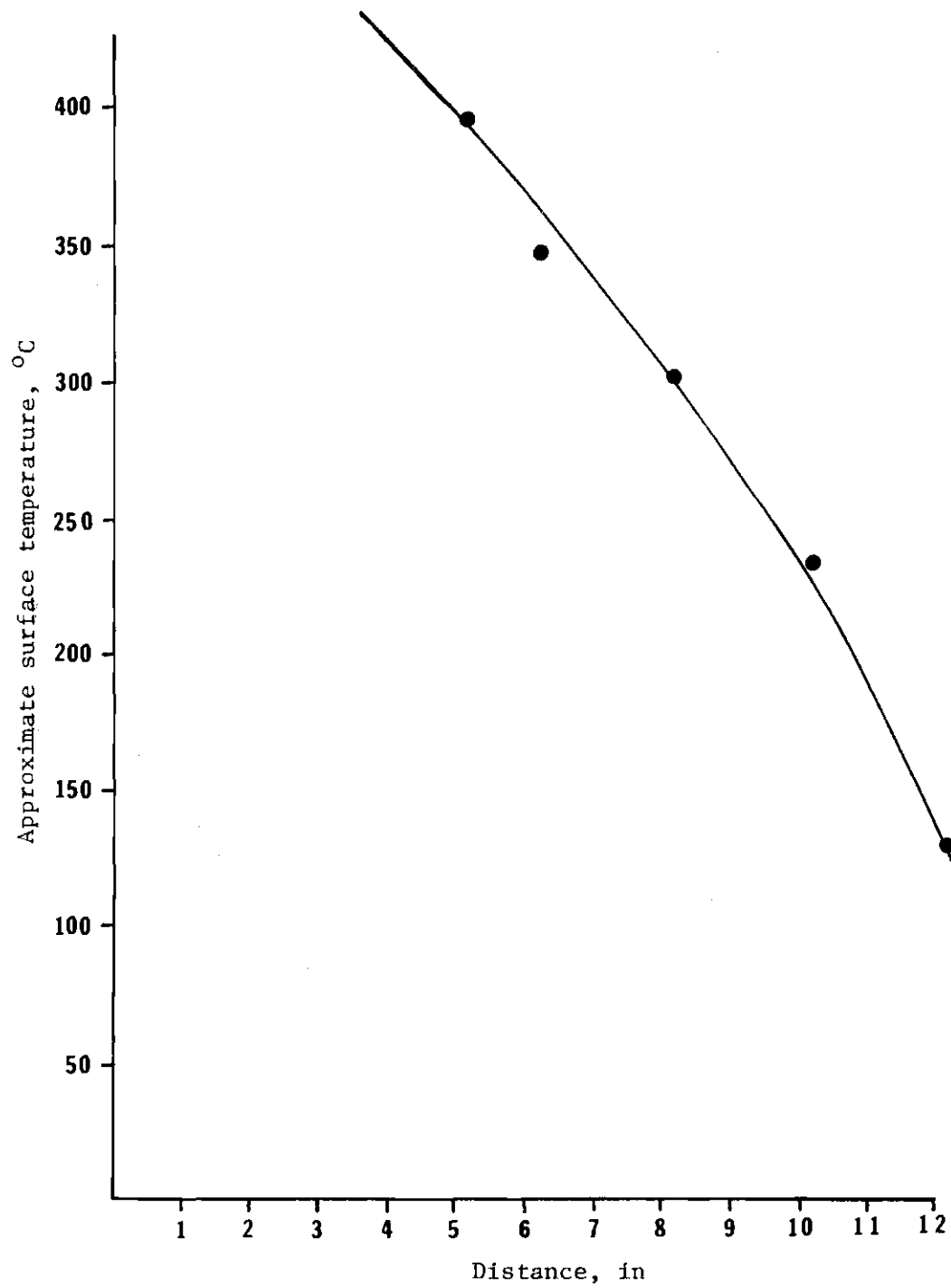


Figure 5. Relationship Between Distance of Lamp and Surface Temperature

the limits tolerable for Teflon.

As before, the situation with potassium hydroxide is quite favorable when using the common commercially available compound containing about 15 percent of water. The material melts quite readily and stays liquid even under moderate radiation.

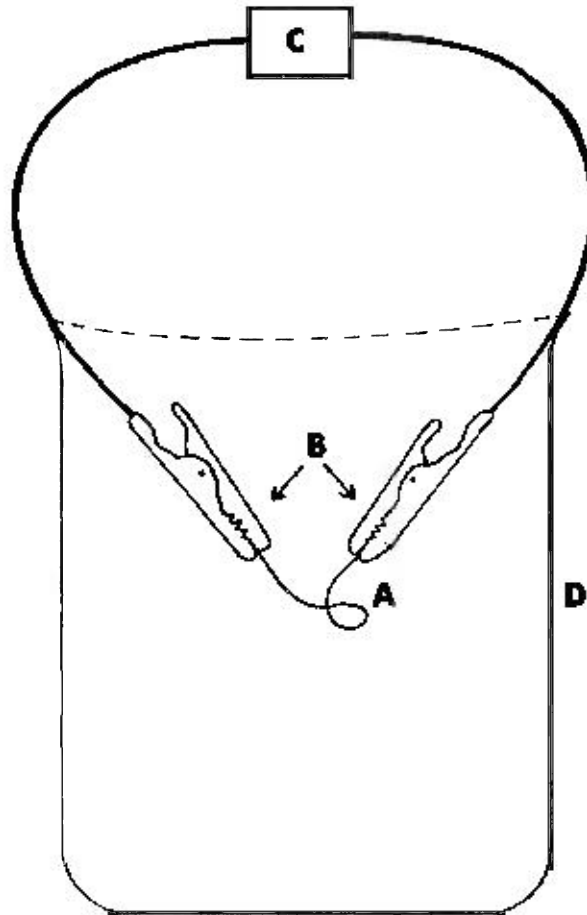
## CHAPTER VI

## SEMI-MICRO AND MICRO APPLICATIONS

Much of what has been discussed thus far has considered problems encountered in trace analysis when performing an opening by fusion. In almost all cases the implication was that macro samples are involved. The extension of the present techniques to semi-micro samples could be accomplished if additional modifications were made to simplify the procedures and further reduce the number of transfer steps.

The apparatus shown in Figure 6 was employed for the opening of a micro sample with sodium peroxide. A small loop of platinum wire (A) is connected to two alligator clips (B) that in turn are connected to a Variac (C). The loop is placed in a small amount of powdered solid sodium peroxide. The voltage is then increased via the Variac, until a portion of the sodium peroxide melts forming a small bead on the loop. The voltage must be increased slowly, otherwise the platinum wire may melt. The voltage is then turned off and the bead is allowed to solidify. Approximately one milligram of sample is placed on the bead. The loop wire assembly is then carefully positioned in the beaker (D). The voltage is then slowly increased to remelt the bead and held there until fusion is complete. The power is then turned off and the loop with the attached bead is disconnected from the clips and placed in a vacuum desiccator where isothermal attack is effected.

Although the preliminary experiments were performed with sodium



A-Platinum Wire Loop; B-Alligator Clips;  
C-Variac; D-Beaker

Figure 6. Apparatus for Micro and Semi-Micro Openings

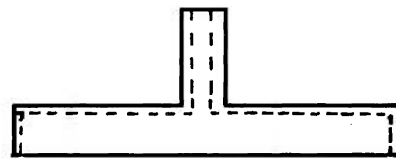
peroxide, the other fluxes such as sodium carbonate and sodium and potassium hydroxide should also be suitable for use with this method.

However, since the platinum loop is in direct contact with the flux some contamination from the platinum will result. This coupled with the obvious superiority of overhead radiation and the use of Teflon, caused this method not to be investigated further.

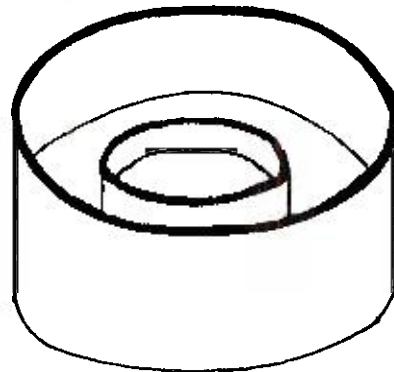
Another method more along the lines of the present investigation involved the use of overhead radiation to perform the fusion in the deepest spot of a Teflon evaporating dish. Here the molten material is held closely together and when the dish is swirled in a rotating fashion the melt is spread over a large area resulting in a cake with increased surface area. The dish with its contents is then placed in a vacuum desiccator and isothermal attack effected.

The situation can be improved even more if all the steps, i.e., fusion and isothermal attack, are carried out in one vessel. A Conway chamber machined from Teflon should allow this to be done. Such a vessel (Figure 7) is fashioned such that the walls of the inner compartment are lower than the outer. The vessel is equipped with two types of lids. One serves merely to seal the vessel; the other (shown in Figure 7) allows reduction of the pressure inside the vessel with the aid of an aspirator.

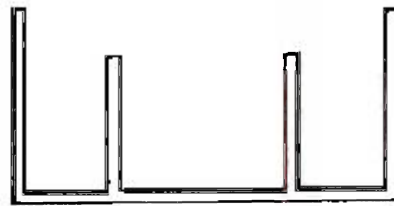
The fusion is performed in the central compartment using overhead radiation. The melt is then solidified and the outer chamber is filled approximately three-fourths full with reagent-grade hydrochloric acid. The second type lid is put in place and the pressure reduced. This method offers all the benefits associated with the use of Teflon, overhead radiation and isothermal attack; it offers the additional advantage in that



Lid



Interior View



Cross-sectional View

Figure 7. Teflon Conway Chamber Used for Openings at Micro Level

this vessel can be used later in the further processing of the sample, such as evaporation and baking.

## CHAPTER VII

## CONCLUDING REMARKS

The purpose of this investigation was to modify existing methods for opening inorganic samples in such a way that they become more suitable for use in trace analysis. The first step was to apply isothermal attack. This combines the purification of the acid and dissolution of the fusion cake into one step; thus, eliminating several transfer steps and thereby reducing the possibility of contamination.

Although the method of isothermal attack was here developed in conjunction with the alkali fusion of silicates, it is not restricted to this application but readily adapts to other materials. Good results were obtained with light metal alloys based on magnesium and aluminum when attacked by hydrochloric acid. Brass drillings reacted completely with isothermally diffused vapors evolved from fuming nitric acid.

The introduction of Teflon and its successful application as a crucible material will prove to be a significant improvement concerning problems encountered with the crucible materials presently being employed. Teflon coupled with overhead radiation is especially attractive for fusions on the micro and semi-micro scale.

## CHAPTER VIII

## CHEMICALS AND EQUIPMENT

Chemicals

All water used in the investigation for preparing and diluting reagents and solutions was obtained from a Barnstead still equipped with a Ventguard filter. The water was then passed through a mixed bed deionizer. All reagents used met ACS analytical reagent specifications. Whenever possible, the fluxes, i.e., sodium carbonate, sodium hydroxide and potassium hydroxide were J. T. Baker "Analytical Reagent" grade salts. All common acid, base, and buffer solutions were prepared from reagent grade chemicals.

Glassware

The usual glassware such as beakers, flasks, etc., were used as needed. For volumetric measurements class A volumetric glassware was used exclusively and without additional calibration.

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PART TWO

THEORY OF FLUOROMETRIC TITRATION CURVES

## CHAPTER I

## INTRODUCTION

The phenomenon of fluorescence was first observed by Monardes in 1565. In 1833, Sir David Brewster noted the red emission from chlorophyll, and in 1852 Sir G. G. Stokes described the mechanism of the absorption and emission process. Fluorescence is one of the most active topics of research in science today as evidenced by the increasing number of papers, reviews, and monographs published each year.

Originally the term luminescence was used interchangeably with fluorescence; however, nowadays luminescence is used in the broader sense to describe any light emission. The various types of luminescence can then be classified according to the means by which energy is supplied to excite the luminescent species.

In general, photoluminescence is the form of luminescence in which molecules are excited by interaction with electromagnetic radiation. In photoluminescence, if the release of electromagnetic energy is immediate (usually about  $10^{-8}$  seconds), the process is called fluorescence; whereas, if the release of energy takes place later than  $10^{-8}$  seconds, the process is called phosphorescence. More recently the classification has been based not on time considerations, but rather on the mechanism involved. In fluorescence the excitation step is between a lower (ground) level and an excited singlet state and from there to a somewhat lower triplet state where the electron remains for some time and

only then upon return to the ground state does emission take place. Thus, the light emission of some material may be delayed and under the old classification be considered as phosphorescence while actually only singlet state transitions are involved and according to the new mechanistic classification is actually a "delayed fluorescence".

Chemiluminescence is the process in which the excitation energy is obtained from the chemical energy released during a reaction, and if the chemical reaction takes place in a living organism, e.g., a firefly, the chemiluminescence is given the particular name, bioluminescence.

When certain crystals, such as sugar, are broken the energy stored on crystal formation is in part released as light and such a process is called triboluminescence.

Other types of luminescence much less commonly encountered are cathodoluminescence and thermoluminescence. The first results from a release of energy produced by cathode rays, while the second occurs when a material existing in high vibrational energy levels emits energy after being exposed to small amounts of thermal energy.

The type of luminescence which is of present interest is fluorescence. The fluorescence normally observed in solutions is called Stokes fluorescence. This is the re-emission of photons which have a longer wavelength (lower frequency) than those absorbed. Fluorescence has provided some of the most sensitive and selective methods of analysis for many substances (II-1).

It is interesting to compare the sensitivity and selectivity of

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References for Part II on page 81.

fluorometry with another area of optical measurement, that of spectrophotometry. Fluorescence has proven superior in both respects.

The main reason for the increased sensitivity is that in fluorometry the detector senses only the sample fluorescence. Thus, the signal-to-noise ratio is extremely large. In spectrophotometric methods the analogous quantity, absorbed radiation, is measured as the difference between the incident and the transmitted beams. This small decrease in the intensity of a very large signal results in a low signal-to-noise ratio with a correspondingly significant loss in sensitivity.

The selectivity of fluorescence is the result of two main factors: (a) there are fewer fluorescent compounds than absorbing ones because all fluorescent compounds must necessarily absorb radiation, but not all compounds that absorb radiation emit; (b) two wavelengths are used in fluorometry, but only one in spectrophotometry. Two compounds that absorb radiation at the same wavelength will probably not emit at the same wavelength.

## CHAPTER II

## FLUOROMETRIC DETERMINATIONS

Theoretical Background

The basis for the fluorometric determination method is a relation shown in equation (1)

$$F = \phi I_0 k(1 - e^{-\epsilon bc}) \quad (1)$$

where  $F$  is the fluorescent intensity,  $\phi$  is the quantum efficiency,  $I_0$  is the incident radiant power,  $k$  is an instrument parameter that contains a geometry factor which depends upon the effective solid angle viewed by the detector and a quantum conversion factor for the detector (that varies as a function of wavelength),  $\epsilon$  is the molar absorptivity,  $b$  is the path length of the cell, and  $c$  is the molar concentration.

Beside concentration, the basic fluorescent intensity-concentration equation indicates that there are three other major factors that affect the fluorescence intensity.

1. Quantum Efficiency

Every species possesses a characteristic property that is described by a number called the quantum efficiency. This is the ratio of quanta of light absorbed to quanta emitted.

$$\phi = \frac{\text{number of quanta emitted}}{\text{number of quanta absorbed}} = \text{quantum efficiency}$$

The greater the value of  $\phi$ , the greater will be the fluorescence for a given intensity of exciting light. Thus, it can be seen that a non-fluorescent molecule is one whose quantum efficiency is zero or so close to zero that the fluorescence is not measurable even though absorption does occur.

## 2. Intensity of Incident Radiation

As seen from equation (1), the amount of fluorescence is proportional to the intensity of the exciting light. This is true as long as all the incident photons are capable of interacting with the absorbing species. However, at high intensities of exciter light the situation is more complex. Then the number of available photons may exceed by far the number of absorbing species, and thus a considerable percentage of the incident light will pass through the solution without chance of interacting. However, such a case will probably be only of theoretical interest because in practice at such high intensities heating of solution and apparatus may become so extensive as to be experimentally unmanageable.

## 3. Molar Absorptivity

In order to emit radiation a molecule must first absorb radiation; and therefore, the higher the molar absorptivity, the better will be the fluorescence intensity of the compound.

If the term  $e^{-\epsilon bc}$  in equation (1) is expanded, the following is obtained,

$$e^{-\epsilon bc} = 1 - \epsilon bc + \frac{(\epsilon bc)^2}{2} + \dots + \frac{(\epsilon bc)^n}{n!}$$

If the value for  $\epsilon bc$  is less than about 0.05, the terms of higher order in the above expression can be dropped and equation (1) reduces to,

$$F = \phi I_0 k \epsilon bc \quad (2)$$

or

$$F = k' c \quad (2a)$$

where  $k'$  includes  $\phi$ ,  $I_0$ ,  $k$ ,  $\epsilon$ , and  $b$ .

Thus, at given  $\epsilon$  and  $b$ , for low concentrations of the fluorescing species, i.e., ( $10^{-4}$  to  $10^{-7}$  F), the situation has been reduced to one comparable to Beer's Law in spectrophotometry. The fluorescent intensity is proportional to the concentration of the fluorescing species; and therefore, a plot of fluorescence versus concentration should be linear.

The results in fluorometry can be adversely affected by several parameters, a few of which will be discussed in the following chapter in conjunction with fluorometric titrations.

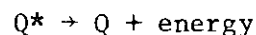
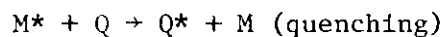
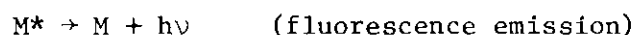
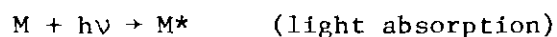
## CHAPTER III

## FLUOROMETRIC TITRATIONS

Several advantages can be gained by switching from a fluorometric determination (where absolute values are measured) to a fluorometric titration (where only changes in fluorescence are measured). Some of these advantages will become obvious when discussing the relevant parameters.

Quenching

In many cases the intensity of the fluorescence is decreased by a competing deactivating process that results from the specific interaction between a fluorophor and another substance present in the system. Such a process is called quenching. The general mechanism for this process can be denoted as follows:



Four common types of quenching (II-2) are observed in fluorescence processes: temperature, oxygen, concentration, and impurity quenching.

1. Temperature Quenching

As the temperature is increased, the fluorescence decreases.

The degree of temperature dependence varies from compound to compound. Temperature effects on fluorescence are a type of excited-state quenching by encounter. The temperature dependence of fluorescence can be compared with that of molecular activity, suggesting that increasing temperature increases molecular motion and collisions, and hence robs the molecule of energy. In a practical sense, temperature control should be exercised for maximum precision and accuracy.

## 2. Oxygen Quenching

One of the more troublesome aspects of fluorometry is the ability of molecular oxygen to quench excited singlet and triplet states of many molecules, especially aromatic hydrocarbons, in solution. The analytical sensitivity can be increased by oxygen removal. This can be accomplished by bubbling an inert gas, such as nitrogen, through the solution for 5 to 10 minutes or, better, by a freeze-thaw cycle.

In some cases, oxygen quenching results from oxidation of the solute. An explanation which is frequently invoked to explain oxygen quenching is concerned with the fact that the ground-state oxygen molecule is a triplet and therefore paramagnetic (II-3). The presence of a paramagnetic species in a solution may increase spin-orbit coupling in any electronically excited molecules in that solution, thereby increasing rates for intersystem crossing. This would have the effect of quenching fluorescence.

## 3. Concentration Quenching

Ideally the fluorescence produced in a cell should be uniform throughout, i.e., the exciting light is evenly distributed along its path. In other words, each potentially absorbing species interacts with

the exciting light. Thus, at low concentrations there is a linear relationship between fluorescence and concentration.

However, at higher concentration the fluorescence produced is not uniform throughout the cell, i.e., the exciting light is not evenly distributed along its path. That portion of the solution near the light source absorbs so much radiation that less and less is available for the remainder of the solution. As a result, considerable excitation occurs at that portion of the solution facing the exciting lamp, but less and less occurs throughout other portions of the cell. This type of concentration quenching causes a fluorescence loss that is called the inner-cell effect (the mathematical considerations for concentration quenching are discussed on page 57 ).

Another form of quenching, which is due to concentration, involves dimer or polymer formation and is called excimer quenching. The excimer has a different electron orientation and a longer emission wavelength than the monomer. Hence, if the fluorescence is measured at the wavelength of the monomer, the emission at the longer wavelength will go undetected. So the observed fluorescence will decrease as the degree of polymerization increases since as this occurs the concentration increases.

#### 4. Impurity Quenching

Since fluorometry is a very sensitive method, impurities at even very low levels may cause serious problems as interferences. These interferences can be in the form of the inner-cell effect, collisional quenching, energy transfer, and charge transfer.

Unless such quenchers are completely matched in effect and concentration in the standards, wrong results in a fluorometric determination

are to be expected. However, such quenching is only of minor concern in a fluorometric titration.

If the above types of quenching occur to such a degree as to lower the fluorescence below practical ranges, neither a fluorometric titration nor a determination would be suitable. However, in a fluorometric titration moderate quenching is tolerable, since it merely changes the slope of the titration curve but has no effect on the location of the end point.

#### Titration Versus Determination

In a fluorometric determination the concentration of a species is determined by comparison of its fluorescence with that of a series of standards. The fluorescence of such standards is measured and then plotted against concentration. The unknown concentration of the species is then read from the calibration curve. Since the relation between fluorescence intensity and concentration of the emitting species is linear only at very low concentrations, one has to operate many times with non-linear calibration curves. This presents a very unfavorable situation. Also, since fluorescence is largely dependent upon the immediate environment, small changes in conditions between the standards and the test solution can result in significant errors when comparing two like concentrations.

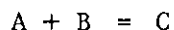
In contrast, in a fluorometric titration no calibration curves are required. Also, the titrant solution is usually prepared from a primary standard or has been standardized. The only requirement in such a titration is that the established conditions remain constant during the course of a titration.

## CHAPTER IV

## COMPUTATION OF FLUOROMETRIC TITRATION CURVES BASED ON COMPLEX FORMATION

Titration curves for a variety of systems can be calculated employing equations of differing complexity. In the particular case of a photometric titration, for example, Goddu and Hume (II-4) have calculated curves for acid-base systems and Flaschka (II-5) has derived equations for titrations based upon complex formation. However, no analogous work has been done in the area of fluorometric titrations. It was, therefore, felt worthwhile to institute a general study and to calculate fluorometric titration curves for systems under a variety of assumed conditions. A PDP 8e mini-computer was available and used to minimize the work required for the task (see appendix A for programs).

The calculations hold for any linear titration based on the simple reaction



with at least one of the involved species being fluorescent so that no indicator is needed. For simplicity of discussion and relation to actual experiments a complexometric titration will be assumed according to the titration equation



The curves will be computed for all three cases; namely, where M, Y, or MY fluoresces. To obtain the required formulae, expressions will be derived by replacing the appropriate species concentrations for  $c$  in equation (1). These derivations are effected as follows.

#### Derivation of Fluorometric Titration Curves

The expression for the relevant equilibrium constant,  $K$ , is

$$K = \frac{[MY]}{[Y][M]} \quad (5)$$

In actual practice  $K$  is not the absolute constant but rather a conditional constant that reflects the effects of any competing equilibria (side reactions) and is related to the absolute constant by factors described and for many cases calculated by Ringbom (II-6).

Material balances for M and Y are

$$C_M = [M] + [MY] \quad (6)$$

$$C_Y = [Y] + [MY] \quad (7)$$

where  $C_M$  and  $C_Y$  are the total concentration of metal and titrant, respectively, present in the titration solution.

Substitution for  $[MY]$  and  $[M]$  in equation (4) gives

$$K = \frac{C_Y - [Y]}{(C_M - C_Y + [Y])Y} \quad (8)$$

expansion yields

$$K(C_M - C_Y) [Y] + K [Y]^2 = C_Y - [Y]$$

and rearrangement yields

$$K [Y]^2 - [Y] \left[ K(C_Y - C_M) - 1 \right] - C_Y = 0 \quad (9)$$

Solving equation (8) for [Y] gives

$$[Y] = \frac{1}{2K} \left\{ [K(C_Y - C_M) - 1] + \sqrt{[K(C_Y - C_M) - 1]^2 + 4KC_Y} \right\} \quad (10)$$

Considering volume change during the titration

$$C_M = C_M^0 \left( \frac{V}{V + b} \right) \quad (11)$$

$$C_Y = C_Y^0 \left( \frac{b}{V + b} \right) \quad (12)$$

where  $C_M^0$  is the original formality of the metal ion in the sample solution,  $C_Y^0$  is the formality of the titrant solution,  $V$  is initial sample volume, and  $b$  is the volume of titrant added.

Substituting equations (10) and (11) into (9) gives

$$[Y] = \frac{1}{2K} \left\{ \left[ K \frac{1}{V+b} (C_Y^0 b - C_M^0 V) - 1 \right] + \sqrt{\left[ K \frac{1}{V+b} (C_Y^0 b - C_M^0 V) - 1 \right]^2 + 4K C_Y^0 b \frac{1}{V+b}} \right\} \quad (13)$$

Analogous formulae for the calculation of [M] and [MY] can be derived as shown below.

In the case for [M], if equations (5) and (6) are subtracted, the equation for [M] is obtained

$$[M] = C_M - C_Y - [Y] \quad (14)$$

An equation for [MY] can be obtained merely by rearranging equation (6)

$$[MY] = C_Y - [Y] \quad (15)$$

The fluorescence intensity is given by equation (1); then upon proper substitution three equations for the particular cases where only Y, M, or MY fluoresces are obtained.

If Y fluoresces

$$F = \alpha (1 - e^{-\beta[Y]}) \quad (16)$$

If M fluoresces

$$F = \alpha \left( 1 - e^{-\beta \left\{ \frac{1}{V+b} (C_M^0 V - C_Y^0 b) - [Y] \right\}} \right) \quad (17)$$

If MY fluoresces

$$F = \alpha \left( 1 - e^{-\beta \left\{ C_Y^0 \left( \frac{b}{V+b} \right) - [Y] \right\}} \right) \quad (18)$$

where  $\alpha = \phi \times I_0 \times k$ ;  $\beta = \varepsilon \times b$ ; and  $[Y]$  as expressed by equation (12).

It should be noted here that in deriving these formulae none of the usual simplifications were made, i.e., the concentration of one species was not neglected when small in comparison to another. Also  $\beta$  was manipulated in such a way that a variety of curves could be obtained for a given set of conditions. Values for the stability constants and concentrations were assumed and then the value of  $\alpha$  was arbitrarily set so that the curves would lay within the same range and thus could readily be compared.

This ranging is achieved in the following manner. Obviously, from equation (1), if  $c = 0$  it follows that  $F = 0$ . As the value of  $\beta \times c$  becomes large, the value of  $e^{-\beta c}$  becomes small and can be neglected with regard to the "one" in the parenthetic portion of equation (1). The equation then reduces to

$$F \cong \alpha \quad (19)$$

Thus all curves will lie between zero and the value of  $\alpha$ . Here  $\alpha$  was set equal to 10; thus all curves lie between 0 and 10 fluorescence units.


Naturally, it would be very interesting to compare the experimental titration curves of an actual complexometric system with those calculated for that system. Since very few metals (e.g.  $\text{Ce}^{3+}$ ) in solution fluoresce and none of the more common complexometric titrants, such as EDTA and EGTA fluoresce, the systems from which to choose an example are limited. A possible alternative would be to titrate the metal directly with the compound which is normally employed as the indicator in a fluorometric titration. However, since very little is known about the majority of indicator systems (i.e., stability constants of metal complexes, molar absorptivities, and quantum efficiencies) and the acquisition of such data would be a very extensive task, this avenue of attack is also restricted.

The description in recent literature (II-6) of a new fluorometric indicator contained pertinent information concerning the stability constants of its complexes with cobalt, copper, and nickel. Therefore, this system appeared to be quite suitable for such a comparative study. Attempts to prepare the monosodium salt of the indicator according to the prescribed procedure failed; and, although such an investigation of a practical system would have been interesting, the time required for such work did not warrant its further pursuit. However, the stability constant given for the copper-indicator complex (i.e.,  $5.0 \times 10^8$ ) was used in the subsequent calculations so as to give some insight into the appearance of curves for the titration of copper.

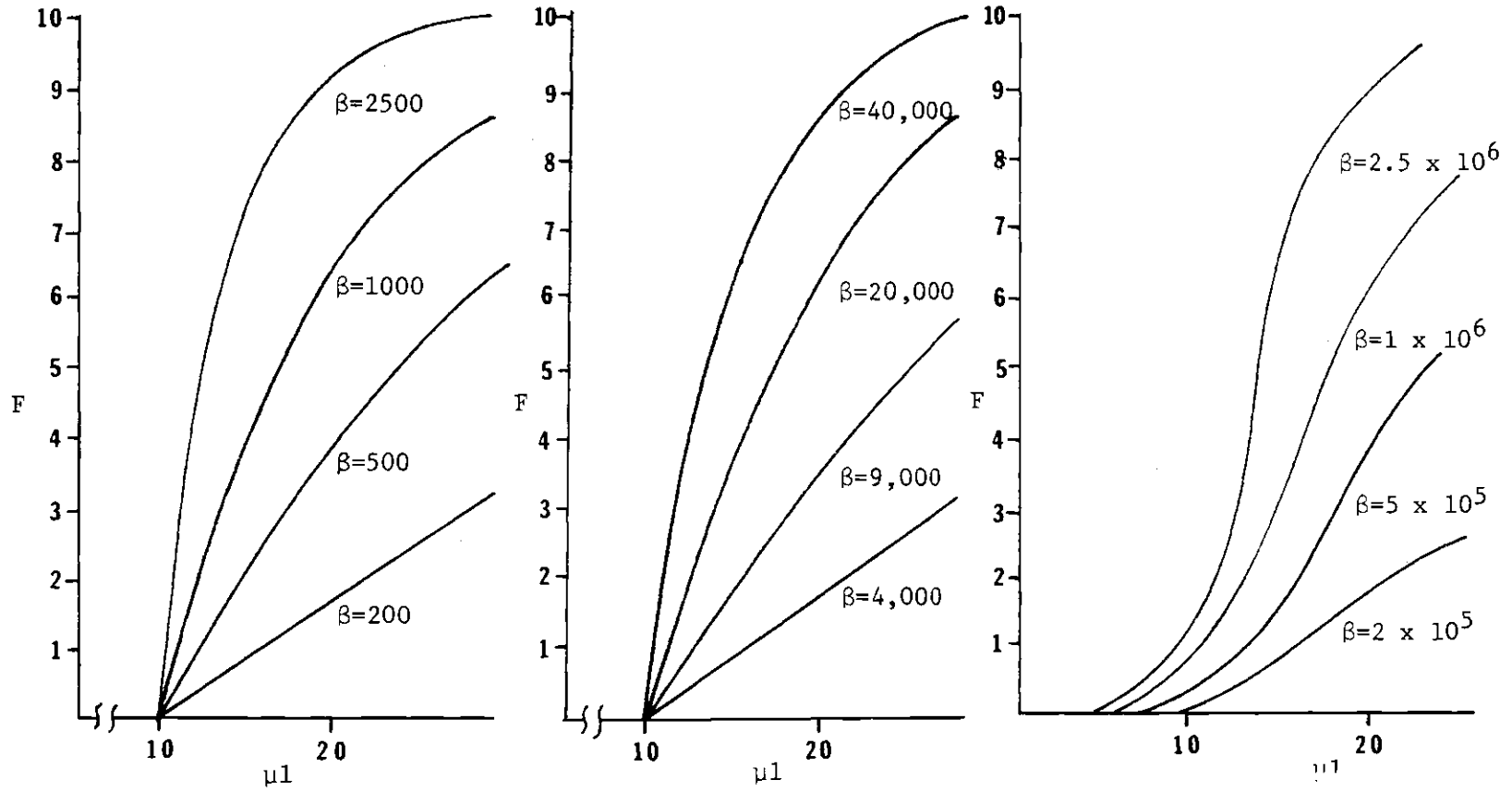
The following three cases, that is, where Y, M, or MY fluoresces, will be handled separately below. The curves discussed in each case are

those where dilution of the sample solution during the titration is negligible, i.e., the concentration of the titrant is far greater (here 1000 times) than that of the species being titrated. Considerations for the effects of and the correction for dilution will be discussed in a later section.

#### Case I: Only the Titrant Y, Fluoresces

In this case, equation (16) is employed to calculate the titration curves. The shape of the curve is  called a reversed "L". The fluorescence remains zero up to the vicinity of the equivalence point and then increases as more titrant is added. Figure 8A shows curves for the titration of  $10^{-3}$  F metal ion with 1.0 F titrant, with different value of  $\beta$ . Since  $\beta$  contains the molar absorptivity of the fluorescing species, it can be appreciated that small values of  $\beta$  give curves having small slopes; whereas, if  $\beta$  is increased the slope of the curve beyond the equivalence point increases.

Since the end point is located by extrapolation of the two straight line segments of the curve, in many instances such location will be made the easier and more precise the larger the value of  $\beta$ . However, in some cases where considerable "equivalence point curvature" (henceforth, abbreviated as e.p. curvature) exists, an increased  $\beta$  will merely improve the appearance of the curve, but will do nothing in aiding the location of the end point. The situation should become clearer upon additional discussion. It should also be noted here that as  $\beta$  is increased, the curve approaches  $\alpha$  asymptotically and bending becomes very noticeable. The mathematical consideration for such



A. Titration of 10 ml of  $10^{-3}F$  Metal with 1.0 F Titrant

B. Titration of 10 ml of  $5 \times 10^{-5}F$  Metal with  $5 \times 10^{-2}F$  Titrant

C. Titration of 10 ml of  $10^{-6}F$  Metal with  $10^{-3}F$  Titrant

Figure 8. Titration Curves where Only Y Fluoresces and  $\beta$  is Varied

$K = 5.0 \times 10^8$ ; Curves Not Corrected for Dilution

behavior has already been discussed.

An important portion of the curve is in the immediate vicinity of the end point. Here depending upon the concentrations and stability constant of the particular system, varying amounts of e.p. curvature will occur. Such curvature results from the fact that when M and Y are present in close to equivalent amounts, i.e., around the equivalence point, a very high degree of dissociation occurs for the complex, MY, which is formed during the titration. Because of the method by which the end point is located certain amounts of e.p. curvature can be tolerated. The region in the vicinity of the endpoint, where e.p. curvature may exist due to extensive dissociation, is not used; instead, the two straight portions far from the equivalence point are extrapolated to an intersect which is then taken as the end point.

Figure 8B shows the curves for the titration of  $5.0 \times 10^{-5}$  F metal with  $5.0 \times 10^{-2}$  F titrant. No e.p. curvature is indicated because the stability constant is rather high and no appreciable dissociation occurs, even though the metal solution is quite dilute. However, in Figure 8C which is the titration of  $10^{-6}$  F metal with  $10^{-3}$  F titrant, if attention is given to the immediate vicinity of the end point, it can be seen that considerable e.p. curvature is encountered. The stability constant is no longer high enough to compensate for the very low concentration of the metal solution being titrated.

The effect of the magnitude of the stability constant on the shape of the curves is shown by Figure 9. Since the interest here is only in the effect of the stability constant, other conditions were set

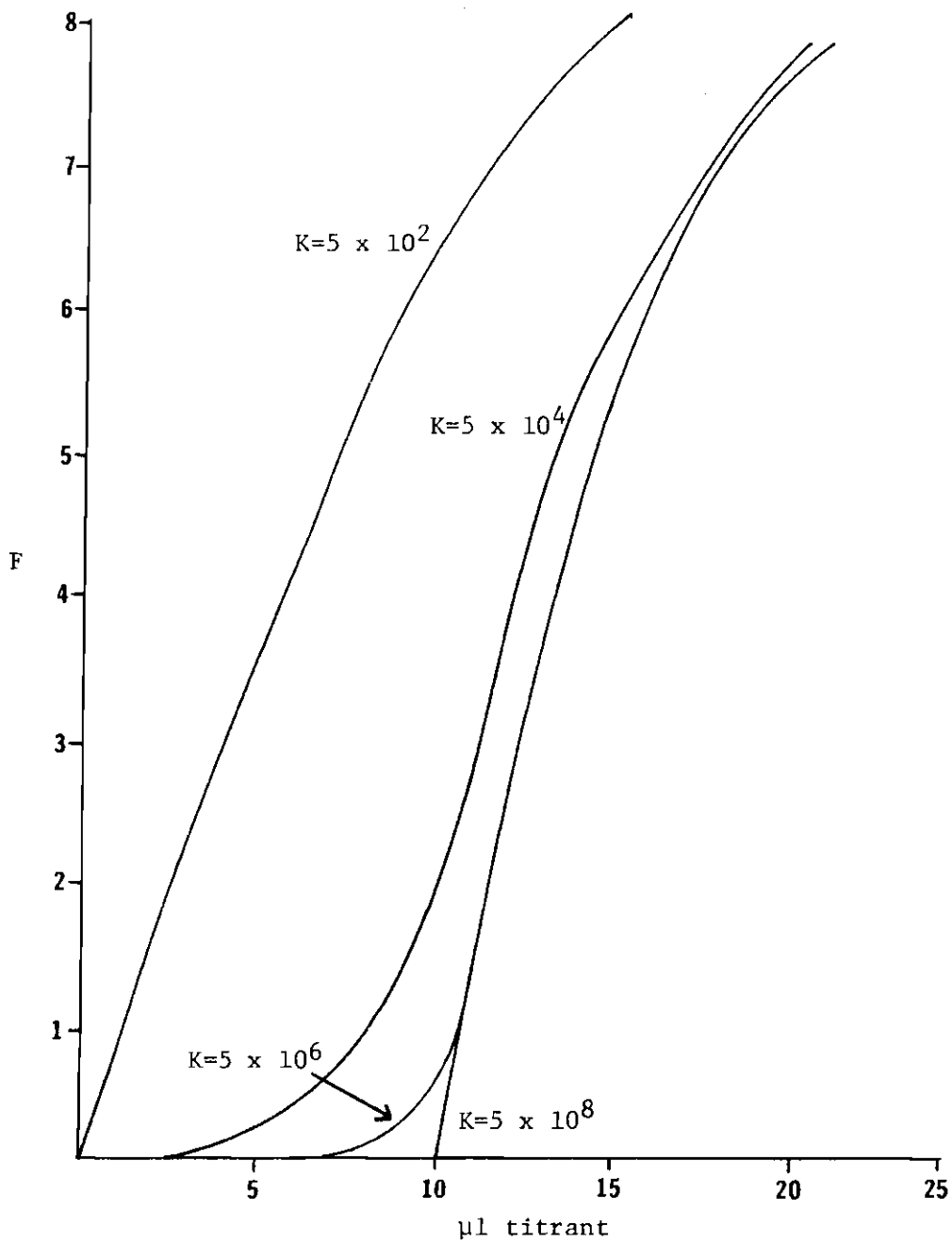


Figure 9. Influence of  $K$  on Titration Curves where Only  $Y$  Fluoresces  
 $\beta = 1500$ ;  $C_M^0 = 10^{-3}F$ ;  $C_T^0 = 1.0F$ ;  $V = 10$  ml

such that no distortion results from the dilution of the solution. Thus, the curves shown in Figure 9 are for the titration of  $10^{-3}$  F metal with 1.0 F titrant, where  $\beta$  is set equal to 1500, and the stability constant is progressively reduced by factors of hundred from its initial value of  $5.0 \times 10^8$  to that of  $5.0 \times 10^2$ .

As can be seen, as the magnitude of the stability constant is decreased, more and more e.p. curvature results until eventually the curve bends in the opposite direction, indicating that very little, if any, complex is being formed.

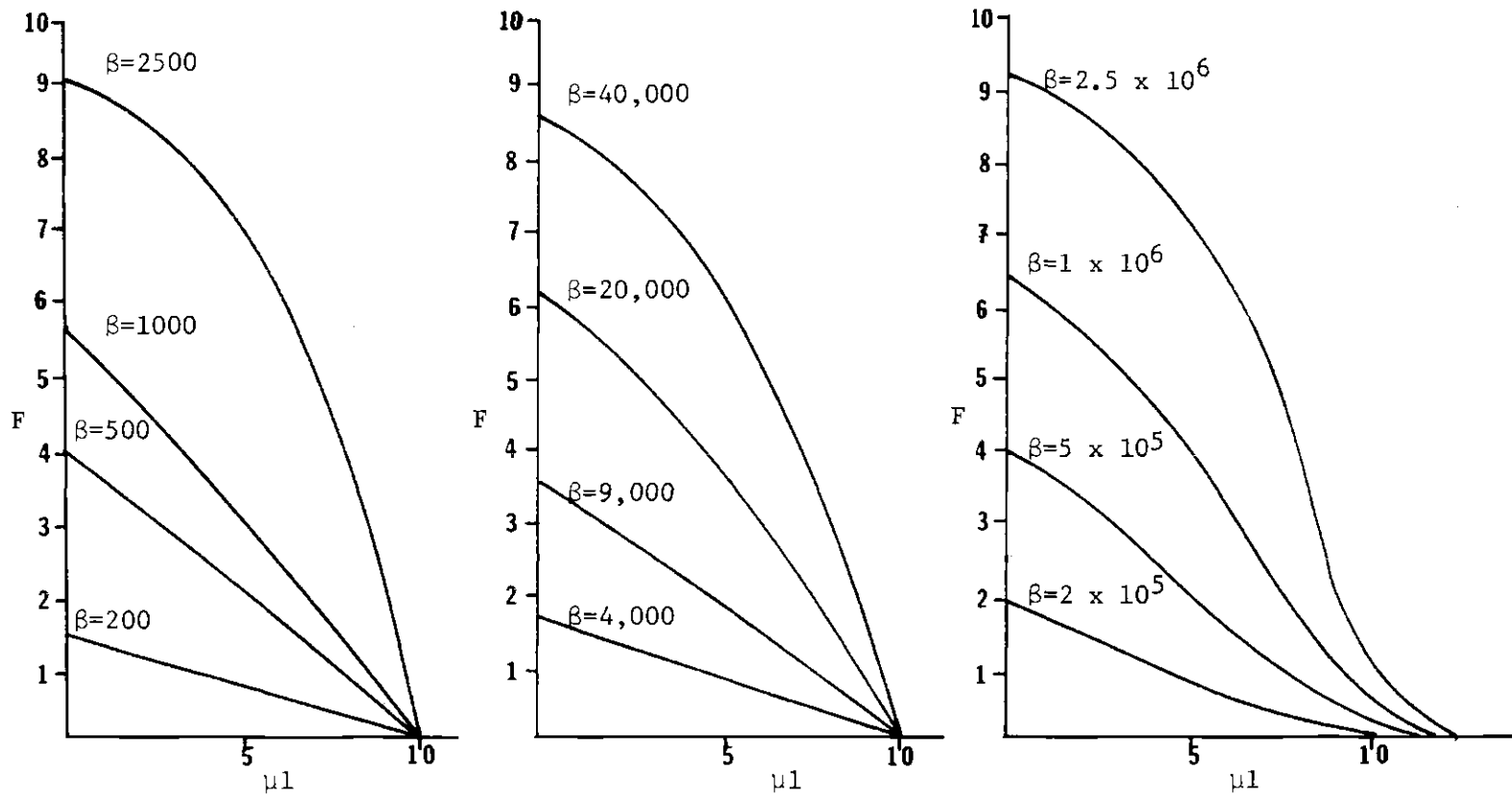
#### Case II: Only the Metal, M, Fluoresces

For this particular case equation (17) is employed to calculate the titration curves. The shape of the curves here is that of an "L". The fluorescence starts out high and then decreases as more titrant is added until it reaches zero.

Since much of what has been said concerning the trends found for Case I also applies here, only brief mention concerning the conclusions which were drawn will be given. Figure 10A shows the curves for the titration of  $10^{-3}$  F metal ion with 1.0 F titrant, where the value of  $\beta$  is varied. The effect  $\beta$  has on the shape of the curve is analogous to that for the previous case; however, its influence on the slope here is on that portion of the curve before the end point.

Also, here reducing the concentration of the metal (with the titrant still always being 1000 greater than that) causes considerable curvature in the vicinity of the end point. This effect can be seen in Figure 10C, which is the titration of  $10^{-6}$  F metal with  $10^{-3}$  F titrant.

The effect of the magnitude of the stability constant on the slope



A. Titration of 10 ml of  $10^{-3}F$  Metal with 1.0 F Titrant

B. Titration of 10 ml of  $5 \times 10^{-5}F$  Metal with  $5 \times 10^{-2}F$  Titrant

C. Titration of 10 ml of  $10^{-6}F$  Metal with  $10^{-3}F$  Titrant

Figure 10. Titration Curves where Only M Fluoresces and  $\beta$  is Varied

$K=5.0 \times 10^8$ ; Curves Not Corrected for Dilution

of the curve is shown in Figure 11. Again the constant has been reduced by a factor of hundred each time from  $5.0 \times 10^8$  to  $5.0 \times 10^2$ .

Case III: Only the Complex, MY, Fluoresces

In this case, equation (17) is employed to calculate the titration curves. The shape of the curves is  $\nabla$ , that is an inverted "L".

Figure 12A shows the curves for the titration of  $10^{-3}$  F metal with 1.0 F titrant at different values of  $\beta$ . At low values of  $\beta$ , the curves are as expected in a linear titration since under these conditions the approximation leading to equation (2a) holds. As  $\beta$  increases, the horizontal portion moves to higher ordinate values. Up to a certain point, the shape of the curves and ease of correctly locating the end point is not effected. However, when the product  $\beta \times c$  becomes too large, equation (2a) no longer holds and distortion of the curves becomes quite noticeable. Eventually, they "hit the ceiling"; that is, quite early in the titration the curves approach the limit value set by  $\alpha$ . Of course, such curves are of no practical value. This distortion is solely due to the fact that the fluorescence intensity and concentration of fluorescing species are related exponentially. Distortion due to dilution (see below) and dissociation because of low stability constants are not in effect, but if present these would obviously worsen the situation.

Examining the curves in Figure 12 shows the danger that the present case poses in an actual practical situation. The operator may assume that the rounding near the equivalence point is due to end point curvature and simply extrapolate upward from the earlier straight por-

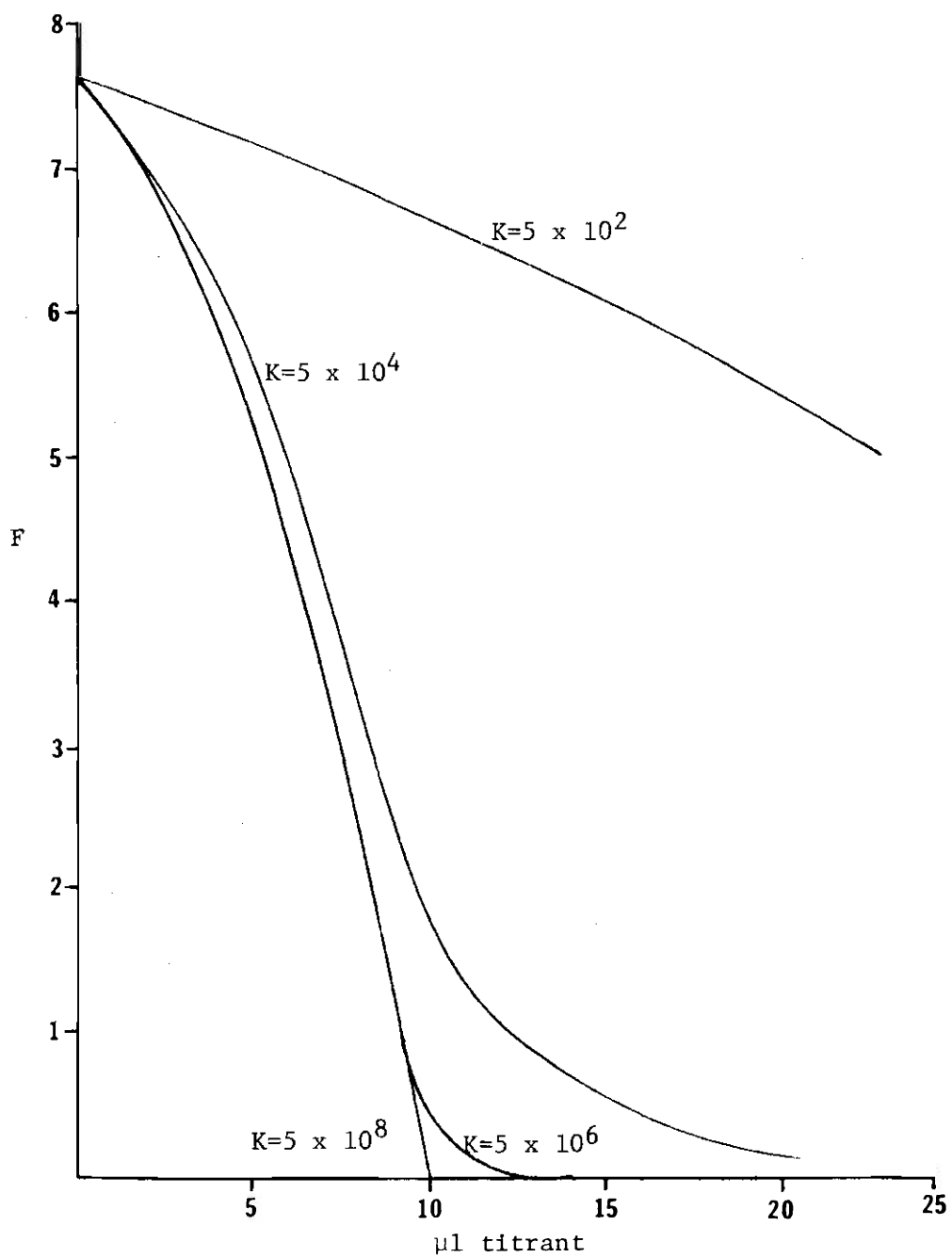
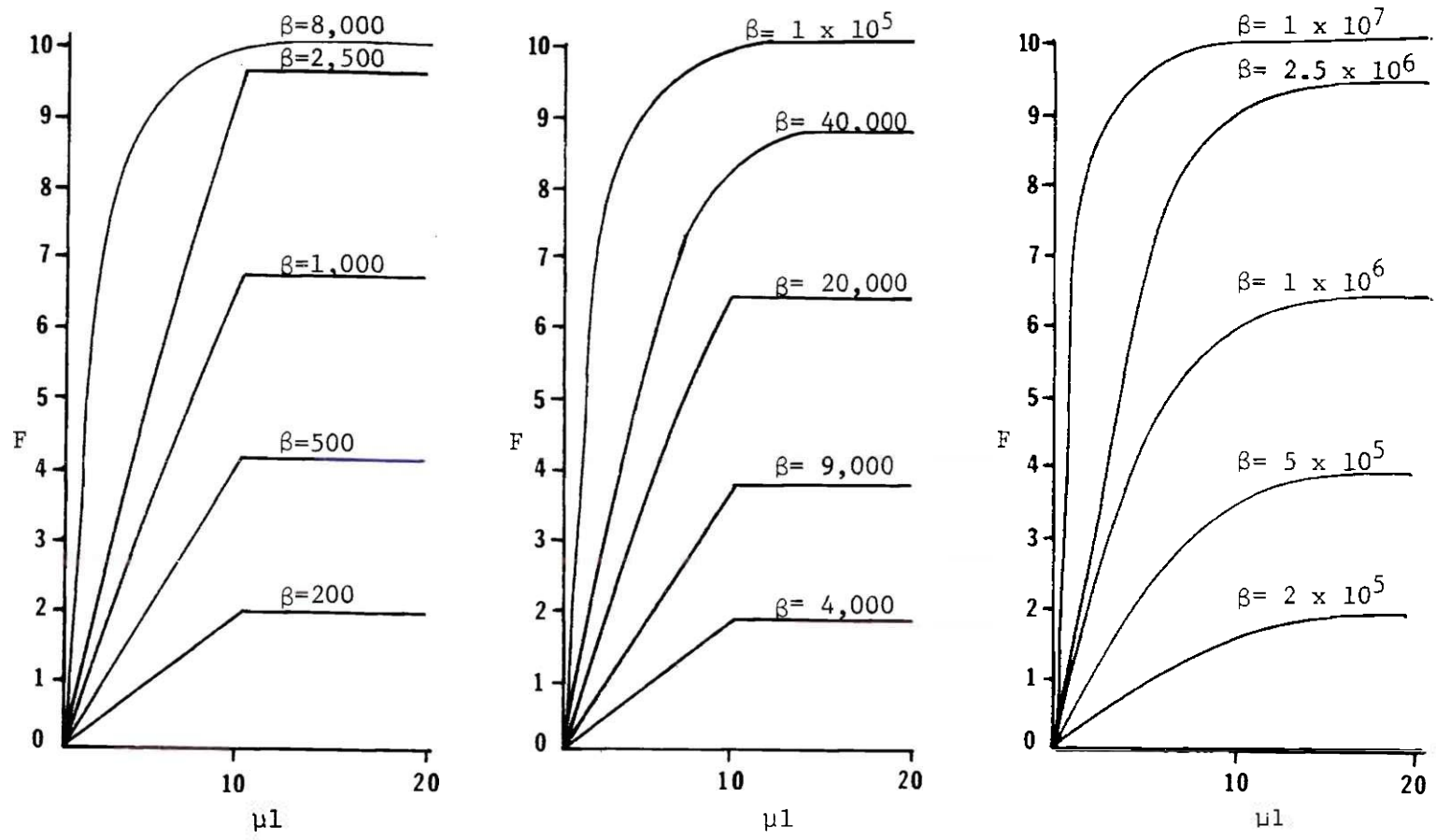


Figure 11. Influence of  $K$  on Titration Curves where Only  $M$  Fluoresces  
 $\beta = 1500$ ;  $C_M^0 = 10^{-3}F$ ;  $C_T^0 = 1.0F$ ;  $V = 10 \text{ ml}$



A. Titration of 10 ml of  $10^{-3}F$  Metal with 1.0 F Titrant

B. Titration of 10 ml of  $5 \times 10^{-5}F$  Metal with  $5 \times 10^{-2}F$  Titrant

C. Titration of 10 ml of  $10^{-6}F$  Metal with  $10^{-3}F$  Titrant

Figure 12. Titration Curves where Only MY Fluoresces and  $\beta$  is Varied

$K = 5.0 \times 10^8$ ; Curves Not Corrected for Dilution

tions. However, it can readily be seen that considerably lower results will then be obtained because the horizontal portion is too low and thus the intercept will be located far to the left, that is, towards too low a volume. Figure 13 shows the influence of the stability constant for a case where the  $\beta \times c$  value per se would not cause a problem. Here as before the stability constant has been reduced by a factor of hundred each time from  $5 \times 10^8$  to  $5 \times 10^2$ .

#### Correction for Dilution

Generally titration curves will depart from strict linearity since the solution volume increases during the titration. Commonly, what is done in such instances is to correct for dilution by multiplying each reading by the factor  $(V + b)/V$ , where  $V$  is the initial volume of the solution to be titrated and  $b$  is the volume of titrant solution added. It should be noted that this factor corrects only for "physical dilution", that is, the concentration goes down since after dilution the same number of particles are present as before dilution only now in a larger volume. There may, however, also be a "chemical dilution". Upon dilution a shift in the equilibrium may occur for certain types of reactions and then the number of relevant particles before and after dilution will be different. Application of the correction factor will not correct for this effect and, depending on the type of equilibrium involved, the overall correction may not fully compensate or may over-compensate for dilution.

The degree of "chemical dilution" depends on the equilibrium constant of the titration reaction and the concentration of the species to be determined. For both parameters the situation worsens as their

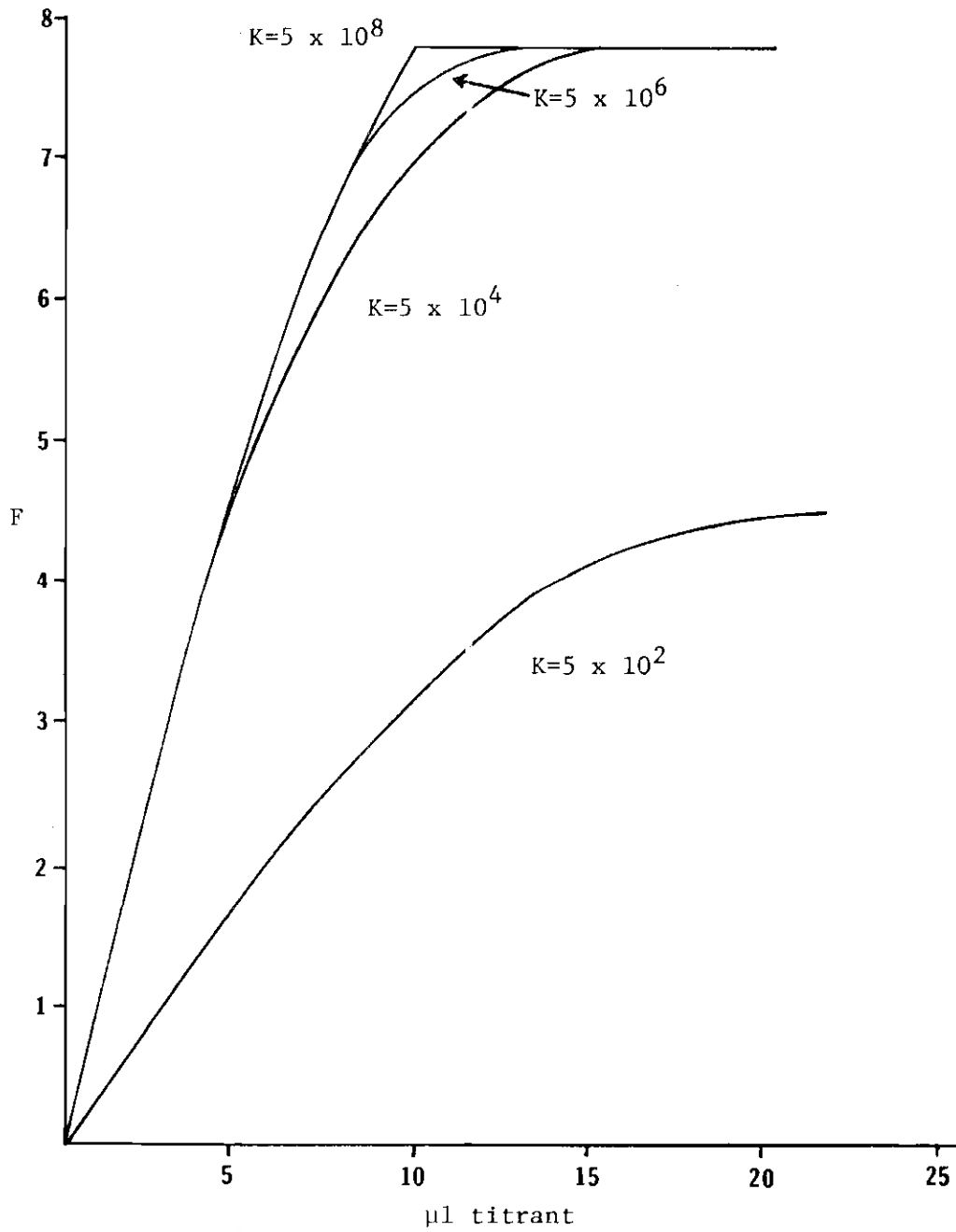


Figure 13. Influence of  $K$  on Titration Curves where Only MY Fluoresces  
 $\beta=1500$ ;  $C_M^0=10^{-3}F$ ;  $C_T^0=1.0F$ ;  $V=10$  ml

values are reduced. Since fluorometric titrations are by nature applied to usually quite dilute solutions, such titrations will be more prone to adverse dilution effects than other titrations, and will thus require special attention.

In linear titrations, such as a photometric one, it is actually not the output reading which is being corrected, but rather the concentration. In the case of a photometric titration where absorbance is directly proportional to concentration, it makes no difference whether the above factor is applied to the absorbance reading or the concentration.

However, the situation in a fluorometric titration is not so simple and straightforward. Only when the absorbance is less than 0.05 and the equation for fluorescence reduces to equation (2a), i.e.,  $F = k'c$ , is it proper simply to multiply the fluorescence reading by the factor  $(V + b)/V$ . In all other cases, when the full equation (i.e.,  $F = \alpha(1 - e^{-\beta c})$ ) has to be taken, the fluorescence is no longer directly proportional to the concentration, but is now related to it exponentially. Thus, it can be seen that multiplying the fluorescence reading by the correction factor is in error. Actually, the concentration of the fluorescing species should be multiplied by  $(V + b)/V$  and then the resulting value used to calculate the fluorescence by equation (1).

In a theoretical consideration this is no problem. However, in practice such a correction is impossible, since the term  $\beta$  (which consists of the molar absorptivity and cell length) must be known; and, more importantly, the concentration of the substance to be determined must also be known. This, of course, is never the case since the titration is

performed to find that concentration. Figures 14, 15 and 16 show curves for Cases I, II and III where just the dilution correction factor has been applied (incorrectly) to the fluorescence reading,  $F$ , and second (correctly) to the concentration in the exponential term.

In particular, in Case II where only  $M$  fluoresces it can be seen when multiplying,  $F$ , by  $(V + b)/V$  a maximum is obtained which indicates that upon applying the correction factor more fluorescence is produced. Obviously, this cannot be the case. However, when the concentration is corrected for dilution and then the fluorescence is calculated, a curve consistent with what is expected is obtained.

Additional consideration of the mathematics and inspection of the curves shows that the situation is not quite as bad as it might appear. For as has been shown there is a small portion of the curve (i.e., where  $\epsilon bc < 0.05$ ) near the end point where correction for dilution by multiplying,  $F$ , by  $(V + b)/V$  is appropriate. In Figure 15 in the present situation for  $\beta = 5000$  the maximum concentration can be  $1 \times 10^{-5} F$ , which is in the region between 9 and 10 ml of titrant added. Thus, the curves for the two methods of dilution correction merge into one curve somewhere between 9 and 10 ml. This is because in this area the fluorescence is directly proportional to concentration.

Of course, the degree to which the dilution problem affects the three cases of fluorescence is different. Where  $M$  or  $Y$  fluoresce the concentration of fluorescing species is quite low in the vicinity of the equivalence point. Then the approximation leading to equation (2a) holds and the correction for dilution can be applied directly to the scale readings. However, warning must be given that when extrapolating

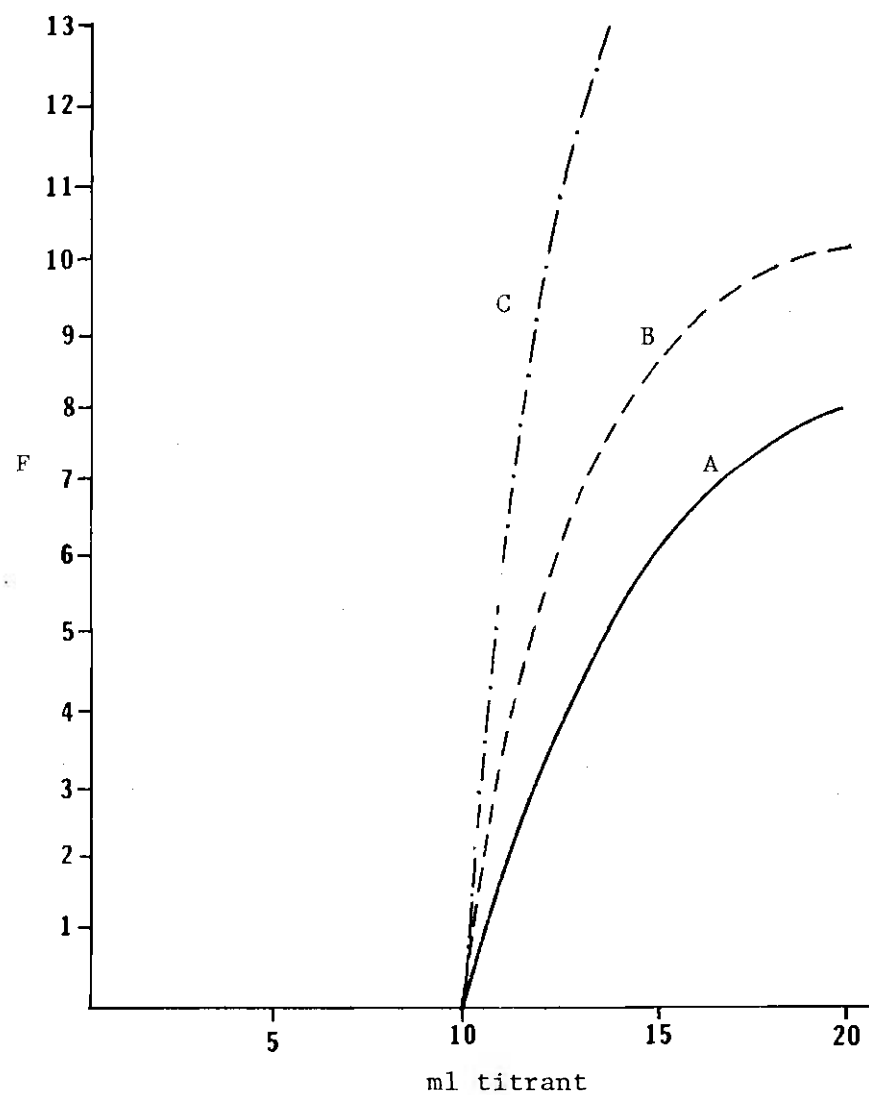


Figure 14. Effect of Dilution Correction on Titration Curves for Case I

- A. Uncorrected
- B. Concentration Corrected for Dilution
- C. Dilution Correction Applied to Fluorescence Reading, F

$$\beta=5000; C_M^0=10^{-3}F; C_T^0=10^{-3}F; V=10 \text{ ml}$$

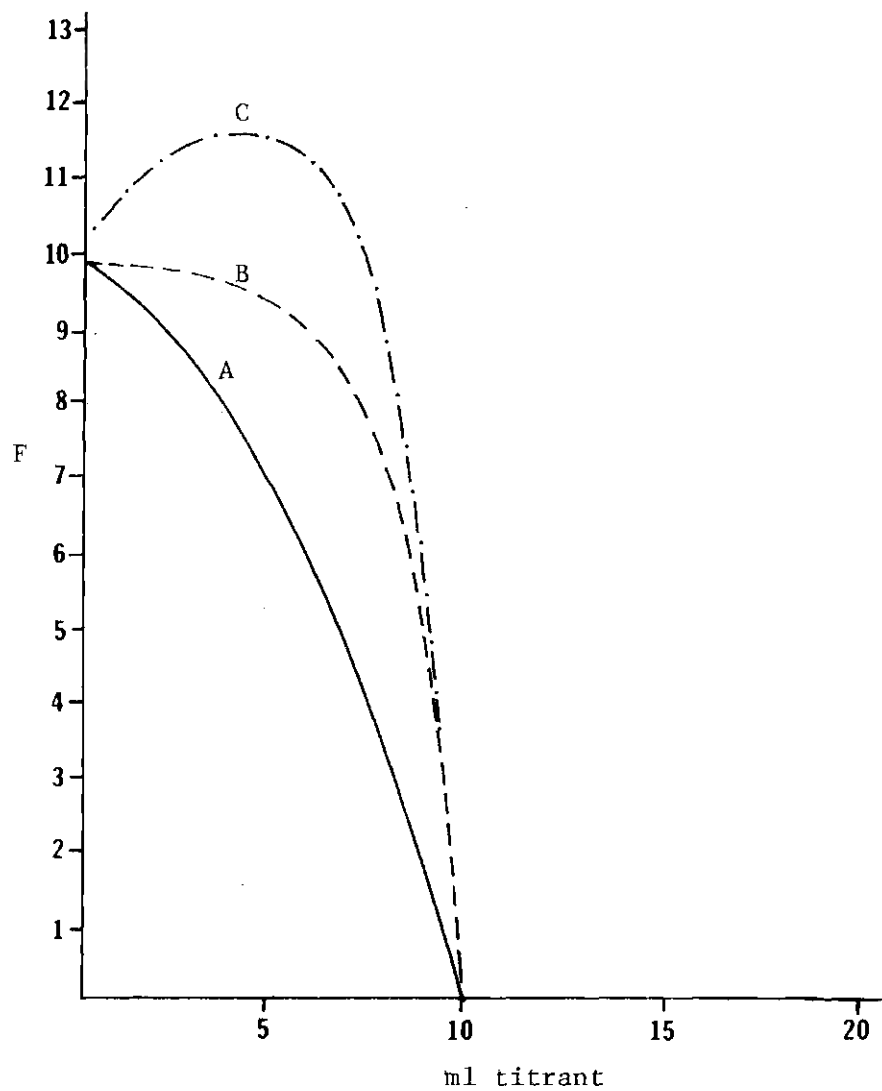


Figure 15. Effect of Dilution Correction on Titration Curves for Case II

- A. Uncorrected
- B. Concentration Corrected for Dilution
- C. Dilution Correction Applied to Fluorescence Reading, F

$$\beta=5000; C_M^0=10^{-3} \text{ F}; C_T^0=10^{-3} \text{ F}; V=10 \text{ ml}$$

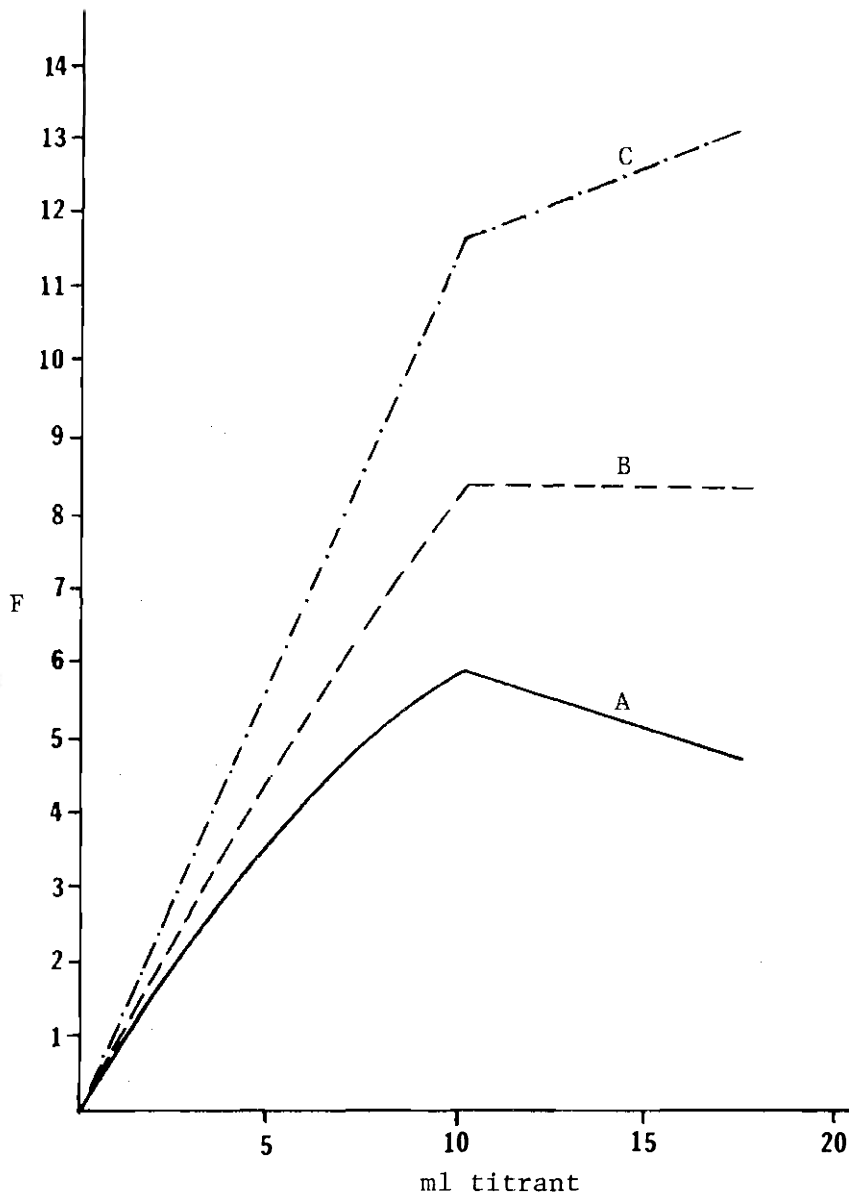


Figure 16. Effect of Dilution Correction on Titration Curves for Case III

- A. Uncorrected
- B. Concentration Corrected for Dilution
- C. Dilution Correction Applied to Fluorescence Reading, F

$$\beta=2500; C_M^0=10^{-3}F; C_T^0=10^{-3}F; V=10 \text{ ml}$$

towards the end point only those portions of the curve not too far from the equivalence point be used. The normal method of using far away portions of the curve as often exercised in other linear titrations is not allowed here because of the danger in using a region where equation (2a) no longer holds.

The most difficult case is where MY is the fluorescing species. Here the concentration of the fluorescing species is high at the equivalence point. Equation (2a) is not applicable; and, consequently, the dilution correction cannot be applied directly to the scale reading. At least one cannot make the decision of applicability a priori.

In practice, the situation could be improved by first making sure through preliminary experiments that the concentration and  $\beta$  are within the limits such that equation (2a) holds. Even after such precautions, the situation may still be troublesome. However, the dilemma could be remedied if the system in Case III was converted to that of Case I or Case II. In the specific instance of a compleximetric titration this is accomplished by first adding the complex former, Y, in sufficient excess such that all of the metal is complexed. The metal is then titrated with another stronger complexing agent, Z, such that if MZ is non-fluorescence the fluorescence will decrease as Z displaces Y forming MZ.

The findings here seem to be of importance for the possible future development of fluorometric titrations. Up until now the main reason for employing such titrations has been their high sensitivity. In almost all cases the concentration of sample solutions involved was low enough to assure the applicability of equation (2a). However, fluorometric

titrations are attractive for another reason; namely, higher selectivity. When titrations are to be applied to practical problems for selectivity reasons, one may well expect that the concentrations employed could be sufficiently high to cause the difficulties just discussed. Thus, it can be seen that extreme care must be applied in fluorometric titrations with respect to dilution. The best conclusion for practical work is to use, whenever possible, rather concentrated titrant solutions in connection with micro or syringe burets and thus avoid the necessity of dilution correction.

## APPENDIX A

The computer programs which follow are written in BASIC RT and were used on a PDP-8e. The computer is provided with the values for the stability constant,  $K$ ; initial volume,  $V$ ; initial concentration of metal,  $C_M^0$  (designated  $M$ ); initial concentration of titrant,  $C_T^0$  (designated  $T$ );  $\alpha$  (designated  $A$ );  $\beta$  (designated  $B$ ); size of each increment to be added,  $D$ ; and the total volume of titrant required,  $N$ .

Program 1 is used to calculate the fluorescence output in the fluorometric titration according to equation (16), i.e. where only the titrant,  $Y$ , fluoresces.

Program 2 is used to calculate the fluorescence output according to equation (17), i.e. where only the metal,  $M$ , fluoresces.

Program 3 is used to calculate the fluorescence output according to equation (18), i.e. where only the complex,  $MY$ , formed during the titration, fluoresces.

In all three programs the following are printed out.

- a) the value of each increment,  $D$ .
- b) the fluorescence output,  $F$ , obtained from the respective equations.
- c) the value,  $C$ , obtained by applying the dilution correction directly to the fluorescence reading,  $F$ .
- d) the value,  $C'$ , obtained by applying the dilution correction to the concentration term in the exponential part of the respective equations for fluorescence.

A listing of each program follows.

PROGRAM 1

Case I, Only the Titrant, Y, Fluoresces

```

10 INPUT K,V5,M,T,A,B,D,N
11 FOR X=0 TO 1 STEP .1 PLOT X,(A*.99)/A\NEXT X
17 F1=A
20 V=V5\V2=V*M\A2=1/(2*K)\A1=A*B
21 D1=0
22 PRINT \PRINT \PRINT
24 PRINT "K=";K\PRINT "VOL=";V5\PRINT "M=";M\PRINT "T=";T
25 PRINT "A=";A\PRINT "B=";B\PRINT "D=";D\PRINT \PRINT
26 PRINT "D"           F           C           C'"
30 PRINT
40 FOR I = D TO N STEP D\D1=D1+D\V1=1/(V+D1)\B1=D1*T
50 S2=((K*V1)*(B1-V2))-1\S3=SQR((S2*S2)+(4*K*B1*V1))
55 Y=A 2*(S2+S3)\R=(V5+D1)/V5\S=Y*A 1
56 Z=-1*B*Y\C=Z\Z=EXP(Z)\Z=1-Z\H=A*Z
57 C=C*R\C=EXP(C)\C=1-C\C=A*C
58 PRINT D1,H,H*R,C\ PLOT D1/N,(H*.99)/F1\NEXT I
59 PRINT
60 INPUT G\IF G=0 THEN 20 \IF G=1 THEN 71 \IF G=2 THEN 72 \IF G=3 THEN 73
61 IF G=4 THEN 74 \IF G=5 THEN 75 \IF G=6 THEN 76 \IF G=7 THEN 77
62 IF G=8 THEN 78 \IF G=9 THEN 79
71 INPUT K\GO TO 60
72 INPUT V5\GO TO 60
73 INPUT M\GO TO 60
74 INPUT T\GO TO 60
75 INPUT A\GO TO 60
76 INPUT B\GO TO 60
77 INPUT D\GO TO 60
78 INPUT N\GO TO 60
79 STOP

```

PROGRAM 2

Case II; Only the Metal, M, Fluoresces

```

10 INPUT K,V5,M,T,A ;B ,D,N
11 FOR X=0 TO 1 STEP .1\ PLOT X,(A*.99)/A \NEXT X
17 F1=A
20 V=V5\ V2=V*M\ A2=1/(2*K)\ A1=A *B
21 D1=0
22 PRINT \PRINT \PRINT
24 PRINT " K=";K\PRINT "VOL=";V5\PRINT "M=";M\PRINT "T=";T
25 PRINT "A =" ;A\PRINT "B=";B\PRINT "D=";D\PRINT \PRINT
26 PRINT " D F C C "
30 PRINT
40 FOR I=D TO N STEP D\D1=D1+D\ V1=1/(V+D1)\ B1=D1*T
50 S2=((K*V1)*(B1-V2))-1\S3=SQR((S2*S2)+(4*K*B1*V1))
53 Y =A 2*(S2+S3) \ R = (V5+D1)/V5
54 W1=D1/(V5+D1) \ W2=V5/(V5+D1)
55 Y=Y+M*W2-T*W1 \ S=Y*A1
56 Z =-1*B *Y\C=Z \ Z =EXP(Z) \ Z =1-Z \ H=A *Z
57 C =C*R\ EXP(C) \ C=1-C \ C=A*C
58 PRINT D1,H,H*R ,C\ PLOT D1/N,(H*.99)/F1 \NEXT I
59 PRINT
60 INPUT G\ IF G=0 THEN 20 \ IF G=1 THEN 71 \ IF G=2 THEN 72 \ IF G=3 THEN 73
61 IF G=4 THEN 74 \ IF G=5 THEN 75 \ IF G=6 THEN 76 \ IF G=7 THEN 77
62 IF G=8 THEN 78 \ IF G=9 THEN 79
71 INPUT K\ GO TO 60
72 INPUT V5\ GO TO 60
73 INPUT M\ GO TO 60
74 INPUT T\ GO TO 60
75 INPUT A\ GO TO 60
76 INPUT B\ GO TO 60
77 INPUT D\ GO TO 60
78 INPUT N\ GO TO 60
79 STOP

```

PROGRAM 3

Case II; Only the Complex, MY, Fluoresces

```

10 INPUT K,V5,M,T,A,B,D,N
11 FOR X=0 TO 1 STEP .1 PLOT X,(A*.99)/A \NEXT X
17 F1=A
20 V=V5 \V2=V*M \A 2=1/(2*K) \A 1=A*B
21 D1=0
22 PRINT \PRINT \PRINT
24 PRINT " K =";K \PRINT " VOL=";V5 \PRINT " M=";M \PRINT " T=";T
25 PRINT " A =";A \PRINT " B =";B \PRINT " D=";D \PRINT \PRINT
26 PRINT " D          F          C          C'"
30 PRINT
40 FOR I=D TO N STEP D \D1=D1+D \V1=1/(V+D1) \B1=D1*T
50 S 2=((K*V1)*(B 1-V2))-1 \S3=SQR ((S2*S2)+(4*K*B 1*V1))
53 Y=A2*(S 2+S3) \R=(V5+D1)/V5
54 W1=D1/(V5+D1) \W2=V5/(V5+D1)
55 Y=T*W1-Y \S *A 1
56 Z =-1* B *Y \C=Z \Z =EXP(Z) \Z =1-Z \H=A *Z
57 C=C*R \C=EXP(C) \C =1-C \C =C*A
58 PRINT D1,H,H*R,C \PLOT D1/N,(H*.99)/F1 \NEXT I
59 PRINT
60 INPUT G \IF G=0 THEN 20 \IF G=1 THEN 71 \IF G=2 THEN 72 \IF G=3 THEN 73
61 IF G=4 THEN 74 \IF G=5 THEN 75 \IF G=6 THEN 76 \IF G=7 THEN 77
62 IF G=8 THEN 78 \IF G=9 THEN 79
71 INPUT K \GO TO 60
72 INPUT V5 \GO TO 60
73 INPUT M \GO TO 60
74 INPUT T \GO TO 60
75 INPUT A \GO TO 60
76 INPUT B \GO TO 60
77 INPUT D \GO TO 60
78 INPUT N \GO TO 60
79 STOP

```

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PART THREE

INVESTIGATIONS IN THE FIELD OF FLUOROMETRIC TITRATIONS

## CHAPTER I

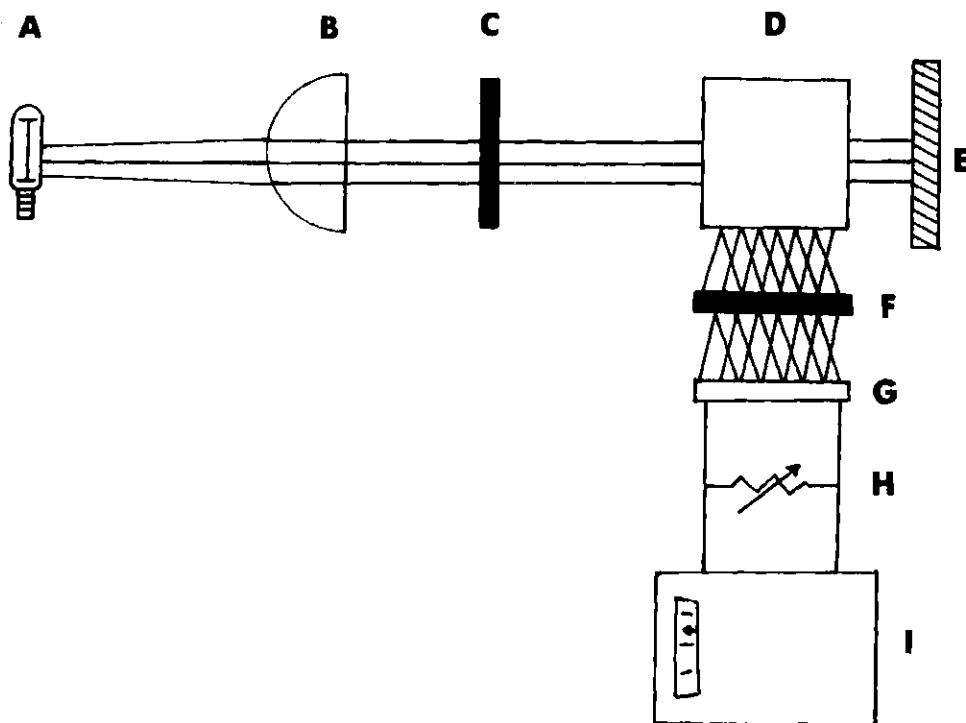
## INTRODUCTION

Fluorometric determinations have become an extremely important weapon in the analytical chemist's arsenal. However, the obvious application of fluorescence to the area of fluorometric titrations has developed slowly. At the beginning fluorometric titrations have been limited to visual end point detection where a sudden change in fluorescence occurs, often employing a "make shift" black box or working in a darkened room. However, in order to make use of a linear fluorometric titration where a gradual decrease or increase of the fluorescence is followed over the whole course of the titration a more sophisticated arrangement is required; one specifically designed as a fluorotitrator would be best. Several such instruments (III-1,2) are commercially available, but are designed for specific situations and thus make the apparatus essentially useless for general work.

Several instruments designed to monitor fluorescent titrations have been described in the literature (III-3-7). These instruments vary greatly in design and approach to meet the necessary requirements. Each has various limitations which restricts its usefulness in monitoring fluorescent titrations. All operate on the same principle as does a fluorometer (see Figure 17), but with additional features to facilitate

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References for Part III on page 111.



A-Excitor Lamp; B-Lens; C-Primary Filter; D-Sample Cell; E-Light Absorbing Background; F-Secondary Filter; G-Photodetector; H-Variable Resistor; I-Meter

Figure 17. Diagram of the Components of a Typical Fluorometer

a titration.

In general, a fluorometer operates as follows:

The desired wavelength of light from the excitor lamp (A) is selected by the primary filter (C) placed between the radiation source and the sample. The wavelength of fluorescent light to be measured is selected by the secondary filter (F) placed between the sample and a photodetector (G) commonly located at a  $90^{\circ}$  angle from the incident beam. The output of the photodetector is amplified and displayed on a meter or recorder. The whole arrangement is, of course, enclosed in a light tight cabinet to exclude ambient light. The necessity of such an enclosure in a fluorometer to exclude ambient light is not too much of a problem for fluorometric determinations, but in the case of titrations causes great inconvenience.

An instrument that does not require complete exclusion of ambient light would be desirable. Such an instrument was employed in the present investigation.

## CHAPTER II

## FLUOROTITRATOR EMPLOYED IN THIS INVESTIGATION

The instrument used here has been developed in the Chemistry Department of Georgia Tech by White (III-8), and has also been described (i.e., the electronics, optics, etc.) elsewhere (III-9). Therefore, only brief comment will be made here concerning those instrumental features important for the performance of titrations.

Ambient Light

The most significant feature of the new fluorotitrator is its operation with the sample exposed to room light. The instrument design is such that very little ambient light reaches the photomultiplier and that portion is corrected for electronically.

Stability

An instrument which is used to follow the change in fluorescence over an entire linear titration can only give good results if that instrument is essentially free from drift such that the entire titration can be performed without a significant change in the set-point. Also, noise should be minimized.

With the new instrument extended periods of operation are no problem since the stability of the instrument is enhanced by the fact that the light source and photomultiplier are constantly monitored as a unit. The stability of the instrument was checked by White and a typical

result showed a drift of 6 scale divisions (based on a scale of 0 to 100) over a three hour period or approximately 2 percent per hour. Since the time required to complete a titration is considerably less, this drift causes no problem.

White's main purpose was to design (i.e., optically, electronically, etc.) and build the fluorotitrator. He evaluated the general performance of the instrument by conducting titrations of copper and calcium with EDTA to a Calcein end point. Both systems employed are well established and for mere preliminary evaluation were quite adequate. However, the studies need to be expanded to test further the versatility of the instrument and to establish its limitations. This was accomplished by investigating another system.

## CHAPTER III

## SYSTEM EMPLOYED IN THIS INVESTIGATION FOR FLUOROMETRIC TITRATION

A number of organic compounds which form complexes with cations in solution with a consequent disappearance or appearance of fluorescence have been employed as indicators in compleximetric titrations (III-10). As indicators these compounds function in a similar manner to metallochromic indicators, i.e., the end point in the titration with a complexing agent is denoted by the change in fluorescence intensity caused by the destruction of the metal-indicator complex. The end point is detected visually. For the majority of the indicators recommended to date, their application has been limited to compleximetry with EDTA as titrant, although, several are useful in titrations with other reagents, e.g., fluoride. One such indicator which has found wide use is Calcein Blue.

Properties of Calcein Blue

Calcein Blue is synthesized by the condensation of 4-methylumbelliferone with formaldehyde and iminodiacetic acid. The indicator was initially prepared independently and concurrently by Eggers (III-11) and Wilkins (III-12), who named it respectively, Umbelliferone Complexan and Calcein Blue. It exhibits a brilliant blue fluorescence below pH 12, but is non-fluorescent at higher pH. Studies have shown that Calcein Blue is useful as an indicator in the EDTA titration of certain elements. In particular Wilkins found that the blue fluorescence is quenched by copper between pH 4 and 10. Also cobalt, manganese, zinc, and mercury

may be successfully determined with Calcein Blue by addition of excess EDTA and back-titration of the excess with copper.

Various papers have appeared describing the use of this indicator in the determination of other metals (III-13,14), e.g., with the alkaline earths. These metals form fluorescent "indicator reversal complexes" at pH's of 12 or higher; whereas, under such conditions the free indicator does not fluoresce. Thus, calcium, barium, and strontium may be titrated directly above pH 12; the end point is marked by the decrease in the fluorescence of the alkaline earth complexes.

As a metallofluorescent indicator Calcein Blue is more efficient than the similar Calcein because the maximum fluorescence of Calcein Blue at 445 nm is produced by excitation at 370 nm, which coincides more closely with the 366 nm emission line of UV sources, employing mercury discharge lamps, than does the much longer excitation wavelength of Calcein.

A more recent paper of interest (III-15) has added additional information on Calcein Blue concerning purity, composition, structure, properties as an acid, and fluorescence as a function of pH.

Besides those already discussed the present study has considered several other metals in conjunction with Calcein Blue, with lead indicating great potential. An investigation of lead here is interesting for several reasons. First, since lead is known to be a health hazard, rapid methods exhibiting good sensitivity and selectivity for its determination, especially in air, are needed. Second, only a few fluorometric methods for lead are known (e.g., the determination of lead as the lead-morin complex) and thus additional research in this area is warranted.

### Preliminary Work

Preliminary experiments have shown that upon addition of a lead salt to a solution of Calcein Blue the intensity of the already existing fluorescence increases, indicating the formation of a complex between lead and Calcein Blue. It was hoped that this fact could then be used as the basis to perform a linear fluorometric titration of lead with Calcein Blue, but the attempts failed because the titration curves obtained were continuously but gently bent over the entire course of the titration and did not allow the location of an end point. The probable reason is that the lead-Calcein Blue complex is too weak to yield a distinctive titration curve. This assumption is also strongly supported by comparison of the experimental curves with those previously calculated for a weak complex system as shown in Figure 13, page 69.

Next, Calcein Blue was used as the indicator in the titration of lead with EDTA, since the requirements with regard to the stability of a complex are much less stringent for an indicator complex than they are for a titration complex (II-16). Of course, the end point in such a titration of lead would not be indicated by a sudden appearance or disappearance of fluorescence, but rather by a decrease in intensity only. It is also important to note here that it was impossible to determine visually whether the change in fluorescence was actually a change in intensity, hue, or both. The facilities needed to determine this were not available and thus for simplicity it was assumed that the change was of the fluorescent intensity.

Such a situation is not good for visual indication and this may be taken as the most probable reason why Calcein Blue has previously not

been reported or considered as an indicator for lead. However, with an instrument such as the one being employed here to follow the titration, a mere change in fluorescence intensity, is no hindrance in establishing the end point.

During the early months of this investigation a puzzling and extremely frustrating situation developed. Very good titration curves were obtained in which the fluorescence decreased as the titration proceeded to a particular level and then remained constant as more titrant was added. Then the behavior of the system changed and curves completely opposite to those were obtained; that is, the fluorescence increased up to a certain level. Since the instrument settings were unchanged, it was felt that the fluorotitrator itself was not responsible. Also, since the lead-EDTA system is well established, it was also ruled out as the possible culprit.

Thus, by the process of elimination, the Calcein Blue had to be the species causing the trouble. When the work was started, the Calcein Blue solution was prepared from the powdered indicator purchased in the standard 1 gm bottle from Baker Chemical Company. When this supply approached exhaustion an order was placed for more reagent, but the indicator received now was from the Fisher Chemical Company. Because both products were labelled "Analytical Grade" no problems were expected. Solutions of the two indicator preparations were used interchangeably and then the inconsistent results occurred.

The two brands of indicator were compared using thin-layer chromatography on Baker-Flex cellulose sheets employing 1-butanol containing 25% ammonia as the mobile phase. The photograph in Figure 18B shows the

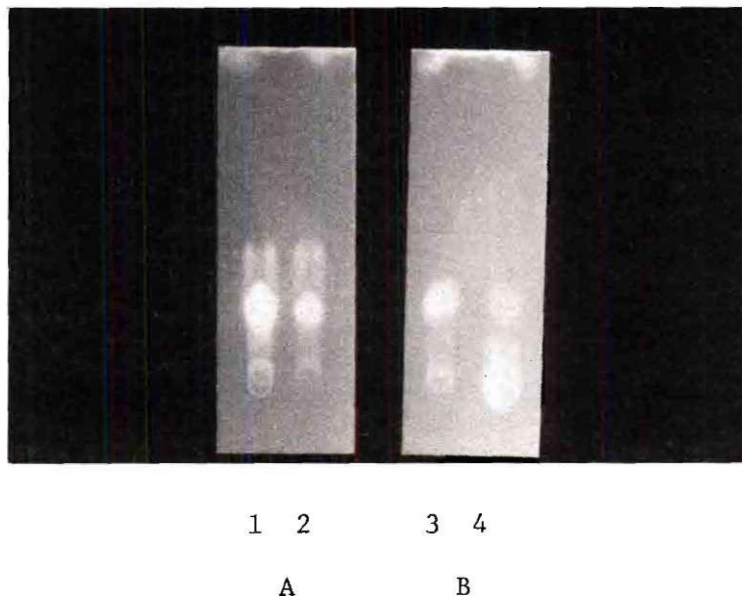


Figure 18. Photograph of Thin-Layer Comparison of Different Brands of Calcein Blue.

- A.1. First Sample of Calcein Blue Obtained from J. T. Baker Chemical Co.
- 2. Second Sample of Calcein Blue Obtained from J. T. Baker Chemical Co.
- B.3. First Sample of Calcein Blue Obtained from J. T. Baker Chemical Co.
- 4. First Sample of Calcein Blue Obtained from Fisher Chemical Co.

The two brands of Calcein Blue were compared using thin-layer chromatography on Baker-Flex cellulose sheets employing 1-butanol containing 25 percent ammonia as the mobile phase. Developing time was 15 minutes. The sheets were illuminated by a blacklight. A Kodak Wratten gelatin filter #2B was placed in front of the camera lens to screen out the ultra-violet light. The time of exposure was three seconds and the F-stop setting was 4.2.

results. A single spot on the left resulted from the Calcein Blue obtained from Baker while a series of spots on the right occurred with the Calcein Blue obtained from Fisher. Since the Baker Brand obviously contained only one component, it was assumed that this was the pure indicator; whereas, the Fisher Brand may have contained impurities such as starting materials or products from subsequent degradation. When tested as visual indicators in common operations, such as the titration of copper with EDTA, both products were satisfactory; but for the investigation intended here Baker material was used exclusively once the situation was clear.

A further Baker product obtained at a later date and of different batch number was tested too and showed consistent behavior. The chromatogram is shown in Figure 18A.

The odd behavior of the two brands of Calcein Blue warrant further investigation; however, this was not in line with the present work and therefore no further investigation was undertaken at this time.

#### EDTA Titration of Lead to a Fluorometric End Point

In using Calcein Blue as the indicator for the titration of lead with EDTA two types of indication can be differentiated according to the nomenclature proposed by Flaschka and Sawyer (III-17).

The first case is a step titration. Here the Calcein Blue is added in an amount very small in comparison to the amount of lead and functions as a true indicator. During the initial part of the titration nothing changes visibly as the free lead is complexed by the EDTA. Then in the immediate vicinity before the equivalence point, the lead begins to be taken away from the indicator and the fluorescence decreases within

a drop or fraction of a drop of titrant added, giving the titration curve the shape of a step, hence the name step indication. The type curve obtained is represented by curve I in Figure 19.

The second case is a slope titration. Here the amount of Calcein Blue added is in excess over that of the lead present and the Calcein Blue does not act as an indicator in the common sense, but rather simply as a fluorogenic agent. A self-indicating system is created. The type curve obtained is represented by curve II in Figure 19. From the first addition of EDTA, lead is taken from the Calcein Blue-lead complex; and, with the fluorescence decreasing, the curve continuously slopes downward over the whole course of the titration until the equivalence point is reached, after which the curve levels off, hence the name slope titration. This gradual change in fluorescence is difficult for the observer to use directly for the end point location. However, when followed by an instrument all the advantages of a self-indicating linear titration are realized as, e.g., averaging of reading, higher selectivity, possibility of straight line portion to be extrapolated and thus less stringent requirements in the stability constants of the complexes involved.

In the present case, where the fluorescence does not completely disappear but merely changes from that of a dark blue to a lighter blue, the eye cannot be used to locate the end point, and thus it must be located with the use of a fluorotitrator.

The situation here is quite analogous to the photometric titration of copper with EDTA in ammoniacal median. The change in color is merely from the dark blue of the ammine complex to the much lighter blue of the EDTA complex. Many of the advantages gained in such a titration can be

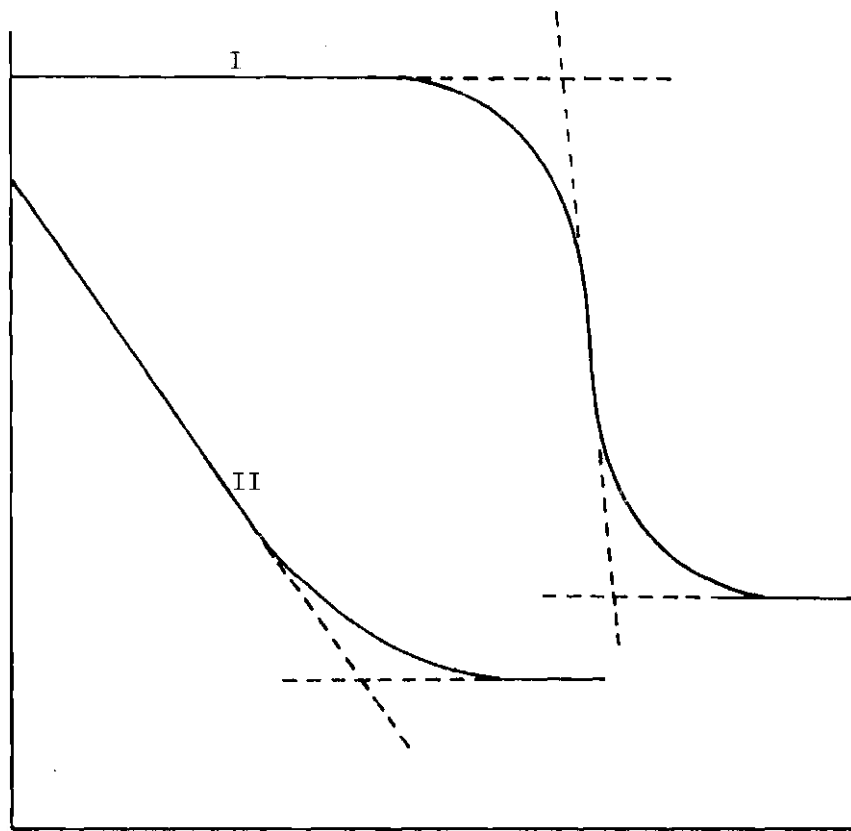


Figure 19. Idealized Photometric Titration Curves for Various Types of Indication

- I. Step-Indication
- II. Self-Indication

beneficially applied here.

#### Procedure

In the present study no primary filter was used and the secondary filter used was an Edmund Scientific Filter #856 with a peak transmittance at 440 nm. The titration vessels employed in this series of titrations were 20 ml scintillation tubes.

The procedure is as follows:

1. Pipet 1 ml of sample into the titration vessel.
2. Pipet 1 ml each of the Calcein Blue solution and pH 4.7 buffer solution into the titration vessel.
3. Finally pipet 5 ml of deionized water into the vessel bringing the volume to a total of 8 ml (Note 1)
4. With the "Set 100" control adjust the instrument to a reading of 50 to 60 scale divisions with the solution to be titrated in the light path.
5. Titrate with the appropriate concentration of EDTA and read the fluorescence output of the solution after each addition of titrant (Note 2).
6. Plot fluorescence versus ml of titrant to obtain the titration curve.
7. Draw the best straight lines through the two branches of the curve, taking the intersection of these two lines as the end point.

Note 1: The minimum volume required such that the solution level is completely above the light beam is 7.5 ml.

Note 2: The solution is stirred after each addition of titrant with a very small magnetic stirring bar. It is necessary that the stirrer be shut off before making the reading; otherwise, the liquid vortex will be in the exciting beam and errors will result.

## CHAPTER IV

## EVALUATION OF THE TITRATION SYSTEM

The results of several titrations of lead are presented in Table 3. These results are within the bounds of acceptability and indicate that good results can be obtained at various levels using the new titrimetric method. A typical titration curve is shown in Figure 20. The "L" shaped curve is as expected. Although the fluorescence does not become zero, it reaches a level due to the fluorescence of the Calcein Blue where it remains. The curve is similar to those calculated for analogous cases in Part II of this thesis. From the titration curve in Figure 20, it can be seen that the expected fluorescence output is linear as the concentration of the fluorescent species is low.

Table 3. Results Obtained from Fluorometric Titration of Lead at Various Concentration Levels

$\mu\text{g Pb, taken}$	$\mu\text{g, found}$	Diff.	% Error
1088	1086	-2	0.18
198.6	194.6	-4	2.0
130.1	129.5	-0.6	0.5
108.8	108.8	0	0
90.5	85.7	-14.8	5
77.6	75.6	-2	2.6
64.6	63.0	1.6	2.5
1.32 <sub>3</sub>	1.28 <sub>9</sub>	-.034	2.6

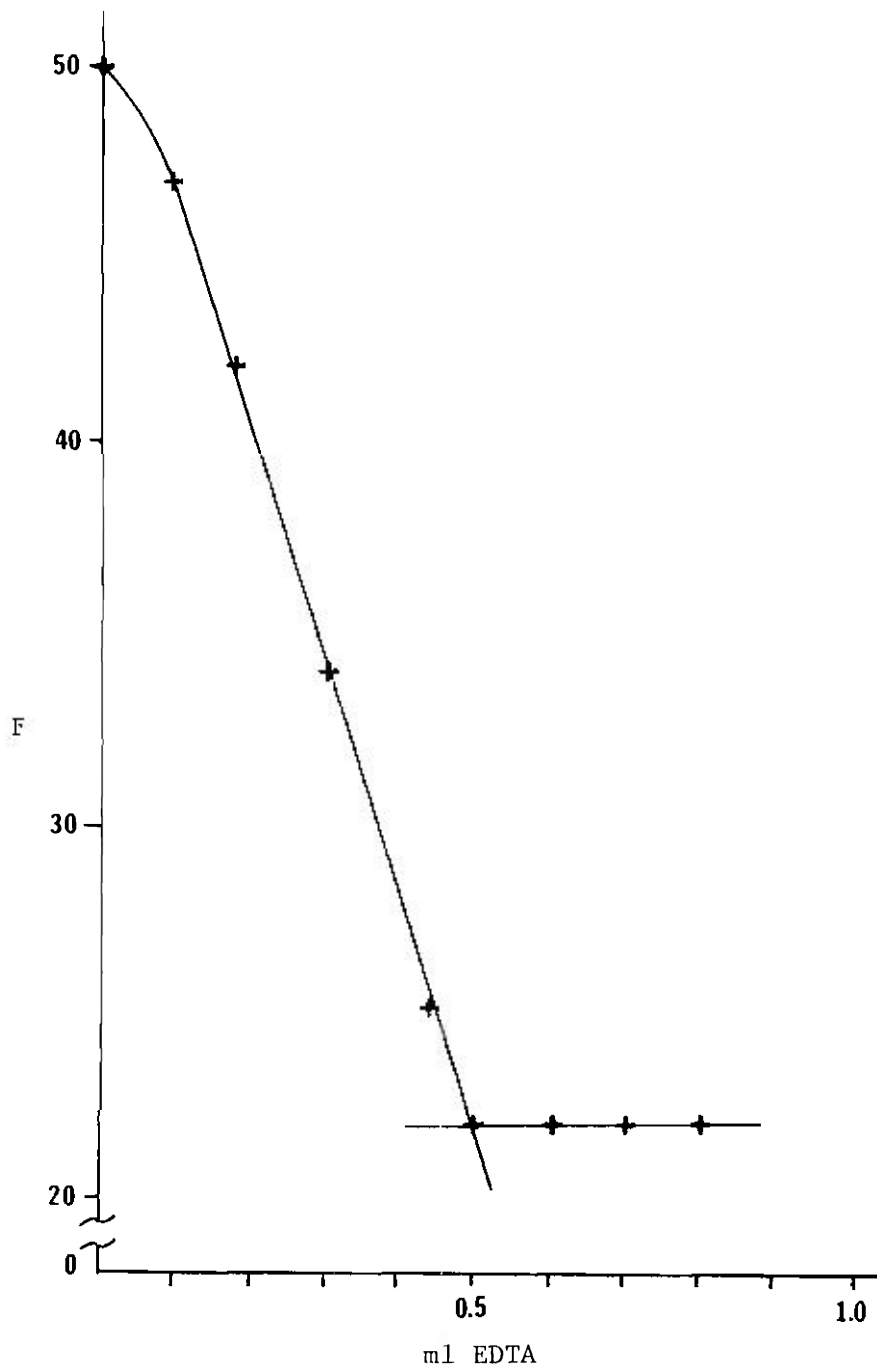


Figure 20. Fluorometric Titration of Lead  
(Titration of 0.5 ml of  $6.24 \times 10^{-4}$  F  
Pb with  $6.08 \times 10^{-4}$  F EDTA in the presence  
of an excess of Calcein Blue to generate a  
self-indicating system)

## CHAPTER V

## EVALUATION OF THE FLASCHKA-WHITE FLUOROTITRATOR

The above curves obtained from the fluorometric titration of lead with EDTA show that the instrument responds linearly as expected within the concentration ranges encountered.

In evaluating the overall performance of the fluorotitrator many aspects beside the actual titration results have been considered.

The initial placement of the instrument on a bench top in the laboratory proved very unsatisfactory. Because of the low concentrations of sought-for substances employed, the introduction of dust was a very aggravating problem. Although, this is not an inherent problem in the instrument itself, such a situation must be considered in an overall evaluation. On the other hand, it is somewhat gratifying to know that very minute dust particles cause such a problem, since it is an indication of the instrument's sensitivity. Since the laboratories as designed are inadequate for trace level work, it was necessary to relocate the instrument in one of the exhaust hoods. This proved to be more satisfactory; however, due to the fact that the hoods are of the negative pressure type in that they draw air in, dust was still somewhat of a problem. The best situation would be a positive pressure or laminar flow hood, but since such facilities were not readily available, other precautions were employed, such as covering the instrument and glassware with a plastic drop cloth when not in use.

The hood was equipped with standard fluorescent lighting. These lights were located directly above the instrument and no appreciable change in a set reading was observed when the overhead lights were switched on and off. However, after the instrument was placed in the hood and operation started large fluctuations of the meter needle were observed when the operator moved back and forth. After much consideration it was found that light from the lamps illuminating the lab and located just in front of the hood was shining directly into the collimating tube positioned before the photomultiplier. By attaching a 5 x 7 index card to the instrument cabinet, so that it shaded the entrance to the collimating tube, the problem was completely solved. It should be noted that the card in no way hampered either physical or visual access to the sample compartment.

Electronically no major problems occurred during the entire eight months of the investigation. The photomultiplier was replaced once during the time of study. This was merely a safety precaution since the original photomultiplier was rapidly approaching the end of its lifetime as stated in the manufacturers specifications. Thus, in order to avoid possible problems later on it was replaced with a new one.

The chopper motor burned out which was probably due to the fact that it was the original motor and possibly prescribed voltage limitations were not strictly adhered to in the preliminary experiments. The original motor operated at 2440 rpm while a replacement of identical make and serial number operated at only 2220 rpm. This difference of 220 rpm caused no problem and realignment after the motor's installation was only minor, indicating the ruggedness of the instrument.

Application of the instrument to field work would be very beneficial. However, the size of the present instrument (58 cm x 45 cm x 21 cm) limits its mobility to a large degree. It must be realized that the instrument built as a prototype was not intended for such applications and was spaciouly designed such that easy access to the contents of the instrument cabinet for the purpose of changing electronic components, aligning the optics, etc., would be no problem. With proper considerations, i.e., printed circuit boards, reduction of space needed for the optics, smaller chopper motor, etc., the instrument could very easily be built to one-half to one-third its present size. With these modifications the instrument could be adapted for field work.

When discussing the effect that varying temperature has upon the performance of the instrument, care must be exercised because the intensity of fluorescence is itself for most systems very temperature dependent. There were times during the investigation when the temperatures in the laboratory varied, due to malfunctions in the heating and cooling system, anywhere from 10 to 15° F above and below normal room temperature. While in operation during these times, no unusual behavior or fluctuations were observed.

As previously mentioned, White (III-8) found in his performance tests a drift of approximately 2 percent per hour. In the present study, shortly after the photomultiplier and chopper motor were replaced, the stability was again checked. This time a typical result showed a drift of 2 scale divisions over a two hour period. Based on an initial setting of 80, this corresponds to a drift of approximately 1 percent per hour, which is slightly better than found by White.

Except for the problem caused by the introduction of dust particles, random fluctuations during operation of the instrument were no problem. Overall the instrument has proven to be very reliable and valuable as a research tool.

## CHAPTER VI

## INTERFERENCE STUDIES

The initial statement of the problem in the present study was to evaluate the versatility of the fluorotitrator. This was accomplished by investigating another system besides the copper and calcium titrations with EDTA employed by White. The results of this investigation are quite gratifying in that the proposed titration seems to be the basis for the development of a new method for the determination of lead.

From the information already known concerning the behavior of various metals with Calcein Blue and EDTA, predictions concerning probable interferences can be made. Thus, any heavy and transition metal which forms a complex with EDTA and/or Calcein Blue will interfere.

Lead is known to form only an extremely weak cyano complex which does not prevent this metal from reacting with EDTA. If cyanide does not block the formation of the lead-Calcein Blue complex, then it should be possible to use cyanide as an agent to mask some of the interferences. Preliminary experiments in which the titration of lead had been performed in the presence of cyanide gave the same titration curve (see Figure 21) as that obtained in its absence. This fact supports the possible use of cyanide as a masking agent in the proposed titrimetric method. However, it must be mentioned that since the pH of the solution is 4.7, a considerable amount of hydrogen cyanide is given off and therefore additional safety precautions must be observed.

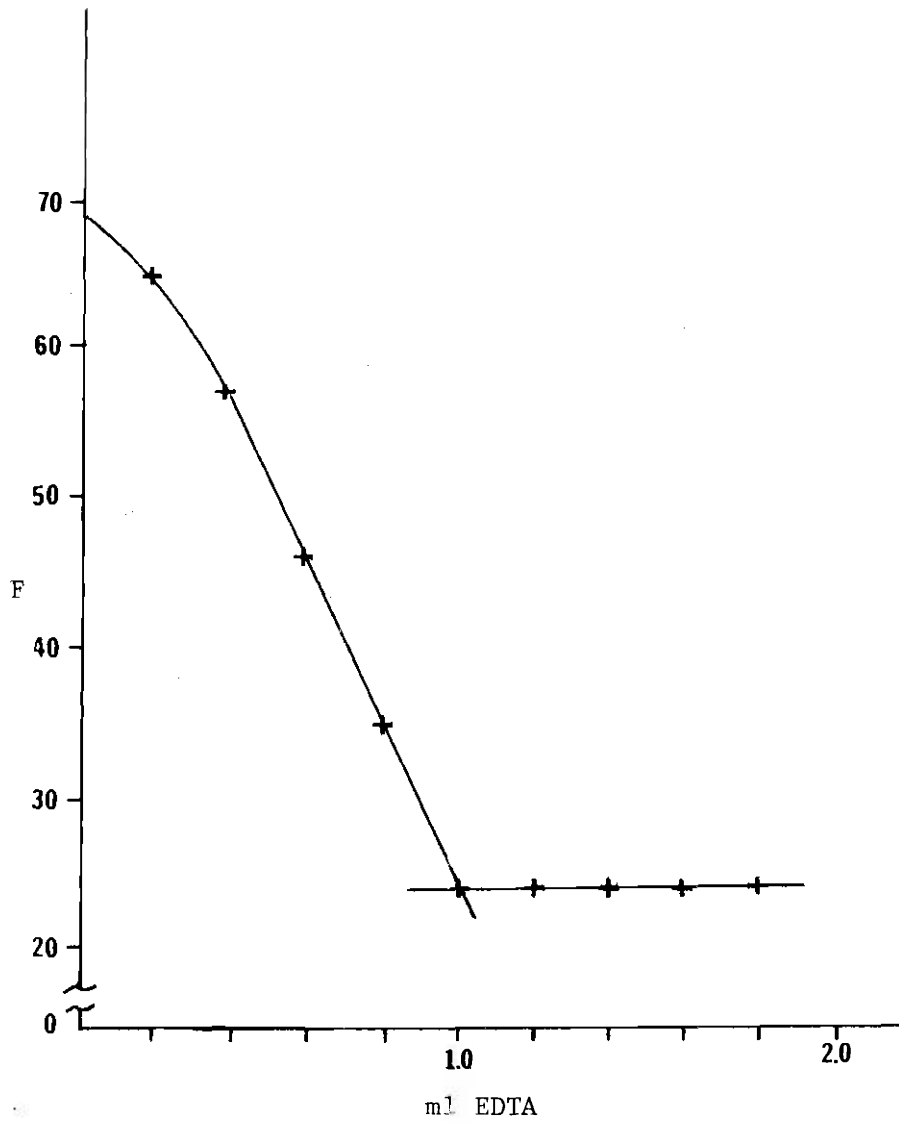


Figure 21. Influence of Cyanide on the Titration of Lead  
(Titration of 1.0 ml of  $6.39 \times 10^{-4}$  F Pb with  
 $6.10 \times 10^{-4}$  F EDTA in the presence of  $9.4 \times 10^{-2}$  F  
potassium cyanide)

An underlying idea in this investigation was the possible use of the proposed method in environmental studies.

Due to the omnipresence of calcium and magnesium in the environment, it would be interesting to know what effects these metals would have upon the titration. Several titrations of lead in the presence of various amounts of these elements were performed. No problems were encountered when the lead-to-calcium or magnesium mole ratio was equal to or less than 1:2. However, at greater molar ratios errors occurred in the titration results. In particular, it was found that the fluorescence increased dramatically near the end point (see Figure 22) when calcium or magnesium were present in sufficient excess. Since calcium and magnesium form only extremely weak complexes with EDTA at this pH, it is felt that this odd occurrence is caused by their interaction with the Calcein Blue. Although such unusual behavior warrants further study, to do so here would be beyond the stated limits of the present investigation.

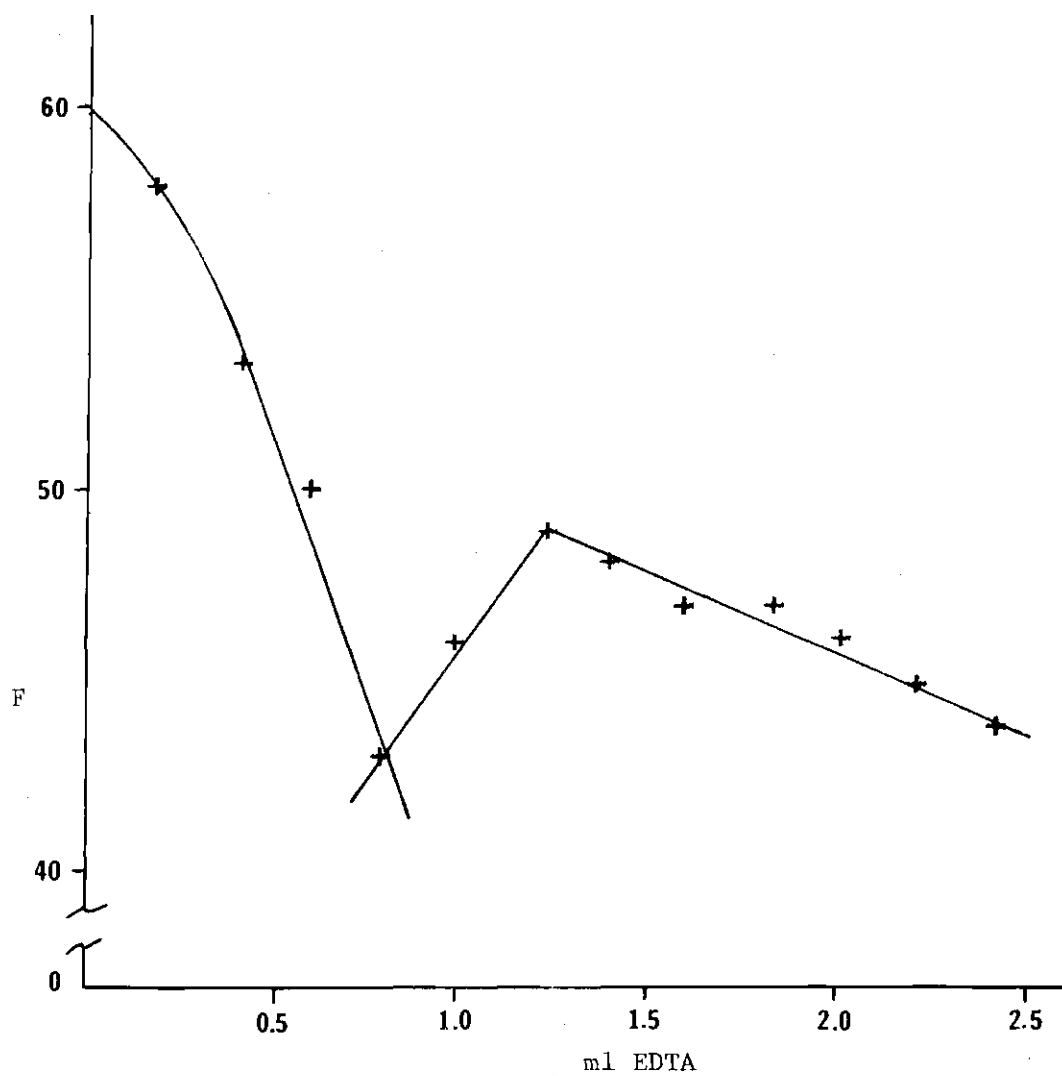


Figure 22. Influence of Calcium on the Titration of Lead (Titration of 1.0 ml of  $6.39 \times 10^{-4}$  F Pb with  $6.10 \times 10^{-4}$  F EDTA in the presence of  $2.6 \times 10^{-4}$  F Ca)

## CHAPTER VII

## CHEMICALS AND EQUIPMENT

Chemicals

All water used in the investigation for preparing and diluting reagents and solutions was obtained from a Barnstead still equipped with a Ventguard filter. The water was then passed through a mixed bed deionizer. All reagents used met ACS analytical reagent specifications. Whenever possible, metal salt solutions were prepared from J. T. Baker "Analytical Reagent" grade salts. All common acid, base, and buffer solutions were prepared from reagent grade chemicals.

Glassware

The usual glassware such as beakers, flasks, etc., were used as needed. For volumetric measurements class A volumetric glassware was used exclusively and without additional calibration.

pH Meter

All pH measurements were made on a Corning Model 7 pH meter. This instrument was standardized with Beckman Standard Buffer at pH 4.01, or a Leeds and Northrup pH 6.86 buffer.

Fluorotitrator

All of the fluorometric titrations were performed on a new fluorotitrator developed and built by White (III-8). The instrument employs a modified double beam system in which the reference signal is used to

compensate for changes in exciter lamp output and fluctuation in the photomultiplier. The chopper system employed blocks light to both the reference and sample beam 163 times each second and triggers an electronic compensation for photomultiplier dark current and any ambient light which might reach the photomultiplier.

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PART FOUR

SOME INVESTIGATIONS OF THE LEAD-PAN COMPLEX

## CHAPTER I

## EXTRACTION BEHAVIOR OF THE LEAD-PAN COMPLEX

During the investigation of the determination of lead by fluorometric titration, consideration was given to the possibility of interferences, and a separation of the lead from other metals contemplated. On small scale and trace level operation, extraction procedures are generally best suited for this purpose. A compound which has been used extensively in this laboratory as an extractant is PAN, i.e., 1-(2-pyridylazo)-2-naphthol. It was felt that with proper masking and adjusting of the pH, lead could be removed, by extraction as the lead-panate, from many of the common metals with which it is associated.

It is mentioned in a review article by Shibata (IV-1) that lead (II) and PAN, in neutral solution, form a red-colored complex that can be extracted into chloroform. However, we were not able to reproduce these findings. The abstract (IV-2) of the original paper by Negoiu and co-workers (IV-3), on which Shibata's statement seems to be based, discusses the formation of the red complex, but makes no mention of an extraction. Since the original paper was not available, it remains open whether the claim on extractability stems from the original investigators or the compiler. The above mentioned references are the only ones related to lead-PAN (analytical or otherwise). This is noteworthy because the

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References for Part IV on page 125.

literature concerning metal-PAN complexes and their application is quite sizable and shows a strong tendency of further growth. Under these circumstances, it seemed interesting to have a closer look at the situation.

The lead does indeed form a complex with PAN as indicated by the red color developed when a few drops of alcoholic PAN solution are added to a buffered lead solution. However, upon shaking the aqueous layer with chloroform, the red color fades and upon phase separation the aqueous layer becomes colorless while the organic layer takes on the color of the free PAN, i.e. orange-yellow. This observation strongly suggests that on extraction, the lead-PAN decomposes and only the free PAN is extracted. Such an assumption can easily be tested by comparing the spectral curve of the organic layer obtained above with that obtained when only PAN is extracted. Such a comparison is presented in Figure 23. The curves are identical except for the small hump in curve B. In order to be sure that the hump and the change in color were not caused by two different forms of the lead-PAN complex, i.e., a red-colored complex in aqueous medium and an orange-yellow complex in chloroform, the following experiments were conducted. First, an absorbance curve of an alcohol-water solution of the lead-PAN complex was run (See Figure 24). It showed an absorbance peak very near the wavelength of the hump. Second, if the lead-PAN complex indeed decomposes, then upon extraction the lead should remain in the aqueous phase, and upon addition of more PAN to this phase the red color of the lead-PAN complex should reappear. The experiment proved this expectation. In fact, even after repeating an extraction on the same solution as many as ten times, the addition of PAN still turned

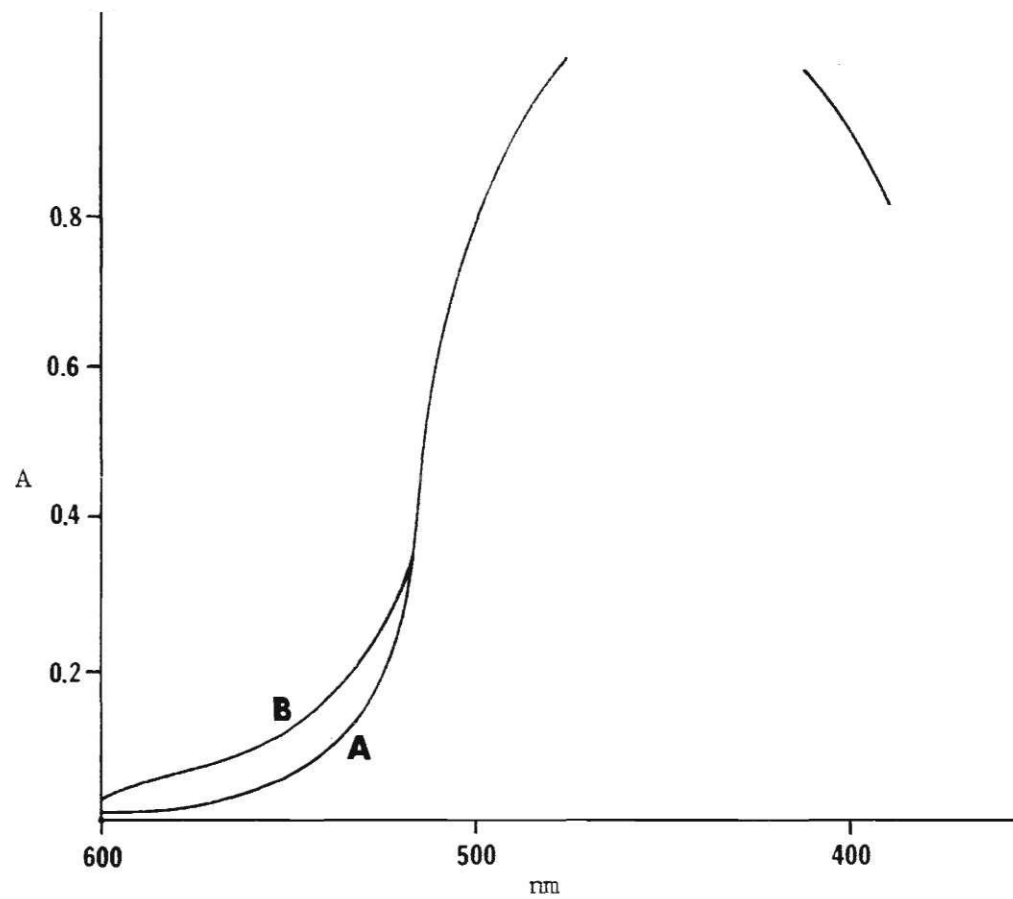


Figure 23. Spectral Curves of the Organic Layers after the Extraction of PAN (A) and Lead-PAN (B)

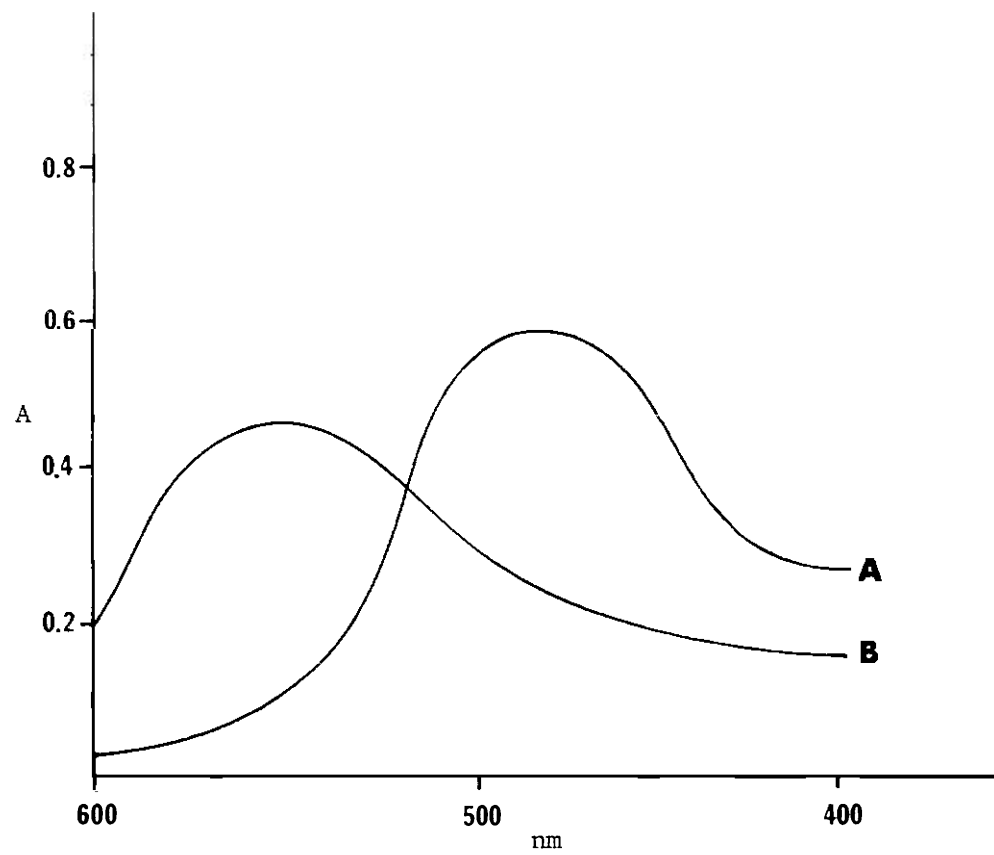


Figure 24. Spectral Curves of PAN (A) and its Lead Complex (B)

the aqueous phase red.

The possibility that such inability to extract the lead-PAN complex could merely be due to the particular organic solvent employed (Chloroform) was checked by using other solvents; namely, methyl isobutyl ketone and carbon tetrachloride. The investigations using these solvents gave identical results as those obtained with chloroform. For further studies methyl isobutyl ketone was used because it is a floater, i.e., it is less dense than water and remains on top of the aqueous layer, and thereby greatly facilitating repeated washing of the organic layer with aqueous media.

Unusual behavior is known to occur with other metal-PAN systems. In particular Flaschka and Weiss (IV-4) found that the cadmium-PAN complex fully extracts from the aqueous phase into the organic solvent, but upon prolonged shaking decomposes. The PAN remains in the organic layer, while the cadmium seemingly completely backtransfers to the aqueous phase.

For an entirely different metal-PAN system; namely, indium-PAN, Zolotov and co-workers (IV-5) observed a similar backtransfer phenomenon. These authors found that preformed In-PAN was completely and rapidly extracted into chloroform. However, if shaking was continued the amount of In-PAN in the organic layer decreased with time until an equilibrium value was reached. From the other direction, that is, shaking an indium containing aqueous solution with a chloroform solution of PAN, equilibrium was also attained, but only after more than an hour of shaking.

Although the unexpected behavior of the lead-PAN system is interesting, it precluded the use of PAN for the separation of lead from

possible interferences.

However, the above findings did present the possible basis for the separation of other metals from lead, for example, zinc.

## CHAPTER II

## SEPARATION OF ZINC AND LEAD USING PAN

Since zinc is commonly found as an impurity in lead, the development of a sensitive and highly selective method for determining zinc in lead matrices would be of practical value and not merely an academic exercise. In light of the fact that in such situations the amount of zinc present is rather minute, the small portion of lead which, as the hump in Figure 23 indicates, is extracted becomes very important. It was felt that possibly a longer shaking time might be beneficial in getting rid of that last trace of lead which is extracted. Of course, before studying this point, it was necessary to assure that long shaking would not cause any backtransfer of the zinc. As can be deduced from Figure 25 this situation poses no problem. The situation for lead, as shown in Figure 26, unfortunately, is not encouraging, because whatever the time of shaking, small amount of lead remains. Several attempts were made to reduce this amount by backwashing with citrate or tartrate solutions, or by adding these complex formers to the initial lead solution. However, none of these experiments were successful. The amount of "residual" lead was, however, reasonably constant and independent of changes in the volume of organic and aqueous phase, pH, and other parameters. Thus, for an actual analysis the situation could be remedied by application of a numerical correction or better by experimental compensation.

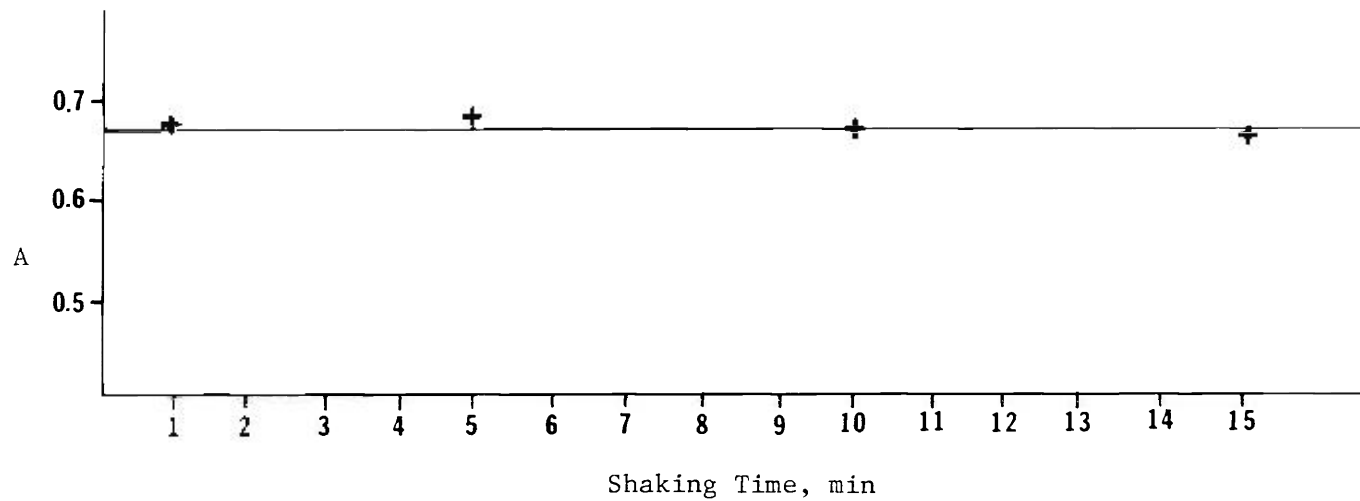


Figure 25. Relation Between Shaking Time and Zinc-PAN Extracted

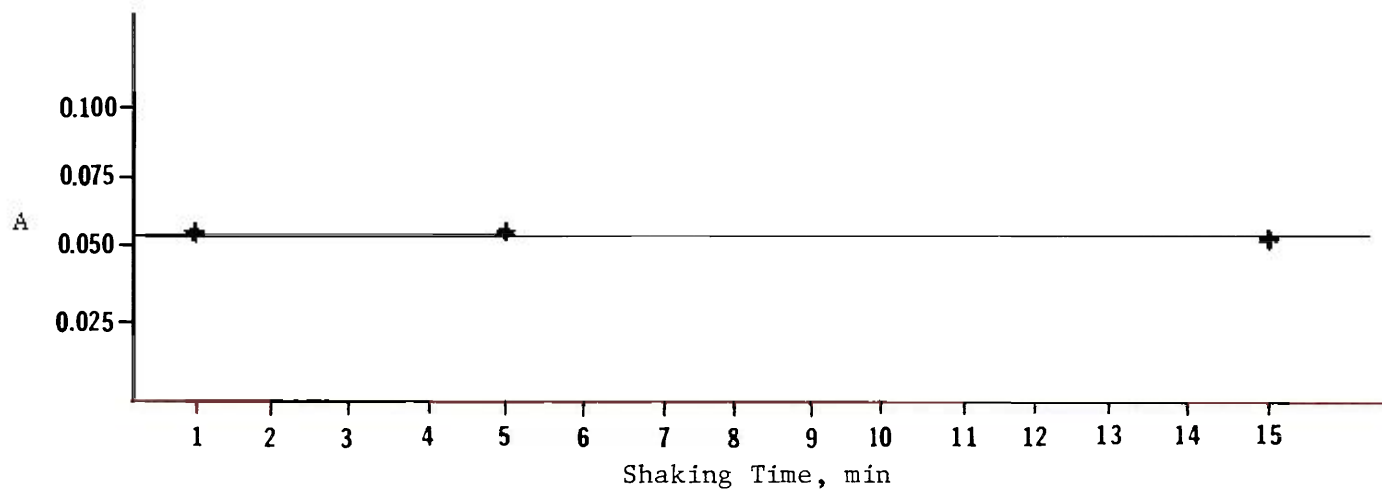


Figure 26. Relation Between Shaking Time and Lead-PAN Extracted

However, the blow that shattered the hope for a good zinc determination came from another direction and made the problem of the "residual" lead irrelevant. The extraction of zinc from lead at molar ratios of up to 1:150 presented no problem and was essentially complete. Then a tendency of low zinc values became noticeable and the zinc deficit grew as the lead-to-zinc ratio increased. Obviously, in the aqueous phase the PAN is bound more and more predominantly by the lead, and zinc is unable to successfully compete for its share. Although the investigation proved interesting, further study was halted since there are established, well working, methods (IV-6) available for situations where the ratio of zinc to lead is 1:150 or even greater. Thus, the findings here are not presented as a possible basis for a new method, but rather as a general contribution to the otherwise remarkably scarce information concerning the lead-PAN complex.

As a final note, it should be mentioned that some metals other than zinc may be able to compete more successfully for the PAN and thus could be determined in the presence of large amounts of lead. However, no experiments, not even preliminary ones, have been conducted in this direction.

## CHAPTER III

## CHEMICALS AND EQUIPMENT

Chemicals

All water used in the investigation for preparing and diluting reagents and solutions was obtained from a Barnstead still equipped with a Ventguard filter. The water was then passed through a mixed bed deionizer. All reagents used met ACS analytical reagent specifications. In every instance possible, metal salt solutions were prepared from J. T. Baker "Analytical Reagent" grade salts. All common acid, base, and buffer solutions were prepared from reagent grade chemicals.

Glassware

The usual glassware such as beakers, flasks, etc., were used as needed. For volumetric measurements class A volumetric glassware was used exclusively and without additional calibration.

pH Meter

All pH measurements were made on a Corning Model 7 pH meter. This instrument was standardized with Beckman Standard Buffer at pH 4.01, or a Leeds and Northrup pH 6.86 buffer.

### Spectrophotometers

The spectrophotometric measurements were carried out on a Bausch and Lomb Spectronic 20.

The absorbance curves were obtained with a Bausch and Lomb Spectronic 505 Spectrophotometer.

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## VITA

Gary Lee Myers was born on July 10, 1949, in Hanover, Pennsylvania to Richard Eugene Myers and Betty Louise Myers. He attended Spring Grove Area High School in Spring Grove and was graduated in June, 1967. In September, 1967, he entered Catawba College in Salisbury, North Carolina.

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In September, 1968, he entered Elizabethtown College in Elizabethtown, Pennsylvania, and was graduated, Cum Laude, with a BS in Chemistry in May, 1971. While attending Elizabethtown College he received the American Institute of Chemists Award for Outstanding Scholastic Achievement.

In September, 1971, he was appointed Graduate Teaching Assistant at the Georgia Institute of Technology. During two quarters he worked under the support of an NSF grant, and then reassumed the position of Teaching Assistant.

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