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LITHIUM ALUMINUM HYDRIDE AS AN ANALYTICAL TOOL IN PHARMACY

by

CARL JOHN LINTNER

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of
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TABLE OF CONTENTS

Page

I

II

TABLE OF CONTENTS (cont.)

III

Part A-Development of the Method

TABLE OF CONTENTS (cont.)

TABLE OF CONTENTS (cont.)

Page

IV

Part B-Application to Pharmaceutical Compounds

TABLE OF CONTENTS (cont.)

Page

TABLE OF CONTENTS (cont.)

TABLE OF CONTENTS (cont.)

V

Part C-Development of the Colorimetric Method

Reagents

TABLE OF CONTENTS (cont.)

INTRODUCTION

At the present there are only a few feasible methods for determining active hydrogens and carbonyl content of unknown substances. The Zerewitinoff method and its modifications are probably most commonly used. Lately lithium aluminum hydride has also been used as a reagent for gasometric determination of active hydrogen. These methods, however, require specialized gasometric equipment not readily available.

The purpose of the present investigation was to develop a volumetric method of analysis based on lithium aluminum hydride as the reagent and a simple electrometric or colorimetric detection of the endpoint. A successful development of a simple volumetric method of determining active hydrogen and carbonyl groups would provide organic and pharmaceutical chemists with a very useful research tool in characterising and establishing structures of natural drugs and other unknown organic compounds. Such a method may also prove to be of considerable value in quantitative analysis of many pharmaceutical compounds.

PAST WORK IN THE FIELD

A search of the literature showed that considerable work has been done on the compound lithium aluminum hydride both for preparatory and analytical purposes. No attempt, however, seemed to have been made to apply the reagent to volumetric method of analysis.

Synthesis of the new powerful reducing agent, lithium aluminum hydride, was first described by Finholt, Bond and Schlesinger.[1] They treated an excess of lithium hydride in absolute ether with an ethereal solution of aluminum chloride with constant stirring. The aluminum chloride was introduced at such a rate that boiling of the liquid in the reaction vessel was continuous. The precipitated lithium chloride and lithium aluminum chloride were separated from the solution by filtration. The filtrate and washings were combined and evaporated under vacuum to yield a 95.4 per cent pure sample of lithium aluminum hydride. The overall yield of the reaction was 85.7 per cent of the theoretical.

$$4\ LiH + AlCl_3 \xrightarrow{ether} LiAlH_4 + 3\ LiCl$$

Addition of further quantities of aluminum chloride yields an ethereal solution of aluminum hydride.

$$3\ LiAlH_4 + AlCl_3 \xrightarrow{ether} 4\ AlH_3 + 3\ LiCl$$

The latter is not stable; it soon deposits a white solid in which the atomic ratio of aluminum to hydrogen still is 3 to 1, but from which the ether cannot be recovered without loss of hydrogen.

Through the use of lithium aluminum hydride new methods, far simpler than any previously available, have been developed for the preparation of hydrides such as silane and stannane and their partially alkylated derivatives. In addition, its use has led to the preparation of previously unknown hydrides such as those of sine and beryllium. The types of reaction by which these results have been achieved are illustrated by the equations:

$$LiAlH_4 + SiCl_4 \xrightarrow{ether} LiCl + AlCl_3 + SiH_4$$

$$LiAlH_4 + 2(CH_3)_2\,SnCl_2 \xrightarrow{ether} LiCl + AlCl_3 + 2(CH_3)_2\,SnH_4$$

$$LiAlH_4 + (CH_3)_2Zn \xrightarrow{ether} LiAl(CH_3)_2H_2 + ZnH_2$$

Reactions such as these usually proceed smoothly at room temperature, and in general give excellent yields of products of high purity.

Experiments with organic compounds indicate that this hydride is capable of reducing carbonyl, carbalkoxy, acyl chloride[2], and nitro groups[7], but is without action on the double bonds of simple olefins[3].

The attractive features of lithium aluminum hydride as a reagent which, combined, place it in a unique position are (1) that it is easily prepared on either a large or small scale from commercially available lithium hydride, (2) that it is indefinitely stable at room temperature, (3) that it is ether soluble, (4) that as compared with other reducing agents, excepting hydrogen, it has a favorable ratio of reducing capacity to mass, (5) that the reactions occur at room temperature, (6) no unusual equipment is needed.

The reaction of lithium aluminum hydride on functional groups of aldehydes, ketones, acid chlorides and acid anhydrides[3] gives reduction to the alcohol stage with extreme rapidity and virtually no side reactions. The yield appears to be limited principally by the efficiency of the procedures employed subsequent to isolating the products. As determined by the hydride consumed, the reductions proceed quantitatively in accordance with the following general equations:

Aldehydes[2]

$$4\ RCHO + LiAlH_4 \longrightarrow LiAl(OCH_2R)_4$$

In general the yields are better with lithium aluminum hydride in the reduction of aldehydes than can be achieved by other methods including the crossed Cannizzaro, catalytic hydrogenation, and the Meerwein-Ponndorf reaction. It lacks the specificity of the Meerwein-Ponndorf reduction for polyfunctional groups containing carboxyl, ester, or nitro radicals. These groups are reduced by the hydride as well as the aldehyde part of the molecule while they are untouched by the Meerwein-Ponndorf. On the other hand, the hydride can be used for amino or hydroxy-aldehydes in which the Meerwein-Ponndorf reaction fails.

Ketones[2]

$$4\ R_2CO + LiAlH_4 \longrightarrow (R_2CHO)_4LiAl$$

Comments above under aldehydes apply also here. Pinacol by-products have not been observed with lithium aluminum hydride.

Anhydrides[2]

$$(RCO)_2O + LiAlH_4 \longrightarrow \tfrac{1}{2}(RCH_2O)_4\ LiAl + \tfrac{1}{2}\ LiAlO_2$$

Acid chlorides[2]

$$2\ RCOCl + LiAlH_4 \longrightarrow \tfrac{1}{2}(RCH_2O)_4LiAl + \tfrac{1}{2}\ AlCl_3$$

Carboxylic acids[4]

$$4\ RCOOH + 3\ LiAlH_4 \longrightarrow (RCH_2O)_4LiAl + 2\ LiAlO_2 + 4H_2$$

The direct reduction by other means is usually poor. Electrolytic reduction and catalytic hydrogenation result in numerous side reactions. In the reduction of hydroxy and amino acids, the hydroxyl and amino groups neither interfere, nor are they removed. This is especially important because in other methods of reduction, certain amino and hydroxyl groups are removed if they are not protected.

ESTERS[2]

$$2\ RCOOR' + LiAlH_4 \longrightarrow \tfrac{1}{2}(RCH_2O)_4LiAl + \tfrac{1}{2}(R'O)_4LiAl$$

Examples of esters which are reduced satisfactorily with lithium aluminum hydride but often not by ordinary methods (e.g. sodium alcohol or catalytic hydrogenation) are hydroxyl and amino esters, substituted malonic esters, and highly conjugated esters.

Amides[6,7]

In general amides are reduced to the amines as shown in the first equation below but some examples of alcohol formation are known. Possible equations are:

$$2\ RCONR_2 + LiAlH_4 \longrightarrow 2\ RCH_2NR_2 + LiAlO_2$$

$$4\ RCONR'_2 + 2\ LiAlH_4 \longrightarrow LiAl(OCH_2R)_4 + LiAl(NR'2)4$$

$$4\ RCONHR + 3\ LiAlH_4 \longrightarrow LiAl(OCH_2R)_4 + 2\ LiAl(R'N)_2 + 4\ H_2$$

Nitriles[7]

$$2\ RCN + LiAlH_4 \longrightarrow (RCH_2N)_2LiAl$$

Aldimines[7]

$$4\ RCH = NR + LiAlH_4 \longrightarrow (RCH_2NR)_4LiAl$$

Aromatic nitro compounds[7]

$$RNO_2 + LiAlH_4 \longrightarrow \tfrac{1}{2}\ R - N = N - R + 2\ H_2 + LiAlO_2$$

This is a simple and sensitive test for the aryl nitro group and is used in the characterisation of the compound.

Aliphatic Nitro Compound[7]

$$2\ RNO_2 + 3\ LiAlH_4 \longrightarrow (RN)_2LiAl + 2\ LiAlO_2 + 6\ H_2$$

Azoxy Compounds[7]

$$2\ RN_2OR + 3\ LiAlH_4 \longrightarrow 2\ R - N = N - R + LiAlO_2 + 2\ H_2$$

Epoxides[7]

$$4\ R(CHCH_2)O + LiAlH_4 \longrightarrow \left[RCH(CH_3)O \right]_4 LiAl$$

Quinones[7]

Quinones are reduced to hydroquinones in excellent yields.

Alkyl Halides[7]

The halogen atoms in alkyl halides may be replaced with hydrogen to form hydrocarbons following the usual order of activity, iodide > bromide > chloride.

$$4\ RX + LiAlH_4 \longrightarrow 4\ RH + LiAlX_4$$

Alicyclic and aryl halides are very unreactive.

Alcohols, glycols, and phenols

$$4\ ROH + LiAlH_4 \longrightarrow LiAl(OR)_4 + 4\ h_2$$

Primary amines[1]

$$2\ RNH_2 + LiAlH_4 \longrightarrow LiAl(RN)_2 + 4\ H_2$$

Secondary Amines[1]

$$4\ R_2NH + LiAlH_4 \longrightarrow LiAl\ (R_2N)_4 + 4\ H_2$$

Carbon to Carbon double bonds are not generally attacked,[3] making possible selective reduction of functional groups in unsaturated compounds. The one class which is an exception to this is represented by an ethylenic nucleus substituted on one side by a phenyl group and on the other by a reducible group, such as carboxyl, nitro, aldehyde, etc. At room temperature in the normal reduction procedure the double bond is reduced, thus cinnamaldehyde is converted to hydrocinnamyl alcohol. Reversing the mode of addition by adding hydride solution to a solution of cinnamaldehyde at a temperature below 10° C., excellent yields of cinnamyl alcohol are obtained. Cinnamyl alcohol in turn was found to be reduced at room temperature to hydrocinnamyl alcohol. Thus there appears to be two stages of reduction, the attack on the double bond being slower than that on the functional group. In general, at room temperature, the reduction of double bonds in alpha-beta unsaturated aliphatic compounds is extremely slow so that good yields of unsaturated alcohols may be obtained. At higher temperatures saturated alcohols may be formed.

Lithium aluminum hydride also reacts with some inorganic compounds.

Water[1]

$$4\ H_2O + LiAlH_4 \longrightarrow LiOH + Al(OH)_3 + 4\ H_2$$

This reaction occurs extremely rapidly and completely. If lithium aluminum hydride is treated with any but very large amounts of water, the resulting reaction is so vigorous that the solid may be heated to incandesence with a resulting loss of hydrogen by thermal decomposition. Exposure of the hydride to moist air causes a relatively slow decomposition.

This is probably due to the formation of a protective coating of aluminum hydroxide.

Ammonia[1]

Ammonia reacts with lithium aluminum hydride to give a quantity of hydrogen which is from 93-99 per cent of that calculated according to the equation:

$$2LiAlH + 5\ NH_3 \longrightarrow \left[LiAlH(NH_2)_2\right]_2\ NH + 3H_2$$

Carbon dioxide[5]

Two different reactions may take place with lithium aluminum hydride and carbon dioxide. With an excess of carbon dioxide, derivatives of formaldehyde are formed, while with an excess of the hydride, methanol derivatives are produced. The latter reaction has been investigated for the preparation of methanol containing isotopic carbon.

$$4\ CO_2 + 3\ LiAlH_4 \longrightarrow LiAl(OCH_3)_4 + 2\ LiAlO_2$$

As shown above many compounds containing active hydrogens react with lithium aluminum hydride to liberate hydrogen gas. A measurement of the gas evolved offers a method of quantitatively determining active hydrogens in a manner similar to the Zerewitinoff[8] procedure but with an advantage over the Grignard reagent.

This use of the Grignard reagent is the basis for the method[9,10] depending upon the quantitative interaction in a closed system of substances containing groups reactive to the Grignard reagent with or without the evolution of gaseous hydrocarbons. Thus substances belonging to the following groups: alcohols, thioalcohols, phenols, thiophenols, acids, amides, sulfonamides, amines and nitro compounds are treated with a known amount of methyl magnesium iodide to yield methane quantitatively. The volume of methane formed is then measured in a suitably constructed methaneometer.

After the evolution due to active hydrogen, the unused Grignard reagent is reacted with a known quantity of aniline and the volume of methane thus generated is again measured. Thus by difference, such groups as react with methyl magnesium iodide without the evolution of methane can be determined by the amount of Grignard reagent consumed.

Zaugg and Horrom[8] used lithium aluminum hydride in a suitable solvent in the Modified and combined Grignard and Quantitative Hydrogenation Apparatus described by Zaugg and Lauer.[11] Their work compared the efficacy of lithium aluminum hydride with methyl magnesium iodide as a quantitative reagent not only for active hydrogen atoms but more particularly for reactive functional groups in organic compounds.

It was found by Zaugg and Horrom[8] that in the case of benzoic acid the hydride reagent is superior to the Grignard reagent for the measurement of both the active hydrogen and carbonyl function. Heating was required for quantitative reaction in reasonable time of the carbonyl group with hydride. Similar conditions with the Grignard reagent, however, did not result in quantitative reaction. Likewise with aniline it was evident that the hydride was a better reagent than the Grignard for the detection of both hydrogens of the amino group. With hindered nitriles, practically no reaction of the Grignard reagent was observed even on long heating. Heating with the hydride produced a result fairly close to the theoretical.

Hochstein[12] used a modified micro-scale Grignard machine to compare lithium aluminum hydride with methyl magnesium iodide. His quantitative studies gave satisfactory results for the determination of active hydrogen in alcohols, phenols, and amines. Benzyl alcohol, n-amyl alcohol, B-naphthol, thio-B-naphthol, 2,4,6, tribenzyl phenol, benzylaniline, diphenylamine and dibenzylamine gave values indicating 1.00 active hydrogens with an average deviation of ±0.015. Phenylethylene glycol, 1,6-hexanediol, hydroquinone, aniline and n-butyl amine gave values indicating 2.00 active hydrogens, with an average deviation of ±0.010.

With lithium aluminum hydride being used in quantitative studies a rapid method for determination of its concentration in solution was needed. Krynitsky, Johnson, and Carhart[13] based their method on the liberation of hydrogen by the hydrolysis of lithium aluminum hydride according to the following equation:

$$LiAlH_4 + 4\ H_2O \longrightarrow LiOH + Al(OH)_3 + 4\ H_2$$

Water was allowed to drop into a solution of lithium aluminum hydride and the hydrogen evolved measured by a manometer. The results of this determination show that the method is reproducible and sufficiently accurate for the quantitative determination of lithium aluminum hydride in ether.

Krynitsky, Johnson and Carhart[13] applied this method to quantitative studies on organic compounds possessing active hydrogens. A known amount of material was added to an excess of lithium aluminum hydride-ether solution at 0° and the liberated gas measured by changes in pressure. The authors found that many of the simple alcohols, phenols, and acids react with lithium aluminum hydride in ether to liberate hydrogen in the expected manner and give results which are in good agreement with the theoretical values. Inconclusive results were obtained with hydroquinone, succinic acid, and terephthalic acid. This may be due to the formation of insoluble material which coats the crystals and inhibits further reaction. Compounds determined by this method included alcohols, acids, amines, amides, nitro and tautomeric compounds. The reaction of lithium aluminum hydride with compounds exhibiting keto-enol tautomerism was rapid and the results indicated that the compounds tested behaved as though they were only partially enolized. With the Grignard reagent similar compounds act as though they exist in the enol form only.

None of the quantitative methods based on lithium aluminum hydride are similar to the volumetric determination proposed here. The only comparable analytical method of this nature is the Karl Fischer titration[14,15] for the detection of water. This method involves a direct titration of the sample with a solution of iodine, sulfur dioxide, and pyridine in methanol, the reagent serving as its own indicator. As dark substances interfere with the colorimetric endpoint, Almy[16] developed an electrometric method. A Beckman pH meter and a platinum tungsten electrode system were used to determine the change in potential. Although the Karl Fischer method is not directly applicable to the determination of alcohols, carbonyl compounds, organic acids, acid anhydrides, etc., it has been adapted to the analysis of these by means of modification which make use of water producing reactions. The major drawbacks to the determination of functional groups by the Karl Fischer method are (1) the method is time consuming, (2) the reactions are not always quantitative, (3) many side reactions are apt to occur.

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PART A

Development of the Method

Introduction

Since the material on this subject has been covered in some detail in the two manuscripts "Electrometric Titration Of Some Functional Groups" and "Electrometric Titration of Alcohol Using Lithium Aluminum Hydride" accepted for publication in SCIENCE and ANALYTICAL CHEMISTRY respectively, they are reproduced herein. However, as may be expected, certain aspects such as the initial difficulties are not treated in these papers. For this reason a report on these aspects are also included in this section.

The first manuscript was written primarily to establish our priority in the development of the method as applied to all types of compounds. The second paper covered the actual development and application of the method mainly to hydroxyl compounds.

Preliminary Studies

The purpose of the initial study was to experimentally test the proposed theoretical ideas regarding abrupt potential change associated with end points of lithium aluminum hydride titrations (see page 26). The first apparatus (shown in Figure 1) was relatively simple. It consisted of silver and platinum electrodes sealed in separate pieces of glass tubing. The silver electrode was essentially isolated by means of a piece of glass tubing and was connected to the reaction mixture by a salt bridge. This apparatus also included a Beckman Model H pH meter. However, this apparatus was soon discarded as the reaction misture diffused into the silver electrode tubing and influenced the potential of the electrode. After several modifications the electrode shown in figure 2 was adopted and used in all future work of the open system method.

As hydrides are powerful reducing agents, it was thought that on the addition of lithium aluminum hydride solution to a solution containing active hydrogens, all hydride would be destroyed until all the hydrogen had reacted. As soon as a slight excess of hydride is present the reduction potential of the system should change markedly. The point of change in potential would then correspond to the endpoint. As preliminary investigations confirmed the anticipated change in reduction potential as shown in figure 3, the method was tried analytically.

The first analytical method tested consisted of a direct titration of a lithium aluminum hydride with a lower alipathic primary alcohol. A lithium aluminum hydride solution in tetrahydrofuran was prepared and the alcohols were dried as described on page 32 in the paper to ANALYTICAL CHEMISTRY.

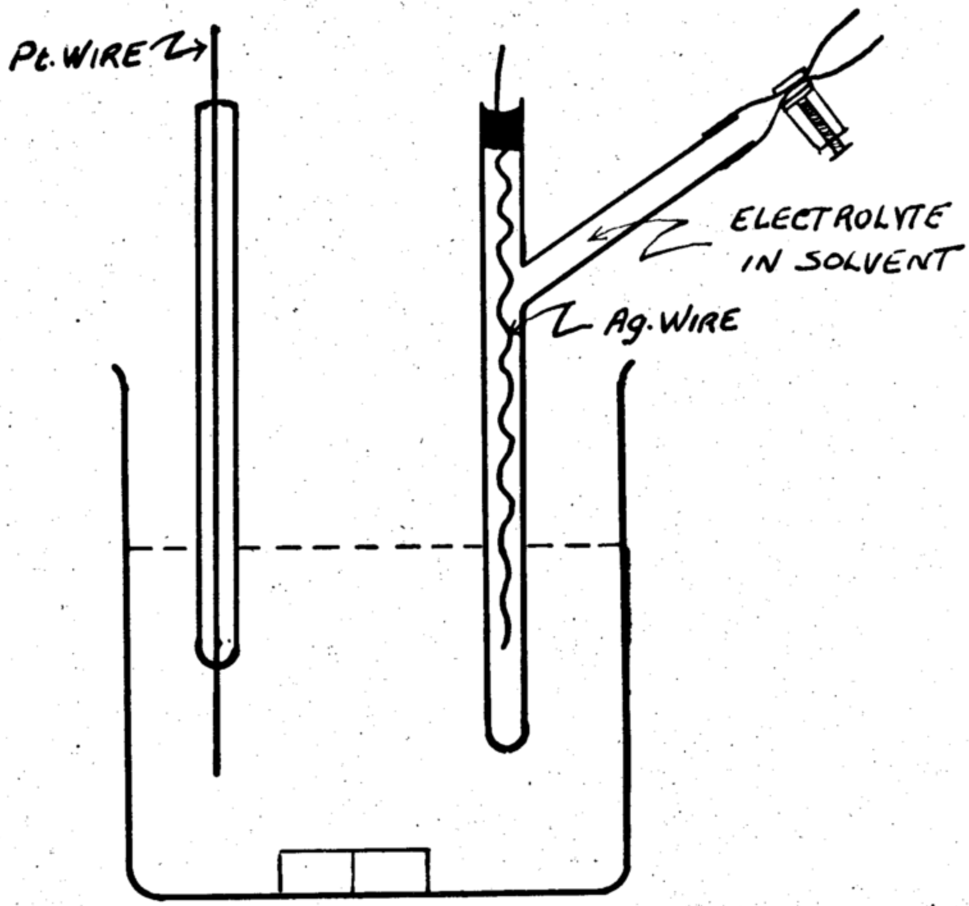

Figure 1 Initial Apparatus Used in Open System

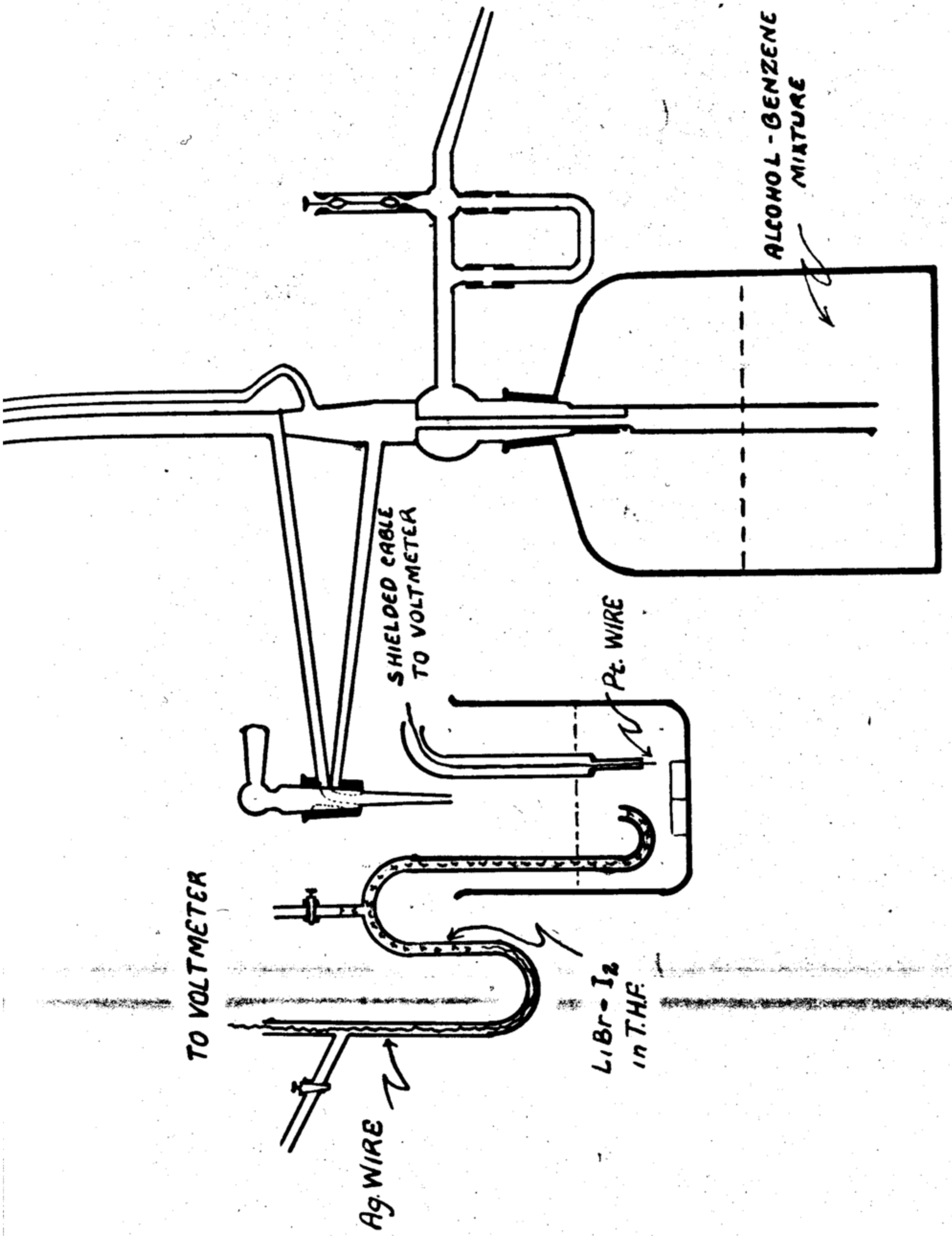

Figure 2 The improved apparatus used in the Open System.

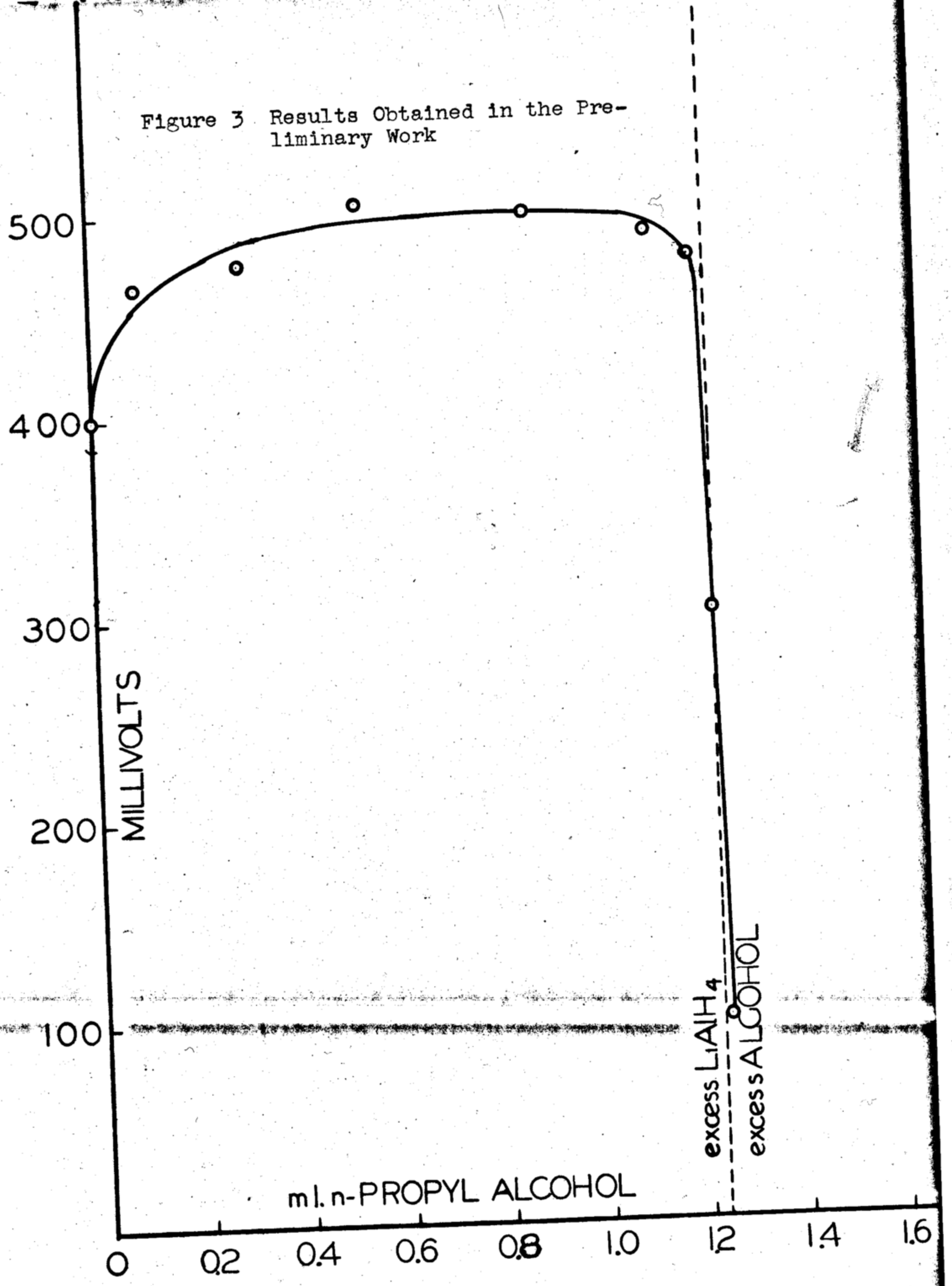

Figure 3 Results Obtained in the Preliminary Work

The procedure employed at this point was as follows: 10.0 ml of the lithium aluminum hydride solution was added to 50 ml of tetrahydrofuran and was agitated for 15 minutes with a magnetic stirrer. This mixture was placed in the apparatus shown in figure 2 and titrated directly with the specified alcohol.

The endpoint was taken as that amount of alcohol added beyond which a sharp change in indicated potential took place.

Results

The results of this method were as follows:

SAMPLE	Ml $LiAlH_4$ Solution	Ml Alcohol Consumed	Gram Molecular Equivalent X 10^3
Methyl Alcohol	10	1.05	26.0
	10	1.00	24.8
	10	1.05	26.0
Ethyl Alcohol	10	1.20	20.5
	10	1.20	20.5
N-propyl Alcohol	10	1.55	20.8
	10	1.60	21.4
Isopropyl Alcohol	10	1.41	18.5
	10	1.41	18.5
N-butyl Alcohol	10	1.15	12.6
	10	1.15	12.6
Isobutyl Alcohol	10	1.20	13.0
	10	1.20	13.0

Discussion and Conclusion

On titrating the methyl alcohol, a thick gel formed that seriously interfered with the electrode reaction. The mixture was too thick to be agitated with the magnetic stirrer. No such untoward reaction was noticed with the other alcohols. It was believed that a constant molecular amount of alcohol would be consumed on titrating a constant volume of lithium aluminum hydride with each of the various alcohols. As the values for n-butyl and isobutyl alcohols were low as determined above, it was believed that they reacted very slow and that during the titration time did not react completely. To overcome this it was decided to add the specified alcohol to an excess of the lithium aluminum hydride solution, allow them to react for a specified amount of time, and then titrate the excess lithium aluminum hydride with a known alcohol.

This method was adopted for all future open system work and was the basis for the work described in the two following manuscripts.

Preliminary Difficulties

Some of the early difficulties of the method included diffusion of the reaction mixture into the salt bridge, stoppage of the opening of the silver electrode, too vigorous reaction of the respective alcohol with the hydride, and drifting of the galvanometer needle. In early work it was attempted to follow the reaction by a Cameron pH meter. However, this instrument could not be balanced quickly enough.

Before a sufficient quantity of tetrahydrofuran was available, dioxane with known limitations as a poor conductor of electricity was tried.

The additional information on the final development of the method and results obtained as mentioned above are treated in the following two papers.

ELECTROMETRIC TITRATION OF SOME FUNCTIONAL GROUPS

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Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Fund.

Although simple titrimetric methods are available for quantitative analysis of many different types of inorganic compounds, relatively few organic compounds lend themselves to rapid volumetric analysis. Carboxylic acids and amino compounds, which can be titrated because of their acid-base property, and olefinic compounds, which can be determined by iodine or bromine titration, represent the main types of organic compounds usually assayed volumetrically. A brief description is given in this preliminary report of a relatively simple electrometric method for the volumetric analysis of several functional groups including -OH, $-NH_2$, R_2CO, RCHO, RCOOR and other easily reducible groups based on lithium aluminum hydride titration.*

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*The use of lithium aluminum hydride for quantitative determination of various functional groups is not new. Hochstein (J. Am. Chem. Soc. 71, 305, (1949), Zaugg and Horrom, Anal. Chem. 20, 1026, (1948), Krynitsky et al. (J. Am. Chem. Soc. 70, 486, (1948), and others have described gasometric methods analogous to the Zerewitinoff method based on measurement of hydrogen gas liberated by the sample on reaction with the hydride. These are, however, not true volumetric methods where a standard solution is added to a stoichiometric endpoint indicated by some visual or electrical change.

Manuscript accepted for publication in ANALYTICAL CHEMISTRY.

The apparatus used, shown in figure 2, consists of a silver electrode, salt bridge, and isolated silver electrode and an automatic burette. Dried and purified tetrahydrofuran is used to fill the salt bridge and the isolated electrode chamber, LiBr being employed as the electrolyte in both cases. In addition a small amount of iodine is added to the electrode chamber to act as a depolarizer. The potential of the electrical cell is most conveniently followed by an indicating potentiometer such as Beckman Type H pH meter.

The procedure employed is as follows: a weighed sample of approximately one gram of the unknown substance to be analyzed is introduced into the reaction vessel. 50 Ml of pure tetrahydrofuran and 20 ml of roughly 0.1 molar lithium aluminum hydride solution in tetrahydrofuran are added in turn and allowed to stand for a few minutes. The excess hydride is determined potentiometrically by adding a standard solution of ethyl or propyl alcohol in dry benzene. The end-point is indicated by a sharp change in the voltage of the electrical cell. The difference between the amount of alcohol consumed during this back titration and that used during a blank run made without the sample represents stoichiometrically the amount of substance in the sample capable of reacting with lithium aluminum hydride.

Typical results are shown in figures 4 and 6. In calculating the molar concentration of various functional types other than an alcoholic hydroxyl, it must be kept in mind that

$$-OH = \tfrac{1}{2}-NH_2 = -R_2CO = -RCHO = \tfrac{1}{2}-COOR = 1/3-COOH$$

More complete details on the development and application of the titrimetric method described will be presented shortly in another publication.

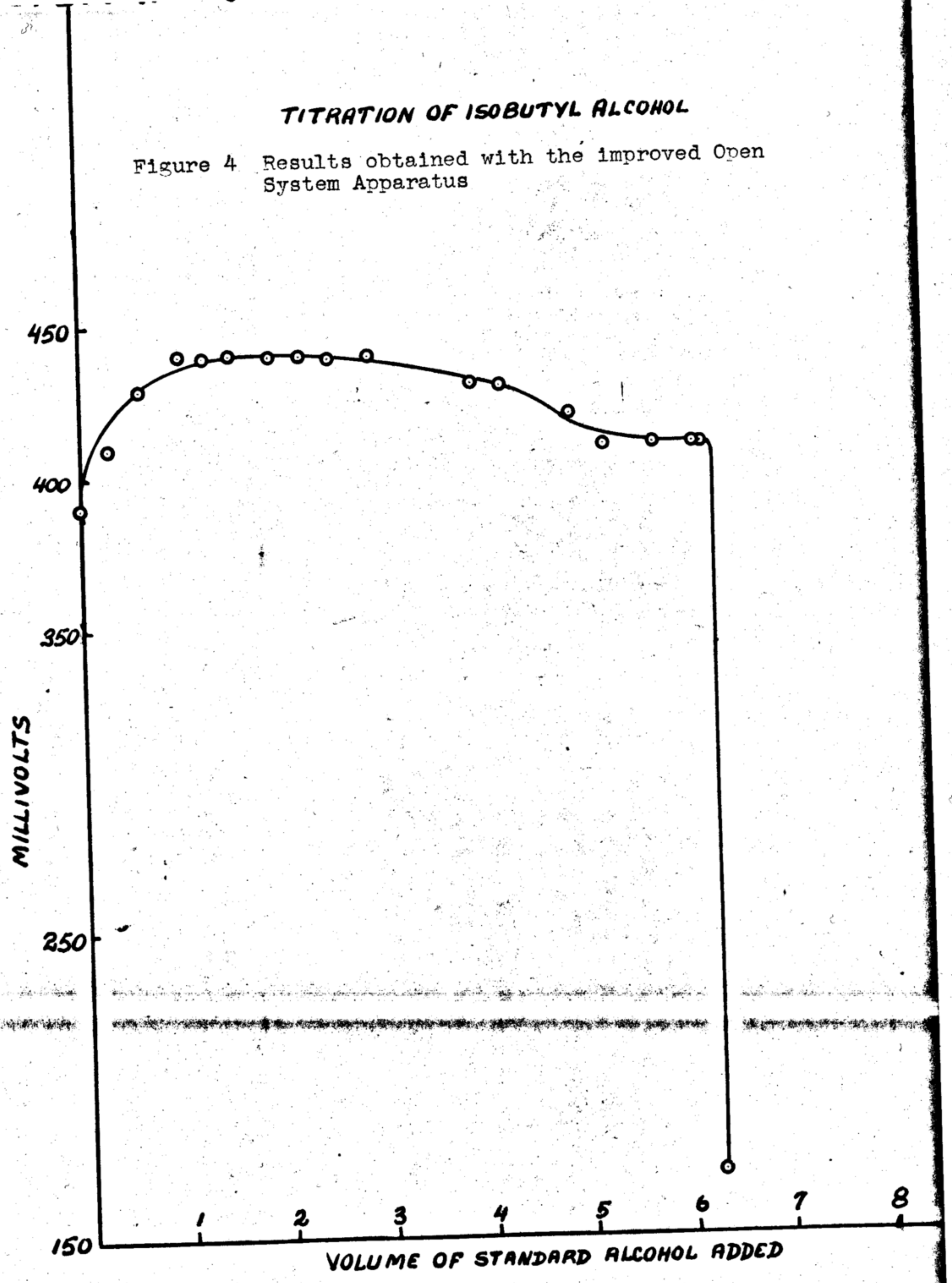

Figure 4 Results obtained with the improved Open System Apparatus

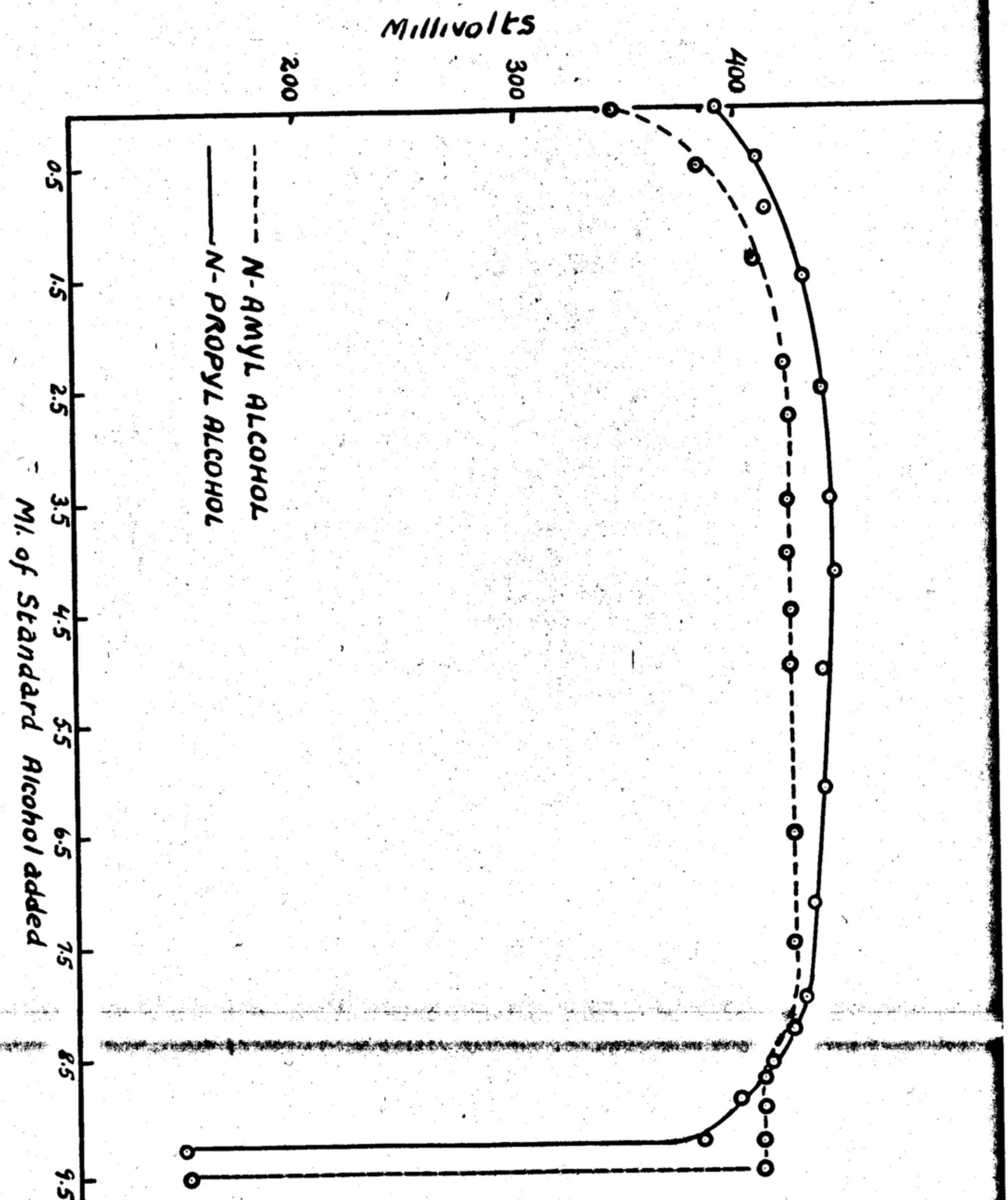

Figure 5 Results Obtained with the Improved Open System Apparatus

ELECTROMETRIC TITRATION OF ALCOHOLS USING LITHIUM ALUMINUM HYDRIDE

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Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Fund.

The possibility of using lithium aluminum hydride solution for the electrometric tritration of several functional groups including hydroxyl, amino, carbonyl and carboxyl in organic compounds was recently pointed out.[1] In this paper the details involved in the development of the method and further experimental results in its application to hydroxyl compounds are presented.

DISCUSSION OF THEORY

The potentialities of lithium aluminum hydride as a reagent for quantitative determination of compounds containing active hydrogen or easily reducible groups have been widely recognized.[2,3,4,5] Nearly all of the methods published have been based on modifications of the Zerewitinoff

Manuscript accepted for publication in ANALYTICAL CHEMISTRY.

method, requiring relatively elaborate gasometric apparatus. The method proposed (hereafter referred to as the Higuchi-Lintner-Schleif method) differs essentially from those of previous workers in that it is a true volumetric method where a standardized solution is added from a burette to a stoichiometric end-point. Such a procedure is, however, feasible only if a suitable means of end-point detection is available.

In the Higuchi-Lintner-Schleif method the stoichiometric point is located by a sudden change in the reduction potential of the system. In titration of a solution of lithium aluminum hydride with an alcohol, for example, the reduction potential of the solution up to the end-point is very high since hydrides are powerful reducing agents nearly in a class with free alkali metals. With introduction of a slight excess of the alcohol, however, the potential drops sharply since now only lithium and aluminum alkoxides which are relatively weak reducing agents are present. This behavior is depicted in

$$4ROH + LiAlH_4 \longrightarrow 4H_2 + LiOR + Al(OR)_3$$

Fig. 4 in the potentiometric titration curve (marked "Blank" in the figure) obtained by titrating a hydride solution with five per cent n-propyl alcohol solution in benzene. The voltage change corresponding to the end-point is large compared to that encountered in most other types of potentiometric titrations. It must be kept in mind that absolute

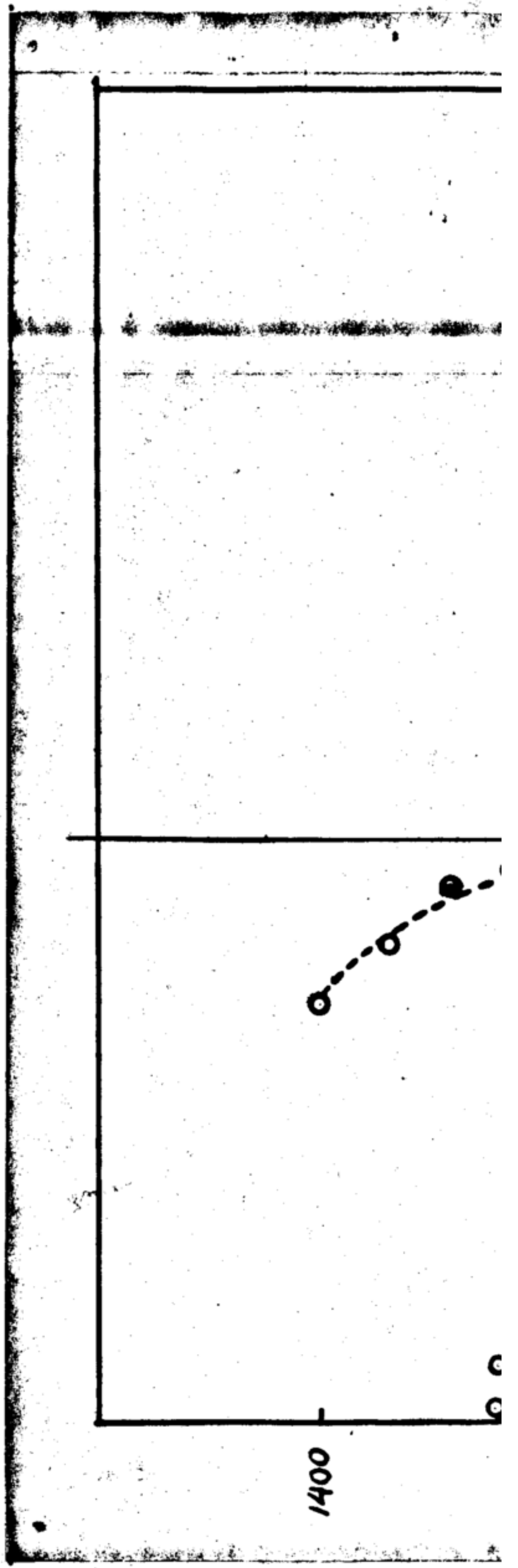

Figure 6 Results Obtai
Apparatus

potential values are not too significant since the electrode reaction at the indicator electrode is in all probability not reversible. The second curve shown was obtained under identical conditions except that a weighed amount of cyclohexanol was added to the hydride before titration, the difference between the two titrations corresponding stoichiometrically to the amount of cyclohexanol added.

DEVELOPMENT OF METHOD

In the development of the method, the problems which presented themselves were (1) selection of a solvent medium suitable for electrometric titration, yet inert toward lithium aluminum hydride; (2) choice of a suitable pair of electrodes; and (3) development of a proper operational procedure for titration. These are discussed in turn.

Selection of Solvent: Several solvents were considered for study. Strictly nonpolar hydrocarbons and ordinary ethers were ruled out because they were too nonconducting. Halogenated hydrocarbons, alcohols, esters, ketones, primary and secondary amines, and other similar functional types could not be employed because of their reactivity to the hydride. Pyridine, because of its excellent solvent properties and ready availability, was one of those seriously considered. It proved unsuitable because of a slow interaction with the hydride. Tetrahydrofuran was finally chosen because of its relatively high polarity, which makes it a good solvent for many salts, and its inertness toward the hydride. Its principal drawback was its tendency to form peroxide with atmospheric oxygen. Although neither aliphatic nor saturated cyclic tertiary amines were studied, it is conceivable that some of these may be superior to tetrahydrofuran.

Electrode System: The choice of a suitable electrode system to measure the reduction potential of the titration mixture offered no special difficulty. Platinum and silver indicator electrodes were tried and found to be effective, the latter giving somewhat more stable results. The selection and design of the reference electrode were governed by the fact that it must be uninfluenced by the changing conditions in the titrating flask but still be in electrical contact with its content. This was accomplished by using an isolated silver electrode surrounded by an electrolyte of lithium bromide and iodine (tetrabutyl ammonium iodide was also added in some cases) in tetrahydrofuran.*

Titration Procedure: In the development of the operational procedure, the employment of standardised hydride solution for direct titration of samples was precluded for several reasons. Higher alcohols, ketones, aldehydes, esters,

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*The addition of iodine is probably superfluous. It was added with the thought that silver iodide may be formed.

lactones, amines, etc., react too slowly to permit titration at a reasonable rate. All these compounds can be titrated by the indirect method.* Moreover, the strength of hydride solution, because of slow reaction with atmospheric moisture and carbon dioxide, slowly changes on standing, necessitating frequent restandardization. Also since the solvent used to dissolve the samples contains some moisture and peroxide, it is necessary to run a blank every day. It is very difficult, furthermore, to dispense the solution from a buret since all stopcock lubricants either are dissolved or react with the potent mixture. For these reasons, the reverse procedure of back titrating an excess of the hydride with a standardized alcohol solution was adopted.

A solution of n-propyl alcohol in benzene was found to be generally satisfactory as the standard. Higher alcohols, as mentioned previously, were generally too unreactive to permit direct titration. The use of methyl alcohol tended to lead to gel formation. Ethyl alcohol was fairly satisfactory except for its high volatility. Branched alcohols were not studied since branching usually results in lower reactivity. Benzene was chosen as the diluent for the standard since it can be dried easily, does not form peroxides as ethers do, and is inert with respect to the hydride. It is, however, by no means an ideal solvent for

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*Evidence for this statement will be presented in this and later papers.

this purpose since its low polarity often results in precipitation of salts from the reaction mixture.

In the remainder of the paper, results of experimental evaluation of the method as applied to hydroxyl compounds are presented.

EXPERIMENTAL

Apparatus: Two different setups were used in studying the feasibility of the method. The first, shown schematically in Fig. 2, was relatively crude and simple, the titration being carried out in an open beaker. The reduction potential was followed by measuring with a Beckman Model H pH meter, the voltage developed across a platinum indicator electrode and a reference electrode as shown in the diagram. Since the low resistance lead of the instrument (which normally connects to the calomel cell) was connected to the reference electrode, it was necessary to make the electrode resistance as low as possible to obtain stable readings. For this reason a fairly large opening was used on the electrode; this, however, resulted in slow cross diffusion of the electrode electrolyte and the beaker content. This apparatus, nevertheless, was employed for most part in the present study because of its simplicity.

The second setup, depicted in Fig. 3, was somewhat more elaborate. An attempt was made in its design to reduce the

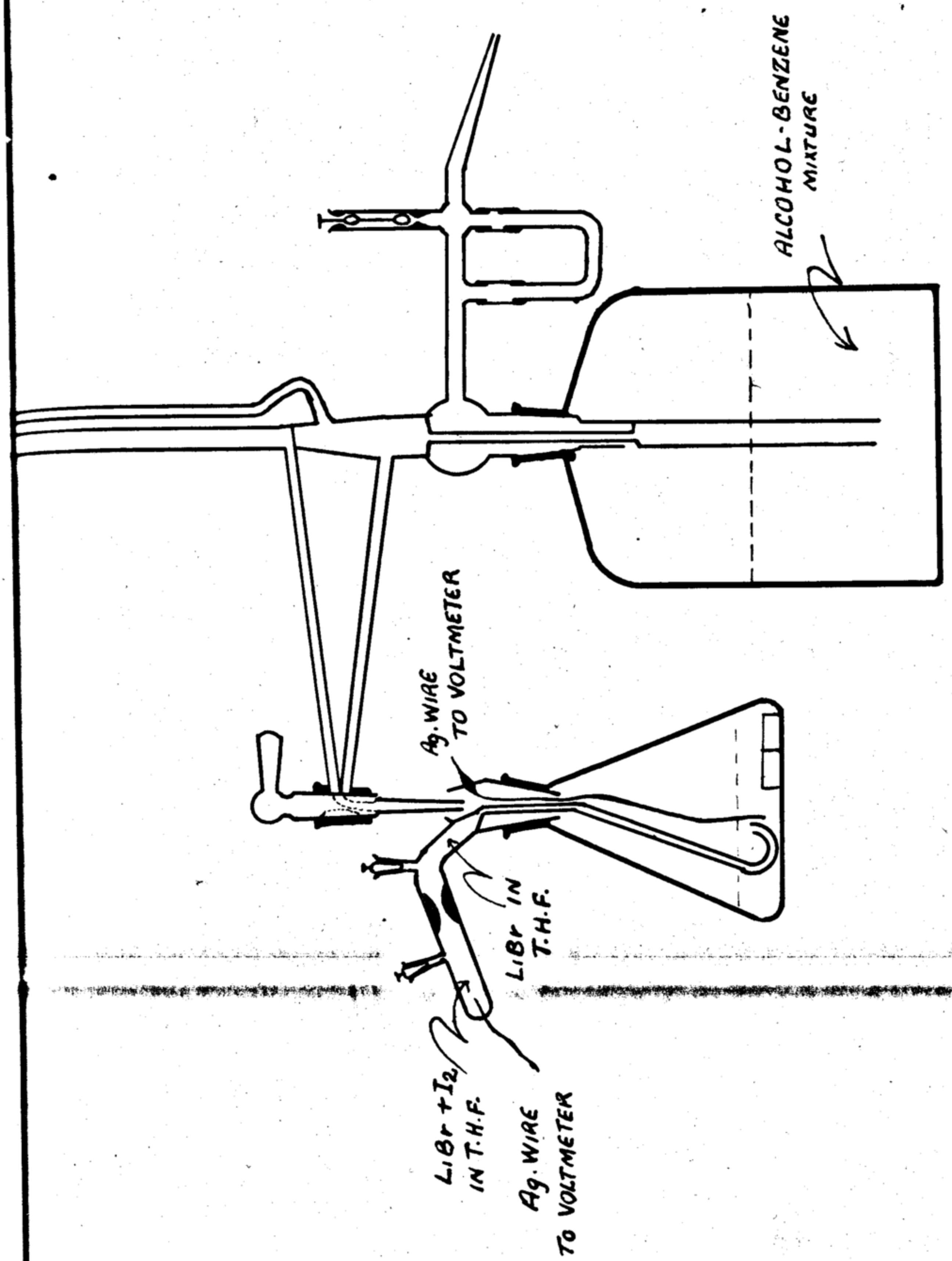

Figure 7 Apparatus Used in the Closed System

moisture pickup noticed with the earlier apparatus. This was done by enclosing the reaction system, the only opening being a small hole to admit the tip of the automatic burette. In addition the reference electrode was redesigned to cut down the exchange of solution between it and the titration mixture. A separate salt bridge chamber was introduced and the tip of the bridge was drawn down to a fine capillary. This change, which resulted in considerable decrease in the conductivity of the electrode, was made possible by connecting the reference electrode to the high resistance lead (which is normally connected to the glass electrode in the usual pH set-up) and the silver indicator electrode to the low resistance terminal of the voltmeter.

Reagents: Reagents and samples used were prepared in the following manner:

Tetrahydrofuran: technical grade solvent as received from Dupont and Co. was purified by distilling twice over lithium aluminum hydride. This procedure was found to be effective in removing water, residual peroxide, inhibitor, and all other impurities which may interfere with the titration. Waste solvent remaining in the titration flask was found to be recoverable in the same manner. Since purified THF forms peroxide rapidly, it was usually purified as needed, no more than a few days supply being distilled at any one time.

$LiAlH_4$ Solutions: a saturated solution of $LiAlH_4$ was prepared by refluxing an excess of the compound with THF (purified). This was allowed to stand overnight and then centrifuged. The clear supernatant solution was poured off and stored in a bottle fitted with a tube of desiccant. The lower concentrations were prepared by dilution with purified THF. On standing for several weeks the solutions usually turned cloudy, but were still usable.

Standard Alcohol in Benzene Solution: n-propyl and ethyl alcohol solutions were employed. In each case the alcohol was first distilled over sodium. The solutions were made up volumetrically, definite volumes of alcohols being added to volumetric flasks and made to the mark with dry benzene (distilled over sodium).

Samples: alcohols, n-butyl, isobutyl, and n-amyl were prppared by drying analytical grade reagents over Drierite for 48 hours. They were then distilled after addition of metallic sodium and stored in glass stoppered Erlenmeyer flasks fitted with a calcium chloride drying tube. Samples so prepared gave no color with anhydrous copper sulfate.

Eastman P-Cresol was freshly distilled and recrystallized, the purified product melting between 34.5-36° C. Octadecyl alcohol, m.p. 58.5-59° C.; cholesterin (Wilson brand), m.p. 148-150° C.; and cetyl alcohol, m.p. 49-49.5° C., were used untreated except for drying at 50° C. in a hot air oven for 48 hourw. Analytical grade phenol, m.p. 40-41° C., was used untreated except for drying by rubbing on a porous plate.

Procedure: The following procedure with minor modifications, was employed in obtaining experimental results reported in this paper. In all cases only oven dried glass ware was used.

Exactly 50 ml. of purified THF and 20 ml. of THF solution of lithium aluminum hydride were pipetted into the reaction vessel (see Fig. 2 and 7). The weighed sample was added and the mixture allowed to react for 15-30 minutes under agitation. The reaction mixture was then titrated potentiometrically with a standard alcohol benzene mixture. The endpoint was indicated by a large change in the voltage on addition of a small amount of the hydride reagent. Within a few tenths of a ml. of the endpoint, it was necessary to wait 10-20 seconds after each addition before taking a reading.

A blank run was made for each set of determinations, identical procedures being used except for the addition of the sample. The number of gram equivalents present in the sample was calculated according to the formula

$$\text{Number of gram equivalents} = \frac{(b - v)M}{1000}$$

where b = ml. of standard alcohol solution consumed by the blank

v = ml. of standard alcohol solution consumed by sample titration

M = molarity of the standard alcohol solution used.

RESULTS AND DISCUSSION

In Table I are listed some results obtained with the apparatus shown in Fig. 2. The agreements between the theoretical and the experimental columns are as good as can be expected with such an open system. Since moisture pickup occurred both in blank and in sample titrations, the values probably reflect certain amount of automatic compensation for this source of error. These runs were made during winter months when relatively dry atmospheric conditions prevailed. As shown in the table, the absolute errors were of the order of a few tenths of a millimole.

Improvement in the accuracy of the method with the revised setup (Fig. 3) is evident in Table II where analytical data for a phenol and several alcohols are presented. Smaller samples and less concentrated standard alcohol solution (5% n-propyl alcohol) were used in obtaining these results. In every case the analytical results were higher than would be expected theoretically. This was probably due to residual moisture in the samples. It must be kept in mind that for a compound with an equivalent weight of 180, 0.05% of moisture would produce approximately 1% high result. Except in the case of t-butyl alcohol, the difference between the theoretical and experimental was usually less than a tenth of a millimole.*

Results of a reproducibility study are shown in Table III. Six samples of cyclohexanol were titrated with the improved apparatus. The mean error for the series is approximately one part in a thousand, comparable to those found with usual good volumetric methods.

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*These analyses were made during the summer months when relative humidity and temperature were both in the nineties.

Table I

Analytical Results with Apparatus Shown in Fig. 2

Sample	Wt Sample	LiAlH$_4$ Sol'n	Std. Alc. Sol. Consumed		Moles Alc. Present			
			Blank	With Sample	Theor.	Found	Difference	% Error
N-Butyl	0.8100	10	8.30 ml	5.15 ml	0.01093	0.01079	-0.0001	1.3
Iso-Butyl	2.6530	20	16.70	6.30	0.0358	0.0357	-0.0001	0.3
N-Propyl	2.2740	20	20.40	9.50	0.0378	0.0374	-0.0004	1.0
N-Amyl	2.5829	20	21.60	12.90	0.0293	0.0298	+0.0005	1.7
Octa-Decyl	2.4934	20	18.70	16.00	0.00907	0.00925	+0.0002	2.0
Phenol	2.5943	20	18.80	10.80	0.0275	0.0274	-0.0001	0.4
Cetyl	2.7342	20	18.70	15.40	0.0113	0.0111	-0.0002	1.8
P-Cresol	1.4927	20	18.80	14.80	0.0138	0.0137	-0.0001	0.7
Choles-	1.3692	20	16.80	15.75	0.00338	0.00360	+0.0002	6.5

Twenty per cent ethanol in benzene solution (by volume)

Table II

Analytical Results with Apparatus Shown in Fig. 7

Sample	Wt Sample	Std. Alc. Sol. Consumed		Moles Alc. Present		Found/Theor.
		Blank	With Sample	Theor.	Found	
T-Butyl Alcohol	.4981	20.33	9.13	.006720	.007496	1.116
	.4860	20.33	9.34	.006562	.007356	(1.121)
Benzyl Alcohol	.5016	20.10	13.14	.004638	.004658	1.006
	.7463	20.10	9.65	.006902	.006994	1.014
Thymol	.2803	20.10	17.20	.001869	.001941	1.038
	.3002	20.10	17.00	.001998	.002074	1.038
Menthol	.4892	5.77	.85	.003131	.003292	1.051
	.4927	5.77	.93	.003153	.003239	1.027
Octadecyl Alcohol	.5526	17.45	14.40	.002043	.002044	1.001
	.2993	17.45	15.78	.001105	.001118	1.011
	.4174	17.45	15.13	.001543	.001553	1.006

Five per cent solution of n-propyl alcohol in benzene.

Table III

Reproducibility Study Using Cyclohexanol

		Std. Alc. Sol. Consumed		Moles Alc. Present			
Sample No.	Sample Weight	Blank	With Sample	Theor.	Actual	Deviation	% Error
62	0.9675	38.65	24.20	.00966	.00967	+0.00001	0.1
13	0.9916	38.65	23.84	.00990	.00991	+0.00001	0.1
26	1.0321	38.65	23.30	.01029	.01028	-0.00001	0.1
10	1.0490	38.65	23.00	.01047	.01047	0.00000	0.0
64	0.9578	38.65	23.35	.00956	.00957	+0.00001	0.1
22	0.9982	38.65	23.75	.00997	.00997	0.00000	0.0

SOURCES OF ERROR

Water is the principal source of error for the method. As was pointed out previously, a trace of moisture can cause a disproportionate error in the analytical result because of the low molecular weight of water. Inadequate drying of samples, wet glassware, and absorption of atmospheric moisture lead to spurious results. Where it is impossible to remove last traces of water, it may be necessary to determine the moisture concentration by Karl Fischer titration.

APPLICATION OF THE METHOD

The method can be employed to advantage in determining concentrations of functional groups both for analytical and characterization work. In this report its application to only hydroxyl containing compounds has been covered. The method is equally applicable to all types of compounds reacting quantitatively with the hydride. As presently developed, it can not distinguish, however, between functional groups containing active hydrogens and those possessing readily reducible groups. Because of slow interaction of the hydride with certain ester linkages, however, these may be differentiated by regulating the reaction time. Principal advantages of the method for studies of this nature are its relative simplicity and rapidity.

The applicability of the method in obtaining a useful chemical constant in the analysis of essential oils is under investigation. The extent of interaction of the hydride with the oils should be a measure of their hydroxyl and carbonyl content.

FUTURE DEVELOPMENTS

The design of the "improved" apparatus is still relatively quite crude as compared to the elaborate setups employed in Karl Fischer titration in keeping out extraneous moisture. It is hoped that interference from this source can be further minimized.

An investigation is presently in progress to study the feasibility of substituting a chemical indicator in place of the electrode assembly. Several compounds, in particular p-amino azobenzene, have been found to change color sharply at the equivalence point. No data are available at the moment on the comparative sensitivity of the electrical and chemical indicator systems. The latter would be of special value in analysis of micro samples.

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PART B

Application to Pharmaceutical Compounds

The development of an electrometric lithium aluminum hydride titration procedure for the quantitative determination of certain functional groups has been described in Part A. In this section results of an investigation studying the feasibility of this method of analysis as applied to various pharmaceutical compounds are presented.

In the first paper the results of nineteen different substances are presented. The average error of the compounds studied was less than two per cent and included amines, acids, lactones, alkaloids, alcohols, aldehydes, esters, and phenols. In all cases the reaction with the hydride followed the predicted stoichiometric relationship.

In addition the analytical data for essential oil and essential oil isolates are presented. The value of this as a criterion for purity and identity of volatile oils is discussed.

The following are manuscripts submitted for publication to the Scientific Edition of the Journal of American Pharmaceutical Association.

APPLICATION OF LITHIUM ALUMINUM HYDRIDE TITRIMETRIC METHOD TO PHARMACEUTICAL COMPOUNDS[+][±]

Carl J. Lintner,[*] Donald A. Zuck, and T. Higuchi

In investigations of either crude or pure pharmaceuticals it is often desirable to know the concentration of functional groups present. Such information is of value not only for purely analytical reasons but is also often necessary for characterization and identification purposes. It is evident that knowledge, for example, of milliequivalences of aldehydic or ketonic grouping present in an unknown sample would prove very useful in many cases.

Such information can be readily obtained for acidic or alkaline groups by the usual alkalimetric or acidimetric

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procedures. Comparable information for alcoholic, aldehydic, ketonic and many other groups is not, however, easily obtainable by the present methods. In this paper, results of application of the HLS method[1,2] which utilises lithium aluminum hydride solution for volumetric determination of these latter functional groups are presented.

Theory

The theoretical basis of the method has been covered in detail in previous papers.[1,2] The method is essentially based on quantitative reactions of lithium aluminum hydride with various functional groups. These are:

Alcohols, glycols and phenols

$$4ROH + LiAlH_4 \longrightarrow LiAl(OR)_4 + 4H_2$$

Carboxylic acids

$$4RCOOH + 3LiAlH_4 \longrightarrow (RCH_2O)_4LiAl + 2LiAlO_2 + 2H_2$$

Primary amines

$$2RNH_2 + LiAlH_4 \longrightarrow LiAl(RN)_2 + 4H_2$$

Secondary amines

$$4R_2NH + LiAlH_4 \longrightarrow LiAl(R_2N)_4 + 4H_2$$

Aldehydes

$$4RCHO + LiAlH_4 \longrightarrow (RCH_2O)_4LiAl$$

Ketones

$$4R_2CO + LiAlH_4 \longrightarrow (R_2CHO)_4LiAl$$

Esters

$$2RCOOR^1 + LiAlH_4 \longrightarrow \frac{1}{2}(RCH_2O)_4LiAl + \frac{1}{2}(R^1O)_4LiAl$$

Anhydrides

$$(RCO)_2O + LiAlH_4 \longrightarrow \frac{1}{2}(RCH_2O)_4LiAl + \frac{1}{2}LiAlO_2$$

Acid chlorides

$$2RCOCl + LiAlH_4 \longrightarrow \frac{1}{2}(RCH_2O)_4LiAl + \frac{1}{2}AlCl_3$$

Amides

$$2RCONR_2 + LiAlH_4 \longrightarrow 2RCH_2NR_2 + LiAlO_2$$

$$4RCONR'_2 + 2LiAlH_4 \longrightarrow LiAl(OCH_2R)_4 + LiAl(NR'_2)_4$$

$$4RCONHR' + 3LiAlH_4 \longrightarrow LiAl(OCH_2R)_4 + 2LiAl(R'N)_2 + 4H_2$$

Nitriles

$$2RCN + LiAlH_4 \longrightarrow (RCH_2N)_2LiAl$$

Aromatic Nitro Compounds

$$4RNO_2 + LiAlH_4 \longrightarrow \frac{1}{2}R-N=N-R + 2H_2 + LiAlO_2$$

In addition to the organic compounds listed above, lithium aluminum hydride reacts with the following inorganic compounds:

Water

$$LiAlH_4 + 4H_2O \longrightarrow LiOH + Al(OH)_3 + 4H_2$$

Ammonia

$$2LiAlH_4 + 5NH_3 \longrightarrow [LiAlH(NH_2)_2]_2NH + 6H_2$$

Metal Halides

$$4MnCln + nLiAlH_4 \longrightarrow MHn + nLiCl + nAlCl_3$$

Carbon Dioxide

$$4CO_2 + 3LiAlH_4 \longrightarrow 3LiAl(OCH_3)_4 + 2LiAlO_2$$

Two different reactions may take place with carbon dioxide. With an excess of carbon dioxide, derivatives of formaldehyde are formed, while with an excess of the hydride, methanol derivatives are produced. The latter reaction has been investigated for the preparation of methanol containing isotopic carbon.

From available evidence most reactions are quantitative with very little side reactions.

The essential difference between the present method and the lithium aluminum hydride methods[3,4,5] that have been previously proposed is in the analysis of lithium aluminum hydride which had undergone reaction. Earlier methods have been based on rather complicated gasometric determinations; in the HLS method use is made of electrometric analysis of of the residual lithium aluminum hydride.

The basis of the electrometric method lies in the fact that hydrides are powerful reducing agents. On addition of lithium aluminum hydride solution to a solution containing alcohol for example, all hydride would be destroyed until all the alcohol had reacted. As soon as a slight excess of hydride is present the reduction potential of the system (as indicated, for example, by a bright platinum or silver electrode) should increase markedly.

Plan of Work

In the present study an investigation of the feasibility of the HLS method for analysis of various typical pharmaceutical compounds has been made. The methods used and results obtained are reported in the following sections.

Apparatus

The apparatus used in this series has already been described in a previous paper.[2] It is shown schematically in Figure 7. It is essentially an electrometric titration assembly with an isolated silver electrode as the reference electrode and a bright silver electrode as the indicator electrode. Because of the unusual nature of the electrolyte used, it is more desirable to connect the reference instead of the usual indicator electrode to the high resistance lead of the indicating voltmeter. Beckman Model H pH meter has been found to be satisfactory for the latter function.

Reagents

Reagents used were prepared in the following manner:

Tetrahydrofuran, which is used as the solvent for the lithium aluminum hydride and also in the electrolytic system, was purified by double distillation over lithium aluminum hydride.

Lithium aluminum hydride solution was prepared by refluxing an excess of the compound with purified tetrahydrofuran. This was allowed to stand overnight and then centrifuged. The clear supernatant solution was poured off and stored in a bottle fitted with a tube of Drierite and Ascarite.

Standard Alcohol-benzene solution was made by adding dry, thiophene-free benzene to dry, analytical grade, normal propyl alcohol. Exactly 50 ml. of the alcohol was made up to one liter in a volumetric flask. The solution was stored in an automatic burette fitted with a Drierite-Ascarite tube.

Procedure

The lithium aluminum hydride solution was pipetted into a 125 ml. glass stoppered erlenmeyer flask. The dried compound to be analyzed was mixed with 25.0 ml. of previously dried tetrahydrofuran. The reaction mixture was then either heated under reflux for 60 minutes or, alternatively, agitated with a mechanical stirrer for 30 minutes. In either case, the mixture was protected by a Drierite drying tube. The electrodes were then inserted and the solution titrated with the 5% n-propyl alcohol solution in benzene. The blank determination was made with the same procedure except for addition of the sample.

The number of gram equivalences of functional group present in the sample were calculated according to the following formula:

$$\text{Number of Gram equivalents} = \frac{(B-V)\,M}{1000}$$

where B = ml. of standard alcohol solution consumed by the blank.

V = ml. of standard alcohol solution consumed by sample titration.

M = molarity of the standard alcohol used.

The following substances were analysed

Aniline - Mallinckrodt - n^{26} = 1.5797 dried by distilling from metallic sodium.

Diphenylamine - M.P. 52-53° dried by rubbing on a porous plate.

Alpha Naphthylamine - M.P. 48-49.5° dried by rubbing on a porous plate.

Dimethylaniline - Baker Chemical Works - n^{25} = 1.5550 was dried by distillation.

Benzoic Acid - analytical grade - M.P. 121-121.5° dried by rubbing on a porous plate.

Salicylic acid - analytical grade M.P. 158-159° dried by rubbing on a porous plate.

Amidopyrine - M.P. 107-109° dried in hot air oven at 90° for 48 hours.

Coumarin - Fritzsche Brothers - M.P. 68-69.5° - dried by rubbing on a porous plate.

Morphine - Merck - recrystallized from alcohol-water mixture, dried in hot air oven at 114° C. for 48 hours. M.P. 252.5-254°.

Cinchonidine - Lehn and Fink - recrystallized from alcohol-water and dried in oven at 114° for 46 hours. M.P. 202.5-20[illegible]°.

Verbenalol - aglucone prepared from verbenalin a glucoside isolated from Verbena Stricta. Courtesy of Dr. L. M. Parks. M.P. 128-130°.

Methyl Salicylate - Merck - n_{26} = 1.5341 dried by distillation.

Benzaldehyde - Merck - n_{26} = 1.5420 dried by distillation.

Vanillin - Fritzsche Brothers - M.P. 81-82° dried by rubbing on a porous plate.

Aspirin - M.P. 135-136° dried in an oven at 102° for 48 hours.

Salol - M.P. 41.5-43° dried by rubbing on a porous plate.

Benzyl Alcohol - n = 1.5368 dried by distillation.

Thymol - Magnus, Mabee and Reynard - M.P. 51-52° dried by rubbing on a porous plate.

Menthol - Merck - M.P. 43-44° dried by rubbing on a porous plate.

ANALYTICAL RESULTS OBTAINED WITH SOME PHARMACEUTICAL COMPOUNDS

Sample	Sample Wt.	Moles Present	Method of Reacting	Vol. of $LiAlH_4$ Soln.	Standard Alcohol Titration Blank	Standard Alcohol Titration Sample	Equivalents of alcohol by sample	Equivalent/ Mole	Predicted Equivalence
Aniline	0.4843	0.005201	X	40.0	39.80	24.70	0.010110	1.94	2
Diphenylamine	0.4703	0.002779	X	10.0	12.85	8.50	0.002912	1.05	1
a-naphthylamine	0.4821	0.003367	Y	20.0	27.31	17.31	0.006693	1.99	2
Dimethylaniline	0.6990	0.005768	Y	40.0	38.65	38.50			0
Benzoic Acid	0.4963	0.004064	X	20.0	22.30	4.68	0.011793	2.90	3
Salicylic Acid	0.5822	0.004215	X	15.0	27.31	2.09	0.16880	4.00	4
Amidopyrine	0.6364	0.002751	X	10.0	19.90	15.76	0.002771	1.01	1
Coumarin	0.4879	0.003338	X	10.0	19.90	5.24	0.009812	2.94	3
Morphine	0.5102	0.001789	X	10.0	18.75	13.45	0.003547	1.98	2
Cinchonidine	0.4931	0.001675	X	10.0	18.75	16.15	0.001740	1.04	1
Verbenalol	0.1125	0.0004977	X	5.0	7.68	3.94	0.002503	5.03	5
Methyl Salicylate	0.2968	0.001951	X	10.0	14.62	5.85	0.005870	3.01	3
Benzaldehyde	0.4831	0.004552	X	10.0	14.71	7.87	0.004578	1.00	1
Vanillin	0.2650	0.001742	X	10.0	19.42	14.17	0.003514	2.02	2
Aspirin	0.5078	0.002818	X	15.0	27.31	6.44	0.013968	4.96	5
Salol	0.5033	0.002349	X	10.0	17.73	7.25	0.007014	2.99	3
Benzyl Alcohol	0.5016	0.004638	X	10.0	20.10	13.14	0.004658	1.00	1
Thymol	0.3002	0.001998	X	10.0	20.10	17.00	0.002075	1.04	1
Menthol	0.4927	0.003153	X	5.0	5.77	0.93	0.003239	1.03	1

X Refluxed 1 hour
Y Stirred 30 minutes

Discussion and Conclusions:

The experimental values obtained by the HLS method agree favorably with the predicted value. The higher values are usually due to low molecular weight impurities. Water, in particular, is an important source of error. Lower values usually indicate an incomplete reaction as with aniline and benzoic acid.

Since the end point obtained by the method is extremely sharp and reproducibility is usually good under proper conditions,[2] even better analytical accuracy appears to be possible with further refinement.

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ELECTROMETRIC TITRATION OF VOLATILE OILS AND VOLATILE OIL ISOLATES USING LITHIUM ALUMINUM HYDRIDE

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Introduction

The analysis of volatile oils and related substances is a highly specialised field of analytical Chemistry which requires diverse physical and chemical procedures. The analytical procedures are employed to characterise oils, to detect spoilage, to detect adulteration and sophistication, and for the general purpose of establishing the identity and validity of these products. Such useful quantitative indices as optical rotation, refractive index, acid number, ester number, etc., which collectively aid in establishing the purity and identity of these substances are usually obtained. The present investigation was undertaken with the supposition that a new useful quantitative index may be obtainable for volatile oils and related substances by application of the new quantitative electrometric titration method employing lithium aluminum hydride. The purpose of the initial study

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was to establish the feasibility of this type of analysis for volatile oils.

The application of the lithium aluminum hydride electrometric titration procedure (HLS method) to the quantitative determination of various functional groups has been described previously,[1,2,3] and allusion has already been made to the possibility of using this procedure in the analysis of volatile oils. It is observed, in the limited amount of data resulting from the use of the HLS method, that alcohols, phenols, aldehydes, ketones, esters and carboxylic acids, compounds which are present in essential oils, react quantitatively with lithium aluminum hydride in the following manner:

Alcohols and Phenols

$$4R\text{-}OH + LiAlH_4 \longrightarrow LiAl(OR)_4 + 4H_2$$

Aldehydes

$$4RCHO + LiAlH_4 \longrightarrow (RCH_2O)_4LiAl$$

Ketones

$$4R_2CO + LiAlH_4 \longrightarrow (R_2CHO)_4LiAl$$

Esters

$$2R\text{-}COOR' + LiAlH_4 \longrightarrow \tfrac{1}{2}(RCH_2O)_4LiAl + \tfrac{1}{2}(R'O)_4LiAl$$

Carboxylic Acids

$$4RCOOH + 3LiAlH_4 \longrightarrow (RCH_2O)_4LiAl + 2LiAlO_2 + 4H_2$$

Lithium aluminum hydride does not as a rule react with ether linkages or ethylenic double bonds. In substances such as cinnamic acid, where the unsaturated bond is definitely activated by conjugation with an aromatic nucleus on one side and a readily reducible group on the other, reduction of the unsaturated linkage is observed.

It is common knowledge that volatile oils consist of mixtures of the type compounds enumerated above together with other types which do not react with lithium aluminum hydride. Volatile oils then should undergo reaction with this agent; and if different lots of a given volatile oil are qualitatively and quantitatively similar (the premise upon which all present indices are based) then a lithium aluminum hydride equivalence of rather constant value for individual oils should be establishable.

Apparatus and Procedure

The description of the apparatus and the preparation of the standard reagents for this procedure have been fully described[2] in previous publications. Techniques consistent with the established procedure were followed in these analyses.

Exactly 50 ml. of purified tetrahydrofuran (THF) and exactly 5 ml., or 10 ml., of THF solution of lithium aluminum hydride were pipetted into the reaction vessel. The weighed volatile oil or isolate was added to this solution and allowed to react for 15 minutes under magnetic stirrer agitation. A standard solution of 5% n-propyl alcohol by volume in benzene was then used in the potentiometric titration of excess lithium aluminum hydride. For each determination a blank run was conducted with the identical procedure except for the addition of the sample. The equivalence of each substance was expressed in milliequivalents per gram of sample.

The following substances were analyzed:

<u>Eucalyptol</u>. Mallinckrodt U.S.P. grade eucalyptol was analyzed after drying by distillation over metallic sodium. The refractive index (n) of this purified substance at 25° was 1.4498.

<u>Methyl Salicylate</u>. Merck methyl salicylate was dried by distillation over metallic sodium ($n_{25} = 1.5341$) and analyzed.

<u>Eugenol</u>. Merck eugenol dried over Drierite for 48 hours ($n_{25} = 1.5375$) was analyzed without further treatment.

<u>Benzaldehyde</u>. Merck U.S.P. grade benzaldehyde was dried by distilling over metallic sodium ($n_{25} = 1.5420$) before analysis.

<u>Thymol</u>. Magnus, Mabee and Reynard U.S.P. grade thymol was dried on a porous plate (M.P. 51-52°) and analyzed without further pretreatment.

Menthol. Merck U.S.P. grade menthol was dried on a porous plate (M.P. 43-44°) and analysed without further pretreatment.

Borneol. A student preparation derived from Oil of Hemlock was recrystallized three times from benzene (M.P. = 208-210°) and analysed without further treatment.

Citronellol. City Chemical Corporation of New York citronellol was dried by standing over magnesium perchlorate for 48 hours (n_{25} = 1.4500) and analysed.

Terpineol. Fritzsche Bros. terpineol was dried by standing over magnesium perchlorate for 48 hours (n_{25} = 1.4798) and analysed without further treatment.

d-carvone. A laboratory-prepared sample* of d-carvone was dried by standing over magnesium perchlorate for 48 hours (n_{25} = 1.4969) and analysed.

Piperonal (Heliotropin). Fritzsche Bros. piperonal was dried on a porous plate (M.P. = 37.37.5°) and subjected to analysis.

l-menthone. Eastman Kodak l-menthone was dried by standing over Drierite for 48 hours (n_{25} = 1.4475) and analysed without further treatment.

Linalool. Fritzsche Bros. linalool was dried by standing over magnesium perchlorate for 48 hours (n_{25} = 1.4689) and analysed.

* Samples furnished by Dr. Gordon Swoboda, now at the School of Pharmacy, University of Kansas.

Limonene. Fritzsche Bros. limonene was dried by standing over magnesium perchlorate for 48 hours (n_{25} = 1.6727) and analysed.

The following volatile oils were each dried over C.P. calcium chloride for 48 hours before analysis:

Selected Peppermint Oil. Fritzsche Bros.

Oil of Spearmint. Fritzsche Bros. U.S.P. grade.

Oil of Rosemary. Fritzsche Bros. U.S.P. grade.

Oil of Lavender. Fritzsche Bros. U.S.P. grade.

Oil of Cinnamon Ceylon Imitation. Fritzsche Bros.

Oil of Cloves. Magnus, Mabee and Reynard U.S.P. grade.

Oil of Lemon, California Coldpressed. Fritzsche Bros. U.S.P. grade.

Oil of Thyme, White Extra Fine. Fritzsche Bros.

Oil of Bergamot F.B., Extra Fine. Fritzsche Bros.

Oil of Litsea Guatemalensis.

Results and Discussion

The results of the analyses on the various volatile oils and related substances are given in Tables I and II. The equivalents per sample are expressed as the fraction of a mole of alcohol to which a given sample is equivalent. The milliequivalent per gram is then readily calculated from the expression

$$\text{M.E. per gram} = \frac{\text{Equivalents per Sample} \times 1000}{\text{Weight of Sample}}$$

The data on the volatile oil isolates shows that for each substance titrated, experimental and theoretical values check reasonably well except for borneol. The method is thus quantitative and a determination of the M.E. per gram on a mixture of such substances as they may occur in volatile oils should serve as a useful index in ascertaining the relative amounts of reactive oxygenated components in a natural product of this type.

The results of the analyses on the volatile oils of Table II indicate the reproducibility of the HLS method, and the possibilities for this procedure in establishing a lithium aluminum hydride equivalence as a quantitative index for purity and identity of these products. The low M.E. per gram in Oil of Lemon and Oil of Orange, for example, is in keeping with the fact that each of these oils contain approximately 90% or more of limonene plus other hydrocarbons which do not react with lithium aluminum hydride. The relatively high M.E. per gram for such oils as Spearmint, Thyme, and Peppermint likewise is in keeping with our knowledge that these oils contain high percentages of esters, phenols, alcohols, and other components which react with lithium aluminum hydride. The data for all of the oils correlates well with our present knowledge of the qualitative and quantitative chemical composition of these products. The HLS method thus provides a simple, readily reproducible procedure for a rapid determination of a chemical index which should be a useful tool in the analysis and identification of volatile oils.

The analytical possibilities of the method are further illustrated in Table III, where results of analysis of several synthetic mixtures of Oil of Lemon and Oil of Spearmint are shown. It is evident that results are reasonably quantitative in nature even at present stage of development of the method.

Table I.

Lithium Aluminum Hydride Equivalence Values for Some Volatile Oil Isolates

Substance Analysed	Sample Weight gms.	$LiAlH_4$ Soln. cc.	Vol. Std. Alc. Sol. Consumed		Theoretical Equivalents per sample	Experimental Equivalents per sample	Equivalents per mole	M.E. per gm.
			Blank	with Sample				
Eucalyptol	0.4815	5.00	7.20	7.20	0.	0.0000	0.00	0.00
Me.Salicylate	0.2968	10.00	14.62	5.85	0.00583	0.005870	3.009	19.77
Eugenol	0.3873	5.00	6.40	2.91	0.002365	0.002336	0.987	6.01
Benzaldehyde	0.4831	10.00	14.71	7.87	0.004552	0.004578	1.005	9.47
Thymol	0.3002	10.00	17.60	14.70	0.001998	0.002074	1.038	6.91
Menthol	0.4927	10.00	15.77	10.93	0.003153	0.003239	1.027	6.57
Borneol	0.3052	5.00	5.77	2.55	0.001979	0.002155	1.089	7.06
Citronellol	0.9048	10.00	17.55	9.00	0.005790	0.005722	0.988	6.32
Terpineol	0.5011	10.00	17.55	12.68	0.003249	0.003260	1.004	6.51
d-carvone	0.4682	10.00	17.35	12.65	0.003117	0.003146	1.009	6.72
Piperonal	0.7365	10.00	17.10	9.80	0.004906	0.004886	0.996	6.63
l-menthone	0.6108	10.00	17.10	10.95	0.003960	0.004116	1.038	6.73
Linalool	0.9420	10.00	17.35	8.10	0.006107	0.006191	1.013	6.57
Limonene	0.9258	10.00	17.35	17.29	0	0.000040	0	0

Table II

Analytical Results for Some Common Volatile Oils

Name	Sample Weight	$LiAlH_4$ Solution	Vol. Std. Alc. Blank	Sol. Consumed with Sample	Equivalents Found	Milli. equiv. per gm.
Oil of Spearmint	0.5357 gms.	10.00 ml.	16.50 ml.	8.55 ml.	0.005321	9.93
" " "	0.5182	10.00	16.50	8.80	0.005154	9.94
Oil of Rosemary	0.5390	10.00	16.50	9.50	0.004685	8.69
" " "	0.4816	10.00	17.94	11.70	0.004176	8.67
Oil of Thyme	0.5102	10.00	16.50	8.55	0.005321	10.43
" " "	0.5267	10.00	16.50	8.10	0.005622	10.67
Oil of Peppermint	0.5164	10.00	13.35	4.25	0.006091	11.79
" " "	0.4112	10.00	17.94	10.68	0.004859	11.82
Oil of Lemon	0.4281	5.00	3.95	3.51	0.002945	0.688
" " "	0.4887	5.00	3.95	3.45	0.0003347	0.685
Oil of Orange	0.7182	10.00	20.33	19.15	0.0007898	1.09
" " "	0.3463	5.00	3.95	3.38	0.0003815	1.09
Oil of Clove	0.5433	5.00	3.95	2.225	0.001138	2.09
" " "	0.3991	5.00	4.70	3.48	0.0008165	2.05
Oil of Bergamot	0.4034	5.00	3.30	1.72	0.001058	2.62
" " "	0.4401	5.00	3.30	1.55	0.001171	2.66
Oil of Lavender	0.4213	5.00	3.30	0.62	0.001794	4.26
" " "	0.4195	5.00	3.30	0.70	0.001740	4.15
Oil of Cinnamon	0.5060	5.00	9.90	3.95	0.003982	7.86
" " "	0.4897	10.00	17.94	12.24	0.003815	7.79
Litsia Guatamalensis Mes	0.5931	10.00	17.35	8.90	0.005656	9.53
"	0.6744	10.00	17.35	7.60	0.006526	9.67

Table III.

Analysis of Synthetic Mixtures of Volatile Oils

	Per cent Actual		Per cent Experimental	
Mixture	Oil of Lemon	Oil of Spearmint	Oil of Lemon	Oil of Spearmint
I	71.58	28.42	71.8	28.2
II	53.26	46.74	52.8	47.1
III	35.58	64.42	35.8	64.2
IV	20.26	79.74	19.6	80.4

Summary

The application of the HLS method of potentiometric titration with lithium aluminum hydride has been illustrated in analyses of volatile oils and volatile oil isolates. The titration of known volatile oil isolates gives experimental values which check well with theoretical values. Titration values on volatile oils correlate well with predicted value based on knowledge of the chemical compositions of these oils. The possible usefulness of the lithium aluminum hydride equivalence as an index for purity and identity of volatile oils has been pointed out.

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PART C

Development of the Colorimetric Method

Introduction

After the development of an electrometric lithium aluminum hydride procedure for the quantitative determination of certain functional groups, it was thought that a colorimetric method would be advantageous. A colorimetric method would eliminate the complex apparatus required in the electrometric procedure and would be more adaptable to micro technique.

Theoretical Basis

A very weak acid is required as the indicator. That is, acids weaker by several pK units than the samples to be analyzed. In addition the chosen indicator must be soluble in the reaction mixture, react slower than the sample, and possess a distinct color change at the endpoint.

Two type structures, the azo and the carbonium ion types, were tried in this series of determinations.

Preparation of Indicators

Commercial grade diphenylamine, fluorene, benzidine, p-aminoazobenzene, diazoamino benzene, N-N' di-B-naphthyl-p-phenylene diamine and N-N' diphenyl-p-phenylene diamine and analytical grade triphenyl methane were used without further treatment.

N-phenyl-p-aminoazobenzene was prepared according to the method of Torrey and MacPherson[1] by diazotizing aniline and then coupling it with diphenylamine in the presence of glacial acetic acid. The product was recrystallized from Skelly B. M.P. 80-81°.

N-methyl-p-amino azobenzene was prepared by the diazotization of aniline followed by coupling with methylaniline. The product was recrystallized once from Skelly B. M.P. 179-180°.

1 azo benzene-N-phenyl-B-naphthylamine was prepared by coupling diazotized aniline with N-phenyl-B-naphthyl-amine[2]. The product was recrystallized once from Skelly B. M.P. 140-141.5°.

Tridiphenyl methane[3] was prepared by the reduction of tridiphenyl carbinol with tin and hydrochloric acid. The product was recrystallized once from chloroform. M.P. 235-237°. The tridiphenyl carbinol was previously prepared by treating p-chlorbiphenyl with diethyl carbonate and metallic sodium[4].

A 0.1 per cent solution of each of the above compounds was prepared in dry benzene and 5 drops of each solution used as the indicator.

Reagents

Reagents used were prepared as follows:

Tetrahydrofuran, which is used as the solvent for the lithium aluminum hydride was purified by double distillation over lithium aluminum hydride.

Lithium aluminum hydride Solution was prepared by refluxing an excess of the compound with purified tetrahydrofuran. This was allowed to stand overnight and then centrifuged. The clear supernatant solution was poured off and stored in a bottle fitted with a tube of Drierite and Ascarite.

Standard alcohol-benzene solution was prepared by adding dry thiophene-free benzene to dry analytical grade normal propyl alcohol. Exactly 50 ml. of the alcohol was made up to one liter in a volumetric flask. The solution was stored in an automatic burette fitted with a Drierite-Ascarite tube.

Cyclohexanol, previously dried by distilling in the presence of metallic sodium, was used as the sample in all of the determinations.

Apparatus

The apparatus used in this series of determinations is shown schematically in figure 8.

Procedure

The lithium aluminum hydride solution was pipetted into a 125 ml glass stoppered erlenmeyer flask. The dried cyclohexanol was mixed with 25.0 ml of previously dried tetrahydrofuran. The reaction mixture, which was agitated with a magnetic stirrer for 30 minutes was protected by a Drierite drying tube. Five drops of the indicator solution was added and the solution titrated with the standard alcohol-benzene solution. A definite color change that remained for 30 seconds was taken as the endpoint.

A blank determination was made with the same procedure except for the addition of the sample.

The number of gram equivalents present in the sample was calculated according to the formula:

$$\text{Number of Gram Equivalents} = \frac{(B-V)\,M}{1000}$$

where B equals the ml. of standard alcohol solution consumed by the blank.

V equals the ml. of standard alcohol consumed by the sample titration.

M equals the molarity of the standard alcohol solution used.

Results and Discussion

The first indicator tried, p-amino azobenzene, gave a color change from orange to yellow. However, this change was very gradual and the endpoint very difficult to detect. As shown in the table on page 71 the results varied and in all cases were lower than the theoretical value expected.

With benzidine a purplish brown color changing to a light brown was produced. This color change, which was reversible, was very gradual and the endpoint hard to detect.

There was no color change produced with diazoamino-benzene and diphenylamine.

An indistinct gradual color change from a dark brown to a lighter hue was produced by 1-azobenzene-N-phenyl-B-naphthylamine.

N-phenyl-p-aminoazobenzene gave a definite reversible color change from orange to yellow but the results as shown in the table were far from the theoretical value expected.

Because of the low values obtained, it was decided to substitute the N-phenyl radical with N-methyl. The reversible color change produced by the N-methyl-p-amino-azobenzene was very satisfactory in that it changed from an orange to a light yellow to orange again before permanently becoming colorless at the endpoint. The endpoint was very easy to see and the final color change was produced by one drop of the standard alochol-benzene solution. The experimental values obtained are close to the theoretical value.

After completion of the work on the azo type compounds, it was decided to try compounds of the carbonium ion type. Triphenylmethane was readily available and was the first tried. Under the conditions of this determination no color change was produced. Tridiphenylmethane was synthesized in order to obtain a more acidic molecule. Again no color change was produced. A quantity of fluorene was available and as it had a pK value in the proper range it was decided to try to follow the titration under ultra violet light. Because atmospheric oxygen affected the color change, a stream of nitrogen gas was bubbled into the reaction mixture. A color change from a dark to a light blue under the ultra violet light was observed. Although this change was reversible, it was a gradual change and unsatisfactory for detecting the endpoint.

At this time a quantity of several rubber antioxidants became available and it was decided to try them as indicators. N-N'-Di-B-naphthyl-p-phenylene diamine and N-N'-diphenyl p-phenylene diamine were used and the titrations again followed by ultra violet light. Both indicators gave a yellow color that turned to a blue. The color change was very gradual and hence unsatisfactory.

Conclusion

The best indicator developed to date, N-methyl p-amino azobenzene, gave results about 96 per cent of the theoretical. This substance exhibits a definite reversible color change that is easy to detect. Although the colorimetric method has not given as accurate results as the electrometric method, the colorimetric method shows definite promise.

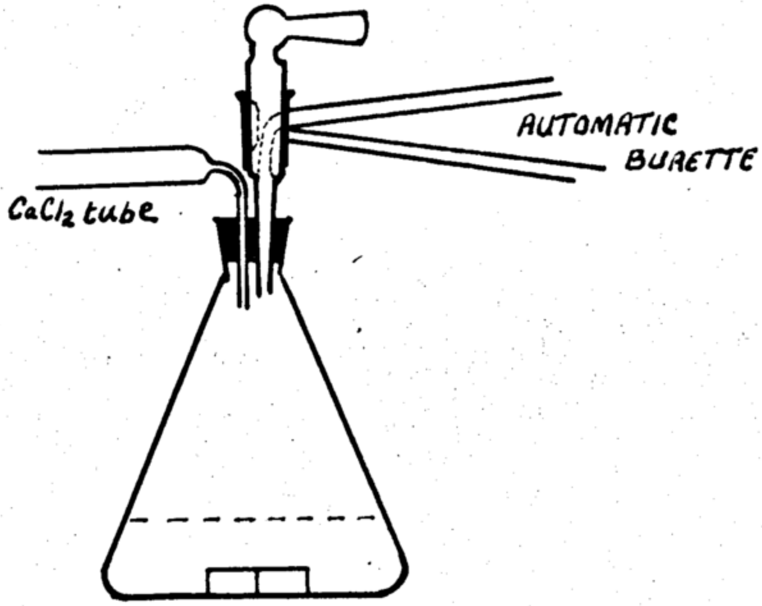

Figure 8 Apparatus Used in the Colorimetric Method

ANALYTICAL RESULTS WITH COLORIMETRIC TITRATION

Indicator	Cyclohexanol		Standard Alcohol Titration		Milli-equivalents found	Mil. equiv./moles	Theoretical M./equiv. moles
	Sample Weight	Millimoles present	Blank	Sample			
p-Amino-Azobenzene	0.3565	3.559	16.65	12.13	3.025	0.849	1.000
" "	0.3216	3.210	16.65	12.57	2.731	0.852	1.000
" "	0.3101	3.096	16.65	12.64	2.684	0.867	1.000
" "	0.3311	3.305	16.65	12.41	2.838	0.858	1.000
N-phenyl-p-amino-azobenzene	0.2293	2.289	5.15	3.20	1.305	0.570	1.000
" "	0.2448	2.444	5.15	2.90	1.506	0.617	1.000
" "	0.2284	2.280	5.97	4.22	1.724	0.514	1.000
" "	0.1727	1.724	5.97	4.56	0.9437	0.549	1.000
N-methyl-p-amino-azobenzene	0.2379	2.375	13.70	10.30	2.276	0.958	1.000
" "	0.2249	2.245	13.70	10.50	2.142	0.950	1.000
" "	0.1036	1.034	14.10	12.60	1.004	0.970	1.000
" "	0.1108	1.106	14.10	12.53	1.051	0.951	1.000

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CONCLUSION

An electrometric method based on lithium aluminum hydride for the quantitative determination of certain functional groups has been developed. This method, which was applied to various pharmaceutical compounds including alcohols, aldehydes, carboxylic acids, ketones, esters, amines, lactones, essential oils and essential oil isolates, gives good results.

In addition a colorimetric method was shown to be feasible. Although the latter method is not as accurate as the electrometric method, the colorimetric procedure shows promise.

SUMMARY

An electrometric and a colorimetric lithium aluminum hydride procedures for the quantitative determination of certain functional groups were developed. Two electrometric methods, the open system and the closed system, were used. The results of the various methods are given in tabular form in the respective sections.

The agreement between the theoretical and experimental values are as good as can be expected with the open system apparatus. The absolute errors of the open system results were of the order of a few tenths of a millimole.

The accuracy of the method was improved with the closed system. With one exception, the difference between the theoretical and experimental values was usually less than a tenth of a millimole.

The electrometric method was applied to various types of pharmaceutical compounds. The experimental values obtained agree favorably with the predicted value. The titration values of volatile oils correlated well with the predicted value based on the knowledge of the chemical composition of these oils.

Eleven potential indicators for the colorimetric method

were investigated. The best indicator tried to date, N-methyl-p-amino azobenzene, gave experimental results 96 per cent of the theoretical. This substance exhibits a definite reversible color change that is easy to detect.

FUTURE WORK

As the design of the "improved" apparatus is still relatively crude as compared to the elaborate set-up used in the Karl Fischer titration, it is hoped to further refine the apparatus.

The chemical kinetics of several reactions of lithium aluminum hydride are to be studied, as well as the determination of the amount of water of hydration in inorganic compounds.

New indicators for the colorimetric method are to be investigated.

As the application of the electrometric method to essential oils has been shown to be feasible, future plans include the study of essential oils from different localities and the development of a new quantitative index for essential oils.

The application of an amperometric method for the detection of the endpoint is scheduled for future work.