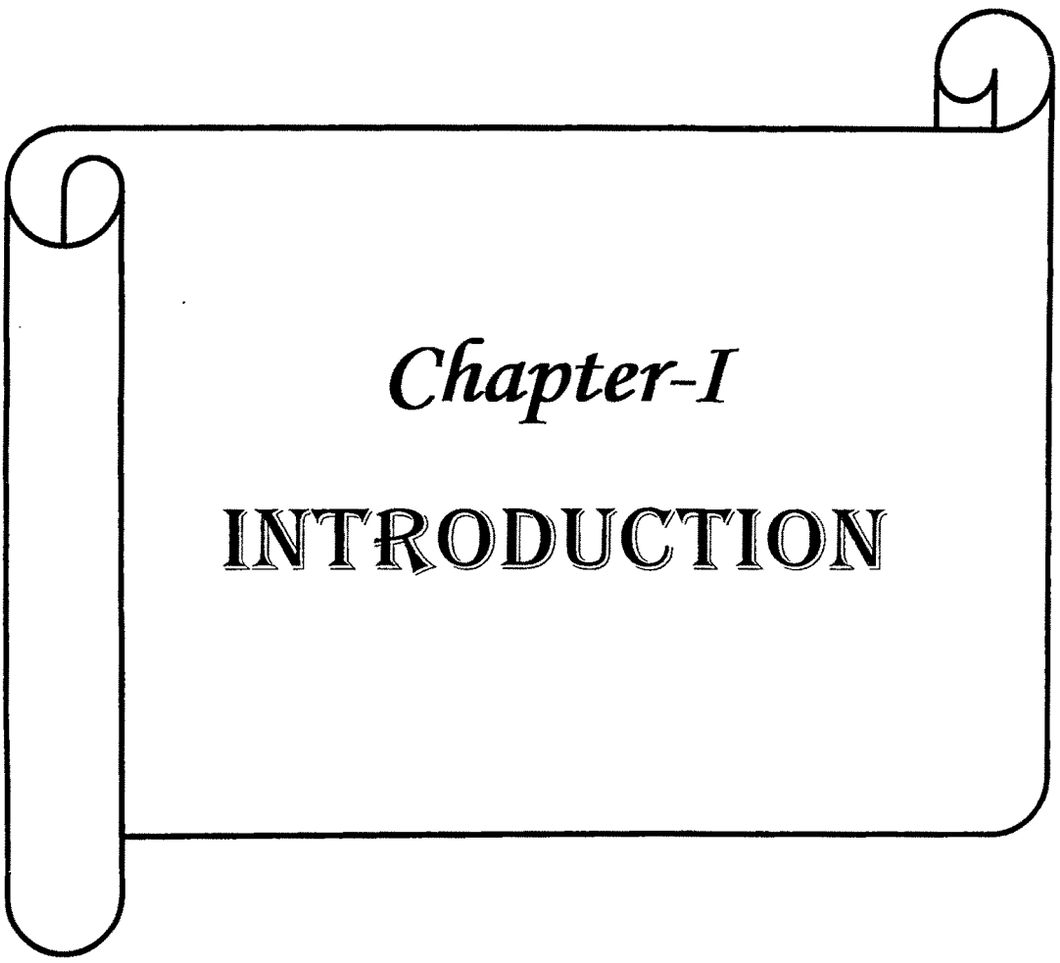

Chapter-I

INTRODUCTION

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INTRODUCTION

Analytical Chemistry may be defined as the science and art of resolution of a sample of matter into its proximate or ultimate part; the determination of its elements or compounds or of the foreign substances. It may contain. The goal of chemical analysis is to provide information about the composition of a sample of matter. In qualitative analysis, information regarding the presence or absence of one or more components of the sample suffice whereas in quantitative analysis the question is: How much of each component or of specified component, is present.

Analysis of organic compounds via the reaction of their functional groups are more fundamental than elemental analysis, because their characteristic properties arise from the functional groups which they contain. A functional group in an organic compound is defined as the atom or group of atoms, including the structural feature like a carbon-carbon double/triple bond, which determine the characteristic properties of an organic compound. Functional group analysis is used to solve various chemical problems like assignment of structure and characteristics of organic compounds, estimation of individual organic compound in a test sample, determination

of trace impurities present in pharmaceutical preparations, natural and industrial products and in analyzing the biochemical samples. It is functional group which imparts particular chemical characteristics to an organic compound. The basis of all quantitative procedures for the estimation of an organic compound/functional group, is the determination of substance formed or consumed by the reaction of the sample with a reagent. Analytical methods are ordinarlily classified according to the property that is observed in the final measurement process. All the methods which are based upon the measurement of mass (gravimetric) and volume (volumetric) are known as 'Classical' or chemical methods of analysis. And the methods which are based upon the measurement of physical property of a substance are known as instrumental analysis.

In gravimetric analysis the substance being determined is converted into an insoluble precipitate, this is collected and dry weighed. In electrogravimetric, electrolysis is carried out and the material deposited on one of the electrodes is weighed.

In titrimetric analysis the substance to be determined, this allowed to react with an appropriate reagent added as standard solution and the volume of soln needed for complete reaction is determined. The common types of reaction which find use in titrimetric are neautralisation reactions, complex forming

reactioons, precipitate forming reactions, oxidation-reduction reactions. Volumetry is concerned with measuring the volume of gas evolved or absorbed in a chemical reaction.

The instrumental methods involve mainly electrical and electronic instruments, they involve measurement of current, voltage or resistance in relation to the concentration of certain species and solution this include voltametry, coulometry, potentiometry and conductometry. Optical methods depend upon the absorption of radiant energy and the measurement of the amount of energy of a particular wave length absorved by the sample or the emission of radiant energy or the measurement of the amount of energy of a particular wave length emitted. Absorption methods are classified as visible, ultraviolet and infra-red spectrophotometry. Atomic absorption spectroscopy involves vapourising the specimen into a flame and then studying the absorption of radiation from an electric lamp producing the spectrum of the element to be determined. The turbidimetry and nephalometry involve measuring the amount of light stopped or scattered. Emission methods involves emission spectrography where the sample is subjected to an electric are and the light emitted is determined. Flame photometry and fluorimetry are similar techniques. X-ray

fluorescence, radioactivity and kinetic methods are also in practice.

On the basis of sample size analytical methods are often classified as:

Macro-dealing with quantities ranging o.1 g - 0.5 g Semi-macro-dealing with quantities ranging

10-50mg ≡ 0.01 to 0.05 g.

Micro-dealing with quantities raning

1-5mg ≡ 0.001-0.005g

Ultra-micro-dealing with quantities

≈100μg = 0.1 mg ≡ 0.0001 g

The term semi-micro is not very apt, referring as it does to quantities larger than micro and it has been proposed that it should be replaced by the term meso. A major constituent is one present in excess of 1 per cent, a minor constituent is present from 0.01 to 1 percent of the sample and a trace constituent is present to an extent of less than 0.01 per cednt of the sample. Using gram as standard unit of weight and its sub-multiples are 1 mg = 10^{-3} g, 1 mcg (microgram)= 10^{-6}g and 1 ng (naogra)= 10^{-9} g. Similarly 1 ml = 10^{-3}L, 1 μL (microlitre) = 10^{-6}L.

The semi- micro, micro and ultra -micro methods of analysis possesses advantage of being economical, fast, accurate and convenient. These methods employ very small

amount of sample, have considerable importance for biochemical analysis and for the study of substances available in very minute quantities as in drugs and other physiologically active substances.

Whatever the method finally chosen for the required determination, it should ideally be a specific method; that is to say, it should be capable of measuring the amount of desired substances accurately. In practice few analytical procedures attain this ideal but many methods are selective, i.e. we can determine one sample in the presence of others. In many instances the desired selectivity is achieved by carrying out the procedure under carefully controlled conditions, particularly with reference to the pH of the solution.

Frequently, however, there are substances present that prevent direct measurement of the amount of a given substances, these are referred to as inferences. The interferences should be separated by suitable techniques.

The function of the analyst is to obtain a result as near to the true value as possible by the correct application of the analytical procedure employed. Statistical methods are used to demonstrate the degree of variability of the results obtained.

Accuracy of a determination may be defined as the concordance between it and the true or most probable value.

Precision may be defined as the concordance of a series of measurement of the same quantity. The mean deviation or the relative mean deviation is a measure of precision. In quantitative analysis the precision of measurements rarely exceeds 1-2 parts per thousands.

Accuracy express the correctness of a measurement and precision the reproducibility of a measurement. Precision always accompanies accuracy but a higher degree of precision does not imply accuracy.

When it is found that the difference in the results of successive determination is small in some cases or large in other cases. The reliability of the result depends upon the magnitude of this difference. It is therefore of interest to enquire briefly into the factors which affect and control the trustworthiness of chemical analysis.

Absolute error of a determination is the difference between the observed or measured value and the true or most probable value of the quantity measured. The absolute error is a measure of the accuracy of the measurements.

Relative error is the absolute error divided by the true or most probable value; it is usually expressed in terms of percentage or in parts per thousand.

The agreement between a series of results is measured by computing their **Mean deviation**. This is evaluated by determining the arithmetical mean of the results then calculating the deviation of each individual measurement from the mean, and finally dividing the sum of the deviations, regardless of sign, by the number of measurements. **Relative mean deviation** is the mean deviation divided by the mean.

This may be expressed in terms of percentage or in parts per thousand.

In analytical chemistry one of the most common statistical terms employed is the **Standard deviation** of a population of observation. This is also called the **Root mean square deviations** as it is the square root of the mean of the sum of the square of the difference between the values and the mean of those values.

The square of standard deviation is called the Variance. A more accurate measure of the precision/relative dispersion known as the coefficient of variation, is given by

$$\text{C.V.} = \frac{S.D. \times 100}{\bar{X}}$$

Where S.D.= standard deviation and $\bar{X}$ = Average or mean value.

Micro analysis is of immense use in the assay of drugs, dyes, paints, natural products, explosives, agricultural chemicals (insecticides, weedicides, soil conditioners) etc. The importance of micro analysis in related scientific areas can be ilustrated by considering its impact on clinical analysis (blood and urine samples) and quality control of pharmaceutical preparations and industrial products.

Drugs are chemical compounds, which exert various physiological effects of therapeutic value. Drugs are used as chemotherapeutic agents in treatment and cure of parasitic disease in vio by direct chemical attack upon the causative invader viruses, fungi, yeast, bacteria, protozoa, heliminths, and other pathogenic organisms, It has been found that a given chemical compound (drug) is specific in its toxicity towards microorganism and it in some way incapacitates a pathogenic invading organism with a minimum effect upon the tissue and physiological process of the host.

Drugs are presented in the form of pharmaceutical formulations. A pharmaceutical formulation may contain a single entity of high purity or a physical mixture of several potent drugs. With the growth of pharmaceutical industry during the last several years, manufacturing trend has been to manufacture more and more complex formulations containing

several drugs with their similar chemical behaviour. In the absence of suitable and simple methods of analysis for complex formulations, the complete analysis may be impractical. Further, due to great variability of formulations to be analysed, proper sampling is extremely important for meaningful results. One should ensure that the selected portion of the sample is the true representative of the whole lot/batch or container. The method to be followed for sampling different types of dosage formulations is as under:

(a) Liquid:

The sample is mixed thoroughly by inverting the container several times. The viscous samples should be mixed for a longer time. One should ensure that sediments are properly dispersed in the liquid before sample is drawn for analysis.

(b)Powder:

The sample should be mixed thoroughly before a portion is taken for analysis.

(c) Tablets:

For reliable assay results, certain number of tablets say 20 tablets, reduced to a fine powder, weighed accurately for estimation and contents, calculated on the basis of average weight.

(d) Capsules:

About 20 capsules are weighed into a small beaker. In case of dry filled capsules, material adhering to the shells is cleaned with cotton. In case of oily capsules, like capsules of Vitamin A and D, sheels are washed with suitable solvent until free from oil. The shells are weighed accurately after drying. The net weight of the contents is obtained by subtracting the weight of shells from the total weight.

CHEMICALS AND REAGENTS:

Reliability of the results partly depends upon the quality of the reagents used in assay procedures. All chemicals required during analysis are of the purest grade (equivalent to Anala R, BDH). The analyst should satisfy himself that the impurities capable of disturbing the accuracy of the method are not present. Special reagents are described under individual method; for common reagents, the analyst may refer to official pharmacopocias.

REFERENCE SUBSTANCES/STANDARDS:

Reference substances are authentic speciments, which have been verified for use as standard for comparion. These reference standards may be obtained from Central Drugs Labouratory, Calcutta, India, British Pharmacopoeia Commission Laboratory, U.K. and Hubert Lando International

Inc., N.Y., USA. However, taking into consideration, the quanitity normally supplied by these repositories and difficulties encountered in obtaining them, it is advisable that quality control labouratory should maintain its own working standards which should be standardised at regular intervals.

PROCEDURES:

Quantity of the test sample should be weighed or measured accurately as specified under the methods, proportionately larger or smaller quantities than the one specified, may be taken provided subsequent steps such as dilutions are adjusted to yield concentrations equivalent to those specified.

CHOICE OF THE REAGENT:

The chemistry of aromatic sulphonyl N-haloamines has evinced considerable interest due to their diverse behaviour[1,2,3]. They are sources of halonium cations, hypohalite species and N-anions which act both as bases and nucleophiles. Due to their greater stability they are used as disinfectant and antiseptic in preference to hypohalites. Considerable progress has been made in analytical chemistry with the introduction of aromatic sulphonyl haloamines as redox titrants[2,3]. N-haloamines react with a surprising range of functional groups, affecting an array of molecular transformations.

Well known member of the N-haloamine are : Chloramine-T, CAT, ($Na^+[p\text{-}Ch_3C_6H_4SO_2NCl]^-$); Dichloramine-T[4], DCT, ($p\text{-}Ch_3C_6H_4SO_2NCl_2$); Bromamine-T[5], BAT ($Na^+[p\text{-}CH_3C_6HSO_2NBr]^-$); Dibromamine-T[6], DBT, ($p\text{-}CH_3C_6H_4SO_2NBr_2$); Chloramine-B[7], CAB, ($Na^+[C_6H_5SO_2NCl]^-$); Dichloramine-B[8], DCB ($C_6H_5SO_2NCl_2$); Bromamine-B[9], BAB ($Na^+[C_6H_5SO_2NBr]^-$); Dibromamine-B[10], DBB ($C_6H_5SO_2NBr_2$); Iodamine-B[11], IAB ($Na^+[C_6H_5SO_2NI]^-$); Iodamine-T[12], IAT, ($Na^+[p\text{-}CH_3C_6H_4SO_2NI]^-$). These oxidants have been extensively used as analytical reagents. Further the toluene analogues are more commonly used than benzene analogues.

Bromamine-B[9](BAB), the sodium salt of N-brome-benzene-sulphonamide ($Na^+|C_6H_5SO_2NBr|^-$) has been introduced as a new oxidimetric titrant. Literature survey s reveals that there is a little application of Broamamine-B reagent as oxidimetric titrant for micro determination of organic compounds. This inspired me to choose Brmamine-B as analytical reagent. In the whole of my work presented in the thesis, Bromamine-B reagent in acidic medium is used as oxidant for the micro estimation of some organic and pharmaceutical compounds.

Preparation and Standardisation of BAT:

Bromamine-B (BAB) was prepared 5 from dibromomine-B(DBB) which in turn prepared by the bromination of chloramine-B (CAB). Recrystalysed CAB (E.Merk, Proanalysis sample) 10g was dissolved in water (200ml) and liquid Br2 (2 ml) was added dropwise from burette with constant stirring of the solution. The golden yellow precipitate of DBB was thoroughly washed with water, filtered under suction and dried in vacum desicator for twenty four hours. The dry sample was found to melt at 92-930C with decomposition. The purity of the sample was checked by determining the concentration of known (prepared) solution (0.1 N) of DBB in glacial acetic acid, iodometrically. About 33g of DBB thus prepared was dissolved in small lots in ice cooled solution of sodium hydroxide (8g of NaOH in 50 mL water). While shaking the contents pale yellow crystals of BAB $Na^{+}(C_6H_5SO_2NBr)^{-}$. H_2O | separates out. The precipitate was filtered under suction, washed with minimum quanityt of water and dried over P_2O_5.

Approximately 0.1 N (0.05M) stock solution of BAB was prepared by dissolving 4 g of the compound in 250 mL distilled water and standardised iodometrically.

Standardisation:

Five mL of Bromamine-B solution (0.05M) are taken in a 100mL Erlenmmeyer flask. The solution is acidified with 2mL of 2N sulphuric acid. Contents are shaken thoroughly and 5 ml of KI (10%) solution is added to it. The reaction mixture is allowed to stand at room temp. for a minute. The liberated iodine is titrated against o.1 N sodium thiosulphate to starch end point. The concentration of Bromamine-B solution was calculated by the litre value of hypo. To cover the interaction of the different solvents a blank experiment was also run repeating all the experimental details except the Bromamine-B, It was also observed that BAB reagent is stable at room temperature for several days when kept in Amber coloured bottle however, for the sake of accuracy the reagent is standardised daily before use.

DETERMINATION OF COMPOUNDS:

For the determination of the reductants (organic and pharmaceutical compounds) following technique was adopted. Aliquots contaning 1-5 mg of the reductant in proper solvent were taken in a 100 mL Erlenmmeyer flask and a calculated excess (5 mL) of 0.1 N BAB solution was added to it. Contents were acidified by 2mL of 2N-H_2SO_4 and allowed to react for

prescribed reaction time. After the reaction was over the unconsumed BAB reagent was determined iodometrically.

REACTIVE SPECIES OF BROMAMINE-B:

On the basis of the reactive species of CAT following equilibria are possible for BAT in acidic medium [13, 14].

$C_6H_5SO_2N.NaBr \rightleftharpoons C_6H_5SO_2NBr^- + Na^+$ - (1)

$C_6H_5SO_2N.NaBr \rightleftharpoons C_6H_5SO_2NHBr + H_2O$ - (2)

$C_6H_5SO_2NHBr \rightleftharpoons C_6H_5SO_2NBr_2 + C_6H_5SO_2NH_2$ - (3)

$C_6H_5SO_2NHBr + H_2O \rightleftharpoons C_6H_5SO_2NH_2 + HOBr$ - (4)

$C_6H_5SO_2NBr_2 + H_2O \rightleftharpoons C_6H_5SO_2NHBr + HOBr$ - (5)

$HOBr + H_2O \rightleftharpoons OBr^- + H_3^-O$ - (6)

It appears from equilibria (1-6) that any one of these species, Bromamine-B, N-bromo-benzenesulphonamide ($C_6H_5SO_2NHBr$), Hypobromous acid (HOBr), dibromaminer-B (C_6H_5NHBr2) and hypobromite (OBr^-) could work as oxidising units. Bishop and Jenningl have reported the concentration of various species present in 0.05 M CAB over a wide range of pH value. Soper 11 reported that HOCL is very small in acidified CAB solution and it is independent of Chloramine-B. Similar conclusion is drawn for HOBr in acidified BAT solution and thus any role of HOBr as an oxidant seems to be unlikely. The remaining species i.e. either $C_6H_5SO_2NHBr$ or $C_6H_5SO_2NBr_2$

appears to be real oxidising species of BAB. It is reported6 that oxidation with BAB proceeds very much like that with CAT. The formal redox potential of the BAB/benzenesulphonamide couple was determined and found to be +1.16V at25±0.02°.This is quite comparable to the value of +1.14V for CAB.

Bromamine-B also acts as oxidant in alkaline medium. By similar argument as in acidic solution of BAB, it may be concluded that the probable oxidising species in alkaline solution of BAB, depending upon the pH of the medium are $C_6H_5SO_2NBr^-$, $C_6H_5SO_2NHBr$, HOBr and OBr^-.

It is observed that bromamine-B undergo a two electron change in its oxidimetric reactions and it get reduced to benzenesulphonamide.

$C_6H_5SO_2NNaBr + H^+ + 2e^- \rightarrow C_6H_5SO_2NH_2 + NaBr.$

The reduced product of BAB, benzenesulphonamide in its oxidimetric reaction with a reductant was identified byu paper chromatography with a reductant was identified by paper chromatography with benzyl alcohol saturated with water as the solvent and 0.5% vanillin in 1% HCl in ethanol as the spray reagent (Rf= 0.91).

Bromamine-B as an Oxidant:

Bromamine-B, has recently been employed for estimation of a few organic compounds. Estimation of hydroquinone, isonitotinicacid hydrazide, dydrazine sulphate, ascorbicacid benyl hydrazine

BROWMAMINE-B AS HALOGENATING AGENT:

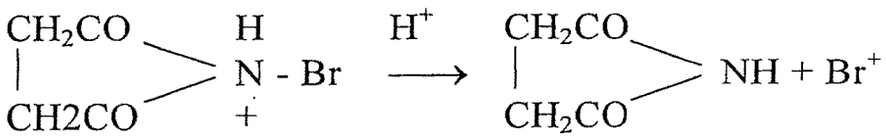
The use of Chloramine-T as halogenating agent has been well established[18,19]. Its use as a source of chloroniumion has been made in the chlorination of phenols and cresols[20-23] anisols[24], aromatic anines[25], phenyl acetate[26] p-amino-benzene sulphonamidesd[27] and naphthols[28]. It has been well established[9,16,17] that BAB behaves like in many of its reactions. Thus BAT may also be used as brominating agent for an array of organic compounds. A general reaction thereby which Bromamine-B liberates bromonium ion (Br^+) can be represented as

$$C_6H_5N.NaBr + H_2O \rightarrow C_6H_5SO_2NH_2 + NaOHBr^+$$

In chlorination of phenols and anilines with CAT, it has been established[29,30] that in acidic solution $C_6H_5SO_2NH2Cl^+$ and $C_6H_5SO_2NHCl^-$ while in alkaline solution $C_6H_5SO_2NHCl^-$ and TsNHCl are present as active species. On the basis of study of kinetics it has been concluded that $C_6H_5SO_2NH2Cl^+$ in acidic and $C_6H_5SO_2NHCl$ in slightly alkaline solution of CAB are

effective chlorinating agent. BAB like CAB gives several oxidising as well as chlorinating species in aqueous solution. The stability of each species depends on the nature and pH of the medium. Thus from above arguments it may be concluded that $C_6H_5SO_2NH2Br+$ and $C_6H_5SO_2NBHr$ are more likely brominating species in acidic and slightly alkaline solution of BAB respectively.

Similar to N-haloamines the use of N-haloimides has also been reported. A survey of literature reveals that N-bromosuccinimide (NBS)[31] is the most widely studied reagent as compared to N-chlorosuccinimide, N-bromosaccharine, N-bromothalimide etc. In acidic medium NBS has been reported to work as effective source of bromonium ion



While comparing the two reagents i.e. NBS and BAB, it has been noticed that BAB has got certain advantages over NBS. The present reagent is more soluble in aqueous medium, works as stronger electrophile and gives free radical free reactions. In BAB only the species $C_6H_5SO_2NH_2Br^+$ works as brominating agent in acidic medium.

Aim of the present work:

After studying various aspects of Bromamine-B reported in literature it is found that the reagent has potentialities of being adopted as an analytical reagent for a variety of simple and medicinal organic compounds. In the present work I have employed this reagent for the micro estimation of phenols and hydrazine derivatives. It was also observed that the reagent can work for the estimation of certain medicinal compounds therefore I have employed the reagent for the estimation of some acridines, purines and anti-hyper-tensive drugs as well. The main object of the work is to give an entirely new method for the estimation of these compounds with the present reagent. In my knowledge this type of work has not been to bring the error of the method within 0.5-1% .For developing suitable reaction condition the study of different variables such as reaction time, concentration of the reagent, reaction medium and reaction temperature are made. In case of every compound the stoichiometry is established. On the basis of the isolation, identification and stoichiometry of reaction, the course of reaction has been explained. In case of anti-hypertensive drugs the method is developed for the pure compounds and employed for its different pharmaceutical preparations i.e. tablets, capsules, injection etc. keeping uniformity with the trend inthe

analysis of pharmaceutical products prevailing at present in standard analytical laboratory recovery experiments were also made by standard drug addition technique. For every estimation the percentage error, SD and CV values were calculated.

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