PHARMACEUTICAL ANALYSIS

Method of Analysis and Assay: Non-Aqueous Titrations

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Non-Aqueous titrations, Types of Solvents, Perchloric Acid, Sodium Methoxide

Introduction

The Bronsted Lowery theory of acid and bases can be applied equally well to reactions occurring during acid base titrations in non-aqueous solvents. This is because this approach considers an acid as any substance, which will tend to donate a proton, and a base as any substance, which will accept a proton. Substances which give poor end points due to being weak acids or bases in aqueous solution will frequently give far more satisfactory end point when titrations are carried out in non-aqueous media. An additional advantage is that many substances, which are insoluble in water, are sufficiently soluble in organic solvents to permit their titrations in these non-aqueous media.

In the Bronsted lowery theory, any acid, (HB) is considered to dissociate in solution to give a proton (H^+) and a conjugate base (B^-) :- where as any base (B) will combine with a proton to produce a conjugate acid (HB^+) :

$$HB \leftrightarrow H^+ + B^-$$
$$B+H^+ \leftrightarrow HB^+$$

The ability of substances to act as acids or bases will very much depend on the choice of solvent system. Non aqueous solvents are classified into the four groups: “aprotic, protophillic, photogenic and amphiprotic.”

Merits of Non Aqueous over Aqueous Titrations: The substances, which are either to weakly acidic or too weakly basics to give sharp end point in aqueous solutions, can easily be titrated with accuracy is non-aqueous solvent.

Advantages of Non Aqueous Solvent over Aqueous Solvent

1) Organic acids and bases that are insoluble in water are soluble in non-aqueous solvent.
2) Organic acid, which is of comparable strength to water, cannot be titrated easily non-aqueous solvent. Bases also follow the same rules.
3) A non-aqueous solvent may help two are more acids in mixture. The individual acid can give separate end point in different solvent.
4) By the proper choice of the solvents or indicator, the biological ingredients of a substance whether acidic or basic can be selectively titrated.
5) Non aqueous titrations are simple and accurate, examples of non aqueous titration are :

Ephedrine preparations, codeine phosphate in APC, tetracycline, teramycin, Anti- histamines and various piprazine preparations.

Solvents for Non Aqueous Titrations

1. Aprotic Solvents : Aprotic solvents include those substances, which may be considered chemically neutral, and virtually un-reactive under the conditions employed. Carbon tetrachloride and toluene come in this group; they possess low dielectric constants, do not cause ionization in solutes and do not undergo reactions with acids and bases. Aprotic solvents are frequently used to dilute reaction mixture.

2. Protophilic Solvents : Protophilic solvents are the substances that possess a high affinity for protons. The over all reaction can be represented as: -

$$HB+S \leftrightarrow SH^+ + B^-$$

The equilibrium in this reversible reaction will be generally influenced by the nature of the acid and the solvent. Weak acids are normally used in the presence of strongly protophilic solvents as their acidic strengths are then enhanced and then become comparable to these of strong acids; this is known as the **levelling effect.**

3. Protogenic Solvents : Protogenic solvents are acidic in nature and readily donate protons. Anhydrous acids such as hydrogen fluoride and sulphuric acid fall in this category, because of their strength and ability to donate protons, they enhance the strength of weak bases.

4. Amphiprotic Solvents : Amphiprotic solvents consist of liquids, such as water, alcohols and weak organic acids, which are slightly ionized and combine both protogenic and protophillic properties in being able to donate protons and accept protons ethanoic acid displays acidic properties in dissociating to produce protons:

$$CH_3COOH \Longleftrightarrow CH_3COO^- + H^+$$

But in the presence of perchloric acid, a far stronger acid, it will accept a proton:

$$CH_3COOH + HClO_4 \leftrightarrow CH_3COOH_2^+ + ClO_4^-$$

The $CH_3COOH_2^+$ ion can very readily give up its proton to react with a base, so basic properties of a base is enhanced, so titrations between weak base and perchloric acid can often be accurately carried out using ethanoic acid as solvent.

5. Levelling Solvents: In general, strongly protophilic solvents are important to force equilibrium equation to the right. This effect is so powerful that, in strongly protophillic solvents, all acids act as of similar strength. The converse occurs with strongly protogenic solvents, which cause all bases to act as they were of similar strength. Solvents, which act in this way, are known as **Leveling Solvents.**

Differential Titrations

Determination in non-aqueous solvents is important for substances which may give poor end points in normal aqueous titration and for substances which are not soluble in water. They are particularly valuable for determining the properties of individual components in mixtures of acids or mixture of bases. These differential titrations are carried out in solvents, which do not exert a leveling effect.

Applications: Although indicators may be used to establish individual end points, as in traditional acid-base titrations, potentiometric methods of end point detection are also used extensively, especially for highly coloured solutions. Non aqueous titration have been used to quantify the mixtures of primary, secondary and tertiary amines, for studying sulphonamides, mixture of purines and for many other organic amino compounds and salts of organic acid.

Do Not Use A Solvent Until You Are Fully Acquainted With Its Hazards And How To Use It Safely.

Some Examples of Non-Aqueous Solvents

A very large number of inorganic solvents have been used for non-aqueous titrations, but a few have been used more frequently than others. Some of the most widely applied solvents systems are discussed below. In all instances pure, dry analytical reagent quality solvent should be used to assist in obtaining sharp end points.

Glacial Ethanoic Acid : Glacial ethanoic acid is the most frequently used non-aqueous solvent. Before it is used it is advisable to check the water content. This may be between 0.1% and 1.0%.

Acetonitrile: Acetonitrile (methyl cyanide, cyanomethane) is frequently used with other solvents such as chloroform and phenol and especially with ethanoic acid. It enables very sharp end points to be obtained in the titration of metal ethanoates when titrated with perchloric acid.

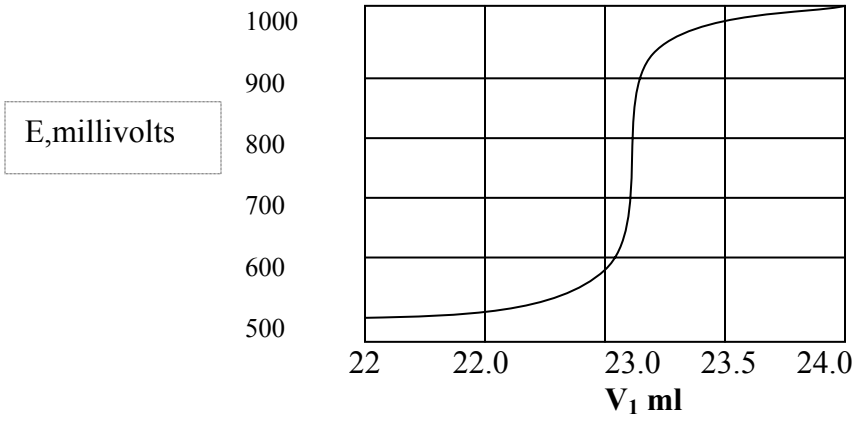
Alcohol : Salt of organic acids, especially of soaps are best determined in mixtures of glycols and alcohols or mixtures of glycols and hydrocarbons. The most common combinations are ethylene glycol (dihydroxyethane) with propan-2-ol or butan-1-ol. The combinations provide admirable solvents for both the polar and non-polar ends of the molecules.

Dioxane : Dioxane is another popular solvent, which is often used in place of glacial ethanoic acid when mixtures of substances are to be quantified. Unlike ethanoic acid, dioxane is not a leveling solvent and separate end points are normally possible, corresponding to the individual components in the mixtures.

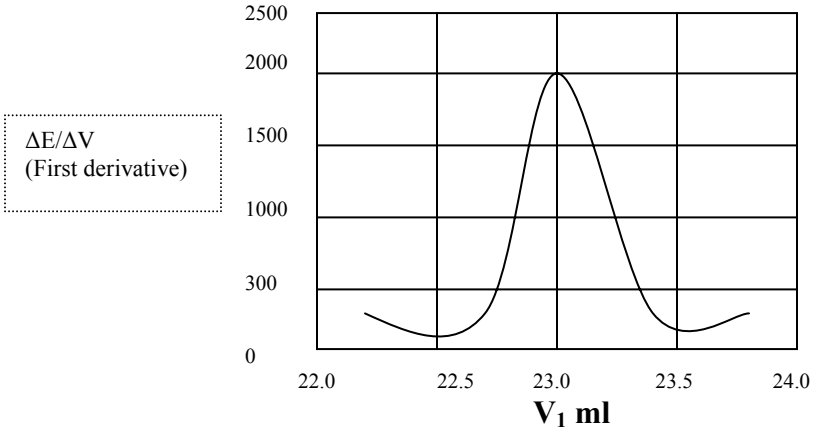
Dimethylformamide: Dimethylformamide (DMF) is a protophillic solvent, which is frequently employed for titrations between, for instance, benzoic acid and amides, although end points may sometimes be difficult to obtain.

Indicators for Non-Aqueous Titrations

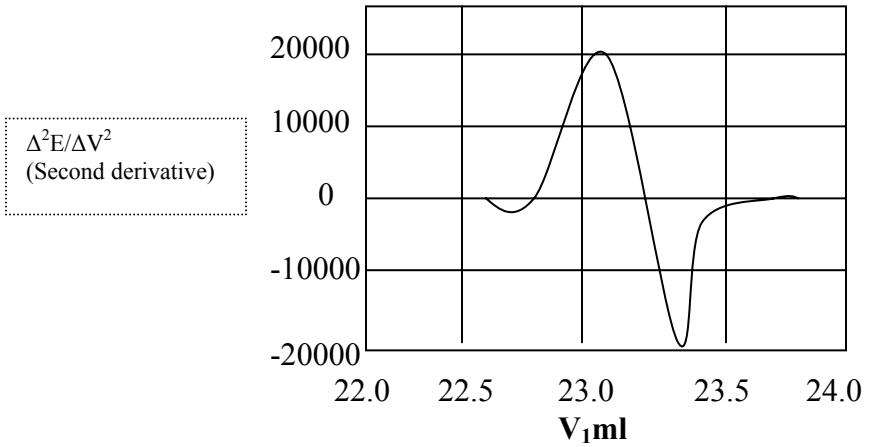
The ionized and unionized or the different resonant forms of indicators are apply equally well for non-aqueous titrations but their colour changes at the end point vary from titration to titrations, as they depend on the nature of the titrant. The colour corresponding to the correct end point may be established by carrying out a potentiometric titration while simultaneously observing the colour change of the indicator. The appropriate colour corresponds to the inflexion point of the titration curve.



a) The part of the experimental titration curve in the vicinity of the equivalence point.



b) The first derivative curve i.e. the slope of the titration curve as a function of V.



c) The second derivative curve i.e. the slope of curve (b) as a function of V.

The majority of non-aqueous titrations are carried out using a fairly limited range of indicators here are some typical example.

1. Crystal Violet: Used as 0.5% w/v solution in glacial acetic acid. Its colour change is from violet through blue followed by green, then to greenish yellow, in reactions in which bases such as pyridine are titrated with perchloric acid.

2. Methyl Red: Used as a 0.2% w/v solution in dioxane with a yellow to red colour change.

3. Naphthol Benzein: When employed as a 0.2% w/v solution in ethanoic acid gives a yellow to green colour change. It gives sharp end points in nitro methane containing ethanoic anhydride for titration of weak bases against perchloric acid.

4. Quenaldine Red : Used as an indicator for drug determinations in dimethylformamide solution. A 0.1% w/v solution in ethanol gives a colour change from purple red to pale green.

5. Thymol Blue: Used extensively as an indicator for titrations of substances acting as acids in dimethyl formamide solution. A 0.2% w/v solution in methanol gives a sharp colour change from yellow to blue at the end point.

Acidimetry in Non-Aqueous Titrations

In order to perform feasible titrations of weak bases, the solvent system should be selected specifically in such a fashion so as to eliminate as far as possible the competing reaction of water for the proton besides enhancing the strength of the basic species.

Titration of Weak Bases by Non Aqueous Titration

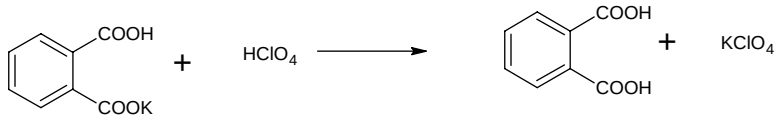
Following points should be considered: -

1. Titrant used.
2. Preparation of 0.1N ($HClO_4$) and it standardization.
3. Solvent used.
4. Practical examples of weak bases along with indicators.
5. Typical example of assay of weakly basic substance e.g. ephedrine HCl.

Titrant used: Solution of $HClO_4$ in either glacial acetic acid or dioxane solution is used for titration of weak bases. Generally $HClO_4$ with a normality of 0.1N to 0.05N is used.

Preparation of 0.1N solution of $HClO_4$ and its standardization: Dissolve 8.5 ml of 72% $HClO_4$ in about 900 ml glacial acetic acid with constant stirring, add about 30 ml acetic anhydride and make up the volume (1000 ml) with glacial acetic acid and keep the mixture for 24 hour. Acetic anhydride absorbed all the water from $HClO_4$ and glacial acetic acid and renders the solution virtually anhydrous. $HClO_4$ must be well diluted with glacial acetic acid before adding acetic anhydride because reaction between $HClO_4$ and acetic anhydride is explosive.

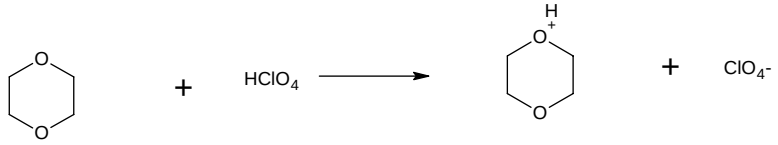
1. Standardization of the above prepared 0.1N $HClO_4$ with A.R. Grade Potassium acid Phthalate.



1 ml of 0.1N $HClO_4$ = 0.020414 gms of potassium acid Phthalate.

To 500 mg of potassium acid phthalate add 25 ml of glacial acetic acid and add few drops of 5% w/v crystal violet in glacial acetic acid as indicator. This solution is titrated with 0.1 $HClO_4$. The colour changes from blue to blue green.

2. Solution in $HClO_4$ in dioxane may be the 2nd titrant, which could be used. It is standardize in the same manner as acetous perchloric acid. High quality dioxane must be used otherwise titrant will become dark. Dioxane can be purified by passing resin or by shaking it with asbestos and then filter it.



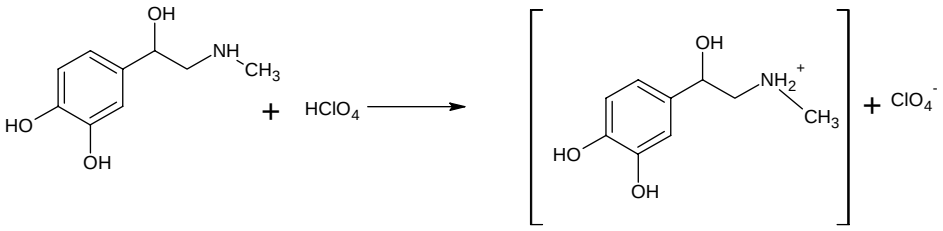
Solvent used : Glacial acetic acid alone or sometimes in combination with some aprotic solvents is often used. The other solvents are $CHCl_3$, benzene, chloro benzene, acetic anhydride and various combinations of these sometime glycohydrocarbon mixtures are also used.

Indicators used: Crystal violet 0.05% w/v in glacial acetic acid, methyl red 0.1% w/v in anhydrous methanol, oracet blue 0.5% w/v in glacial acetic acid.

Practical Examples of Weak Bases: It includes adrenaline acid tartarate, erythromycin strerate, metronidazol tartrate methyldopa, noradrenaline, orphenadrine citrate, prochlorperazine maleate etc.

Asssay of Adrenaline: In general, the reaction-taking place between a primary amine and perchloric acid may be expressed as follows:

$$R.NH_2 + HClO_4 \rightarrow [R.NH_3]^+ + ClO_4$$



Hence, 183.2 g of $C_9H_{13}NO_3 \equiv HClO_4 \equiv H \equiv 1000$ ml N

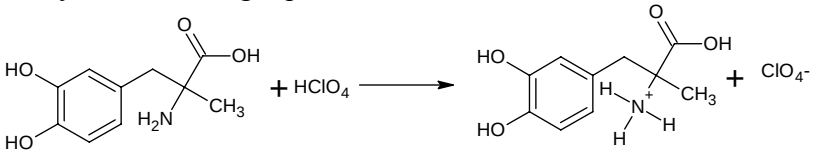
or 0.01832 g of $C_9H_{13}NO_3 \equiv 1$ ml of 0.1 N $HClO_4$

Procedure: Weigh accurately about 0.3 g of sample into a 250 ml conical flask; add Glacial acetic acid (50 ml), warm gently, if necessary. Cool and titrate with 0.1 N perchloric acid using crystal violet or oracet blue B as indicator.

Calculations: The percentage of adrenaline present in the sample is given by:

$$\%\text{Adrenaline} = \frac{\text{X ml} \times \text{Normality (Calculated)} \times 0.01832 \times 100}{\text{N(Given)} \times \text{Wt. of sample(in gm)}}$$

Assay of Methlyldopa: The specific reaction between methyldopa and perchloric acid is expressed by the following equation:



Hence, 211.24 g of $C_{10}H_{13}NO_4 \equiv HClO_4 \equiv H \equiv 1000$ ml N

Or 0.02112 g of $C_{10}H_{13}NO_4 \equiv 1$ ml of 0.1 N $HClO_4$

Material Required: Methyldopa 0.2 g ; anhydrous formic acid : 15 ml ; glacial acetic acid : 30 ml ; dioxane : 30 ml ; 0.1 N perchloric acid and crystal violet solution.

Procedure: Weigh accurately about 0.2 g of sample and dissolve in a mixture of 15 ml of anhydrous formic acid, 30 ml of glacial acetic acid and 30 ml of dioxane. Add 0.1 ml of crystal violet solution and titrate with 0.1 N perchloric acid. Perform a blank determination and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 0.02112 g of $C_{10}H_{13}NO_4$.

Calculations: The percentage of methyldopa present in the sample is given by :

$$\% \text{ Methyldopa} = \frac{\text{X ml} \times \text{Normality (Calculated)} \times 0.02112 \times 100}{\text{N(Given)} \times \text{Wt. of sample(in gm)}}$$

Cognate Assays: Following table enlists the various cognate determinations using different indicators but employing the same titrant i.e., 0.1 N perchloric acid:

S.No.	Name of Substance	Indicator Employed
1.	Adrenaline	Crystal violet
2.	Bisacodyl	α-Naphthol benzein
3.	Pyrimethamine	Quinaldine red
4.	Ergometrine maleate	Crystal violet
5.	Ethambutal hydrochloride	-do-
6.	Guanethidine sulphate	-do-
7.	Isoprenaline sulphate	-do-
8.	Levodopa	Oracet Blue-B
9.	Mepyramine maleate	Crystal violet
10.	Metronidazole	α -Naphtol benzein
11.	Metronidazole benzoate	Brilliant green
12.	Quinidine sulphate	Crystal violet
13.	Salbutamol sulphate	Oracet Blue-B

Titration of Halogen Acid Salts of Bases

In general, the halide ions, namely: chloride, bromide and iodide are very weakly basic in character so much so that they cannot react quantitatively with acetous perchloric acid. In order to overcome this problem, mercuric acetate is usually added (it remains undissociated in acetic acid solution) to a halide salt thereby causing the replacement of halide ion by an equivalent amount of acetate ion, which serves as a strong base in acetic acid as shown below:

$$2R.NH_2.HCl \quad \leftrightarrow \quad 2RNH_3^{+} + 2Cl^{-}$$

$$(CH_3COO)_2 Hg + 2Cl^{-} \rightarrow \quad HgCl_2 + 2CH_3COO^{-}$$

undissociated undissociated

$$2CH_3COOH_2^{+} + 2CH_3COO^{-} \leftrightarrow \quad 4\ CH_3\ COOH$$

Assay of Amitriptyline Hydrochloride:
Materials Required: Amitriptyline hydrochloride : 1.0 g ; mercuric acetate ; crystal violet; 0.1 N perchloric acid ; glacial acetic acid.

Procedure: Weigh accurately about 1.0 g of sample and dissolve it in 50 ml of glacial acetic acid, warm slightly, if necessary, to affect the solution. Cool, add 10 ml of mercuric acetate solution, two drops of crystal violet solution and titrate with 0.1 N perchloric acid to a green end-point. Perform a blank determination and make any necessary correction.

Each ml of 0.1 N perchloric acid is equivalent to 0.03379 g of $C_{20}H_{31}ON.\ HCl$.

Equations:

$$2\ C_{20}H_{31}ON.\ HCl \leftrightarrow 2\ C_{20}H_{31}NOH^{+} + 2Cl^{-}$$

$$(CH_3COOH)_2\ Hg + 2Cl^{-} \rightarrow HgCl_2 + 2CH_3COO^{-}$$

$$2\ CH_3COOH_2^{+} + 2CH_3COO^{-} \leftrightarrow 4CH_3COOH$$

Calculations:

$$C_{20}H_{31}ON.\ HCl \equiv Cl^{-} \equiv CH_3COO^{-} \equiv HClO_4 \equiv H \equiv 1000\ ml\ N$$

or $337.9\ g\ C_{20}H_{31}ON.\ HCl \equiv 1000\ ml\ N\ HClO_4$

or $0.03379\ g\ C_{20}H_{31}ON.\ HCl \equiv 1\ ml\ of\ 0.1\ N\ HClO_4$

Assay of Methacholine Chloride:
Materials Required: Methacholine chloride: 0.4 g; glacial acetic acid: 50 ml; mercuric acetate solution: 10 ml; 0.1 N perchloric acid and crystal violet solution.

Procedure: Weigh accurately about 0.4 g, previously dried and stored in a vacuum desiccator, and dissolve in 50 ml of glacial acetic acid, add 10 ml of mercuric acetate solution, one drop of crystal violet solution and titrated with 0.1 N perchloric acid to a blue-green end point. Perform a blank determination and make any necessary correction.

Each ml of 0.1 N perchloric acid is equivalent to 0.01957 g of $C_8H_{18}ClNO_2$.

Equation:

$$\mathbf{2\ [CH_3COOCH(CH_3)CH_2N^{+}(CH_3)_3].2Cl^{-} + Hg(OOCCH_3)_2 + 2HClO_4 \rightarrow}$$

$$\mathbf{2\ [CH_3COOCH(CH_3)CH_2N^{+}(CH_3)_3].2ClO_4^{-} + 2HOOCCH_3 + HgCl_2}$$

Mercuric acetate*:* It is essentially added to prevent the interference of the hydrochloric acid, which is displaced through the formation of the relatively un-ionized $HgCl_2$, thereby making a predominant shift in the equilibrium so that the titrimetric reaction is quantitative.

Blank Titration: It is usually carried out to account for the possible reaction of atmospheric moisture with the titrant perchloric acid and also to check the titrant being employed to bring about the blue-green end-point.

Calculations: The percentage of methacholine chloride in the sample may be calculated by the following expression:

$$\% \text{ Methacholine chloride} = \frac{\text{X ml} \times \text{Normality (Calculated)} \times 0.01957 \times 100}{\text{N(Given)} \times \text{Wt. of sample(in gm)}}$$

Cognate Assays: The following estimation of various pharmaceutical substances can also be carried out by the aforesaid procedure.

Table: Acidimetric Assays: Non-aqueous Titrations with Perchloric Acid using Mercuric Acetate and different Indicators

S.No.	Name of Substance	Indicator Employed
1.	Amantadine hydrochloride	Crystal violet
2.	Chlorpromazine hydrochloride	Methyl orange
3.	Clonidine hydrochloride	α -Naphthol benzein
4.	Cyproheptadiene.HCl	Crystal violet
5.	Dehydroemetine.HCl	-do-
6.	Ephedrine hydrochloride	-do-
7.	Imipramine hydrochloride	-do-
8.	Isoprenaline hydrochloride	Crystal violet
9.	Lignocaine hydrochloride	-do-
10.	Morphine hydrochloride	-do-
11.	Morphine sulphate	-do-
12.	Phenylephrine hydrochloride	-do-
13.	Phenytoin sodium	α -Naphthol benzein
14.	Promethazine hydrochloride	Methyl orange
15.	Thiabendazole	Crystal violet

Alkalimetry in Non-Aqueous Titrations

A plethora of weakly acidic pharmaceutical substances may be titrated effectively by making use of a suitable non-aqueous solvent with a sharp end-point. The wide spectrum of such organic compounds include: anhydride, acids, amino acids, acid halides, enols (viz., barbiturates), xanthenes, sulphonamides, phenols, imides and lastly the organic salts of inorganic acids.

However, a weak inorganic acid e.g., boric acid, can be estimated conveniently employing ethylenediamine as the non-aqueous solvent.

Preparation of 0.1 N Potassium Methoxide in Toluene-Methanol:
Material Required: Absolute methanol, dry toluene, Potassium metal.
Procedure: Add into a dry flask, a mixture of methanol (40 ml) and dry toluene (50 ml) and cover it loosely. Carefully add freshly cut pieces of potassium metal (5.6 gm) to the above mixture gradually with constant shaking. After complete dissolution of potassium metal, add enough absolute methanol to yield a clear solution. Toluene 50 ml is added with constant shaking until the mixture turns hazy in appearance. The process is repeated by the alternate addition of methanol and benzene until 1 litre of solution is obtained, taking care to add a minimum volume of methanol to give a visible clear solution.

Preparation of 0.1 N Sodium Methoxide: It is prepared exactly in a similar manner as for 0.1 N Potassium Methoxide, using 2.3g of freshly cut sodium in place of potassium.

Preparation of 0.1 N Lithium Methoxide: It is prepared as for 0.1 N Potassium Methoxide, but using 0.7 g of lithium in place of potassium.

Standardization of 0.1 N Methoxide Solution
Material Required: Dimethylformamide (DMF): 10 ml; thymol blue (0.3% in MeOH); 0.1 N lithium methoxide in toluene methanol; benzoic acid: 0.6 g.

Procedure:. Transfer 10 ml of DMF in a conical flask and add to it 3 to 4 drops of thymol blue and first neutralize the acidic impurities present in DMF by titrating with 0.1 N lithium methoxide in toluene-methanol. Quickly introduce 0.06g of benzoic acid and titrate immediately with methoxide in toluene-methanol.

Caution: Care must be taken to avoid contamination of neutralized liquid with atmospheric carbon dioxide.

Equations: The various equations involved in the above operations are summarized as stated below:

(i) $Na + CH_3OH \rightarrow CH_3ONa + H^{\uparrow}$

Interaction between sodium metal and methanol is an exothermic reaction and hence, special care must be taken while adding the metal into the dry solvent in small lots at intervals with adequate cooling so as to keep the reaction well under control.

(ii) $H_2O + CH_3ONa \rightarrow CH_3OH + NaOH$
$H_2CO_3 + 2CH_3ONa \rightarrow 2CH_3OH + Na_2CO_3$

The clear solution of sodium methoxide must be kept away from moisture and atmospheric CO_2 as far as possible so as to avoid the above two chemical reactions that might ultimately result into the formation of turbidity.

(iii) $C_6H_5COOH + H\text{—}CON(CH_3)_2 \leftrightarrow HCON^{+}H(CH_3)_2 + C_6H_5COO^{-}$ ------1
DMF

$CH_3ONa \leftrightarrow CH_3O^{-} + Na^{+}$ ----------2

$$HCON^{+}H(CH_3)_2 + CH_3O^{-} \rightarrow HCON(CH_3)_2 + CH_3OH \text{ ----------3}$$

Summing up: $C_6H_5COOH + CH_3ONa \rightarrow C_6H_5COONa + CH_3OH$

Step 1: It shows the solution of benzoic acid (primary standard) in DMF,
Step 2: It depicts ionization of sodium methoxide,
Step 3: It illustrates the interaction between the solvated proton and the methylated ion.

In summing up, the net reaction between the water in the solvent (DMF) and the titrant is equivalent to the volume of sodium methoxide consumed by DMF or may be considered as a blank determination.

N/10 KOH in Methanol
Dissolve 5.6 gm of anhydrous KOH in 1000 ml of anhydrous methanol. This titrant is not as powerful as others. Its main disadvantage is that it reacts with acidic functional groups and produces a molecule of water, which would affect the sensitivity of titration.

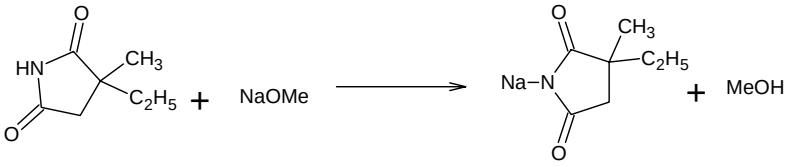
Standardisation : All these titrants are usually standardized against standard benzoic acid AR-Grade. A sufficient amount of benzoic acid which would give a titrate value of 20-30 ml is transferred in a dry flask and dissolved in 25 ml dimethylformamide, 2-3 drops of 0.5% thymol blue indicator in dry methanol is added to the solution. A blank titration is also per formed in the solvent to account acidic impurity in dimethylformamide and the correction is made accordingly.

Assay of Ethosuximide
Materials Required: Ethosuximide: 0.2 g ; dimethylformamide : 50 ml ; azo-violet (0.1 % w/v in DMF) : 2 drops ; sodium methoxide 0.1 N.

Procedure: Weigh accurately about 0.2 g of the sample, dissolve in 50 ml of dimethylformamide, add 2 drops of azo-violet solution and titrate with 0.1 N sodium methoxide to a deep blue end point, taking precautions to prevent absorption of atmospheric carbon dioxide. Perform a blank determination and make any necessary correction.

Each ml of 0.1 N sodium methoxide is equivalent to 0.01412 g of $C_7H_{11}NO_2$.

Equations:



Calculations:
Therefore, 141.17 g $C_7H_{11}NO_2 \equiv NaOMe \equiv H \equiv$ 1000 ml N

0.01417 g $C_7H_{11}NO_2 \equiv$ 1 ml 0.1 N NaOMe

Cognate Assays : The following determinations as stated in the table may be carried out effectively by using 0.1 N sodium methoxide using appropriate indicator or potentiometrically:

Table: Alkalimetric Assays: Non-Aqueous Titrations using Lithium Methoxide/Sodium Methoxide either Potentiometrically or Titrimetrically

S.No.	Name of Substance	Indicator Employed
1.	Acetazolamide	Potentiometric determination
2.	Bendrofluazide	Azo violet
3.	Allopurinol	Thymol blue
4.	Mercaptopurine	-do-
5.	Amylobarbitone	Quinaldine Red
6.	Nalidixic acid	Thymolphthalein

Tetrabutylammonium Hydroxide
The alkalimetry in non-aqueous titrations may also be carried out efficiently by using tetrabutylammonium hydroxide along with an appropriate indicator.

Preparation of 0.1 N Tetrabutylammonium Hydroxide in Toluene-Methanol
Materials Required: Tetrabutylammonium iodide: 40 g; absolute methanol: 90 ml; silver oxide: 25 g; dry toluene: 150 ml.

Procedure: Carefully dissolve 40 g of tetrabutylammonium iodide (Bu_4 NI) in 90 ml of absolute methanol, add to it 20 g of finely powdered purified silver oxide and finally shake the mixture thoroughly for 1 hour. Centrifuge about 2-3 ml of the resultant mixture and test for iodide in the supernatant liquid. In case, it gives a positive test, add about 2 g more of silver oxide and shake for an additional period of 30 minutes. The said method may be repeated until the supernatant liquid obtained is completely free from iodide. The mixture thus obtained is filtered through a fine sintered glass filter and finally rinse the container with 3 portions, each of 50 ml of dry toluene. These washings may be added to the filtrate and the final volume is made upto 1 litre with dry toluene. The clear solution should be kept duly protected from both CO_2 and moisture during storage.

Equation:

$$2Bu_4NI + Ag_2O + H_2O \rightarrow 2Bu_4NOH + 2AgI$$

Tetrabutyl-Ammonium Iodide — Tetrabutyl ammonium hydroxide

List or Applications
Substances that can be titrated by non-aqueous are acid halide, anhydrides, weak acids like amino acids enols like barbiturate, xanthans, phenols, pyrroles, sulphonamide, and organic salt of in organic acid etc.

Precautions : Following points should be considered:-
Moisture and CO_2 have to be excluded, water being weakly basic would compete with perchloric acid and sharpness of end point would be lost, therefore, moisture contents should be less that

0.05%. The presence of CO_2 affects basic solvent like Dimethyleformamide, ethylene diamine and pyridine as they adsorb CO_2 from air.

- ✓ During preparation of Perchloric acid it must be well diluted with acetic acid before adding the acetic anhydride to prevent the formation of explosive acetyl per chlorate.
- ✓ Do not use a solvent until fully acquainted with its hazards and how to use it safely.

Suggested Readings:

1. K A Conners, A Text Book of Pharmaceutical Analysis, Wiley-Intersciences, New York.
2. A H Backett and J B Stenlake, Practical Pharmaceutical Chemistry, Vol.I and II, The Athlone Press of The University of London.
3. Pharmacopoeia of India, Govt.of India, Ministry of Health, Delhi.
4. J Bassett, R C Denney, G H Jeffery, J Mendham, Vogel's Textbook of Quantitative Inorganic Analysis, The ELBS and Longman, London.