11 THERMOANALYTICAL ANALYSIS

CONTAINS :

11.1. INTRODUCTION

Thermoanalytical methods essentially encompass such techniques that are based entirely on the concept of heating a sample followed by well-defned modified procedures, such as : gravimetric analysis, differential analysis and titrimetric analysis. In usual practice, data are generated as a result of continuously recorded curves that may be considered as 'thermal spectra'. These thermal spectra also termed as **'thermograms**, often characterize a single or multicomponent system in terms of :

(*a*) temperature dependencies of its thermodynamic properties, and

(*b*) physicochemical reaction kinetics.

Broadly speaking the thermoanalytical methods are normally classified into the following three categories, namely :

(*i*) Thermogravimetric Analysis (TGA),

(*ii*) Differential Thermal Analysis (DTA), and

(*iii*) Thermometric Titrations.

All the above mentioned techniques shall be discussed briefly with specific reference to their theory, instrumentation, methodology and applications wherever necessary.

11.2. THERMOGRAVIMETRIC ANALYSIS (TGA)

11.2.1. THEORY

A large number of chemical substances invariably decompose upon heating, and this idea of heating a sample to observe weight changes is the underlying principle of thermogravimetric analysis (TGA). However, TGA may be sub-divided into *two* heads, namely :

(*a*) Static (or Isothermal) Thermogravimetric Analysis, and

(*b*) Dynamic Thermogravimetric Analysis.

11.2.1.1. Static Thermogravimetric Analysis

In this particular instance the sample under analysis is maintained at a constant temperature for a period of time during which any changes in weight are observed carefully.

11.2.1.2. Dynamic Thermogravimetric Analysis

In dynamic thermogravimetric analysis a sample is subjected to conditions of predetermined, carefully controlled continuous increase in temperature that is invariably found to be linear with time.

11.2.2. INSTRUMENTATION

The essential requirements for an instrument (Figure 11.1) meant for thermogravimetric analysis are, namely :

(*a*) A high-precision balance,

(*b*) A furnace adequately programmed for a linear rise of temperature with time, and

(*c*) A sensitive recorder.

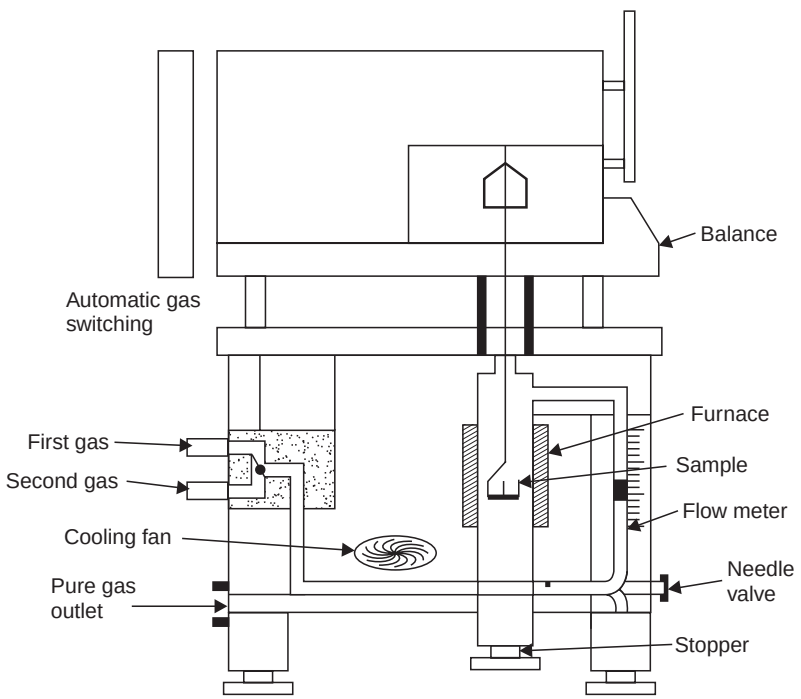

Figure 11.1 : Thermobalance for Thermogravimetric Analysis in a Controlled Atmosphere upto 1000°C.

11.2.2.1. Balances

They are usually of *two* types :

(*a*) **Null-point Type :** It makes use of an appropriate sensing-element which aptly detects any slightest deviation of the balance beam and provides the application of a restoring force, directly proportional to the change in weight, thereby returning the beam to its original null-point. The restoring-force is subsequently recorded either directly or with the aid of a transducer.

(*b*) **Deflection Type :** It is essentially based on either a conventional analytical balance consisting of helical spring, cantilever beam and strain gauze or a torsion analytical balance involving the conversion of deviations directly into a record of the weight change.

11.2.2.2. Furnace

The furnace must be designed in such a fashion so as to incorporate an appropriate smooth input thereby maintaining either a fixed temperature or a predetermined linear-heating programme (*e.g.*, 10°C-600°C per hour).

The temperature control of the furnace is satisfactorily achieved via a thermocouple mounted very close to the furnace-winding. The maximum operational temperature may be obtained by using different thermocouples as indicated below :

S.No.	Specifications	Max. Temp. (°C)
1.	Nickel-Chrome (Nichrome)	1100
2.	Platinum-Rhodium	1450
3.	Graphite-Tube Furnace*	> 1500

*Control and measurement of temperatures are critical and problematic.

11.2.2.3. Recorder

The recording device must be such so as to :

(*i*) record both temperature and weight continuously, and

(*ii*) make a definite periodic record of the time.

11.2.3. METHODOLOGY

The '*thermogram*' for calcium oxalate monohydrate ($CaC_2O_4.H_2O$) is presented in Figure 11.2. The successive plateaus correspond to the anhydrous oxalate (100-250°C), calcium carbonate (400-500°C), and finally calcium oxide (700-850°C). In other words, these plateaus on the decomposition curve designate *two* vital aspects, namely :

(*a*) clear indication of constant weight, and

(*b*) stable phases within a specified temperature interval. The chemical reactions involved may be summarized as follows :

$$CaC_2O_4.H_2O \xrightleftharpoons{100\text{-}250^\circ C} CaC_2O_4 \xrightleftharpoons{400\text{-}500^\circ C} CaCO_3 \xrightleftharpoons{700\text{-}850^\circ C} CaO$$

[Stage 1] [$+H_2O\uparrow$] [Stage 2] [$+CO\uparrow$] [Stage 3] [$+CO_2\uparrow$]

11.2.3.1. Interpretation of Thermogram

In the thermogram (Figure 11.2), which vividly illustrates the thermogravimetric evaluation of $CaC_2O_4.H_2O$, it is ensured that the weight of this product decreases in several stages, namely :

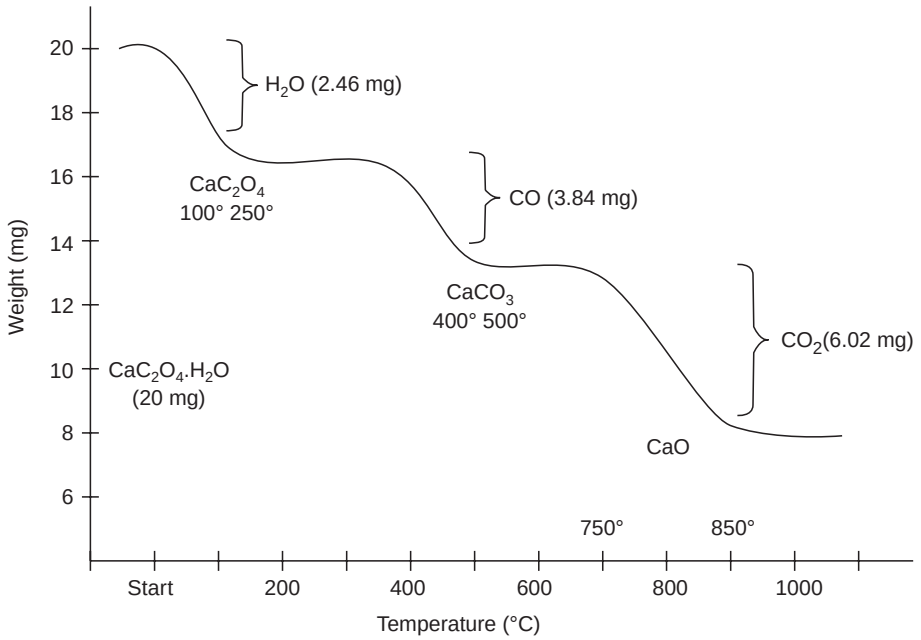

Figure 11.2 : Thermogravimetric Evaluation of Calcium Oxalate Monohydrate heated at the rate of 6°C min^{-1}.

Stage 1 : The water of hydration (or crystallization) from calcium oxalate monohydrate is lost which corresponds to 2.46 mg (12.3%) equivalent to 1 mole of H_2O in the temperature range 100-250°C.

Actually, the 12.3% weight loss that took place between 100-250°C should correspond to 12.3% of the original formula weight for $CaCO_3\ H_2O$ (FW = 146). Hence, the product being lost has a formula weight of $0.123 \times 146 = 17.958$ ($\simeq$ 18.0), and it corresponds to H_2O.

Stage 2 : One mole of carbon monoxide is evolved subsequently from calcium oxalate, corresponding to 3.84 mg (19.2%) in the temperature range 400-500°C.

The 19.2% weight loss that occurred between 400-500°C should correspond to 19.2% of the original formula weight of 146. Therefore, the product being given out has a formula weight of $1.92 \times 146 = 28.0$, that corresponds to CO.

Stage 3 : Finally, a mole of CO_2 is evolved from calcium carbonate that corresponds to 6.02 mg (3.01%) in the temperature range 700-850°C.

The weight loss amounting to 3.01% which took place in the range 700-850°C must, in fact, corresponds to 3.01% of the original formula weight of 146. Therefore, the product being evolved has a formula weight of $0.301 \times 146 = 43.946$ ($\simeq$ 44), and it corresponds to CO_2.

It is quite evident that in a multicomponent system wherein more than one component exhibits weight variations and that too at different temperature regions, the composition of the original compound may be estimated as depicted in Figure 11.2.

In a situation whereby an inert material is present along with a pure substance, from the generated thermogram the composition of the mixture may be derived from the percentage weight variation which takes place relative to the percentage weight variation observed with the pure compound (A), by employing the following expression :

$$\text{Component A (wt \%)} = \frac{\text{\% wt. change for mixture}}{\text{\% wt. change for pure compound A}} \times 100\ (\%)$$

11.2.4. APPLICATIONS

The most broadly based application of the thermogravimetric analysis (TGA) has been visualized and exploited in the investigation of analytical methods, such as :

(*i*) Determining appropriate forms for many elements,

(*ii*) Screening and testing of substances which may be used as potential analytical standards (primary standard), and

(*iii*) Direct application of the technique in analytical assays.

A few typical applications of TGA are, namely :

(*a*) Plateaus for hydrates are sometimes based on the initial water content (*i.e.*, water of crystallization). It has been observed that in humidified air at low heating rates, hydrates usually give rise to good plateaus.

Example : Dehydration of sodium tungstate 28-hydrate [Na_2WO_4.28 H_2O (5 : 12)]

Experimental parameters* :

A. Humidified air, 300°C/hour,

B. Humidified air, 150°C/hour,

C. Humidified air 10°C/hour,

D. Room air, 10°C/hour,

Sample weight : 0.5000 g ;

n = Moles water per $5Na_2O$, 12 WO_3

(*b*) Analysis of flue-gas scrubber system in environmental analysis.

The flue-gas emitted from a coal-fired-power-plant is subjected to scrubbing by the aid of wet limestone to get rid of sulphur dioxide (SO_2) as completely as possible. TGA helps in monitoring the system by carrying out the analysis of the products resulting from the scrubbing process, that mainly consist of (*i*) $CaCO_3$; (*ii*) $CaSO_3$. $CaSO_3$. 1/4 H_2O, and (*iii*) $CaSO_4$. $2H_2O$.

The thermogram obtained from TGA provides the following valuable informations which suggests the decomposition occurring at three distinct stages

S.No.	Conversion		Wt. Loss Region (°C)	Wt. Loss (%)	Due To
	From	To			
1.	$CaSO_4.2H_2O$	$CaSO_4$	100-200	66	$2H_2O$
2.	$CaSO_3.CaSO_4.1/2\ H_2O$	$CaSO_3.CaSO_4$	420	31	$1/2H_2O$
3.	$CaCO_3$	CaO	630-800	03	CO_2

thereby causing the loss due to two moles of water, half-a-mole of water and one mole of CO_2.

(c) The stepwise degradation of organic polymers has received adequate attention which has broadened the in-depth knowledge of polymer chemistry. In this specific instance the sample is either heated under vacuum or in an inert atmosphere (of N_2).

(*d*) The thermogravimetric data may be employed to evaluate the kinetic parameters of weight variations in reactions.

* After, Newkirk A.E., **Anal. Chem., 32** 1560 (1960).

11.3. DIFFERENTIAL THERMAL ANALYSIS (DTA)

11.3.1. THEORY

The difference of temperature between the sample under estimation and a thermally-inert reference material is continuously recorded as a function of furnace temperature in differential thermal analysis (DTA). In actual practice both TGA and DTA are regarded as complementary techniques whereby information gathered by the usage of one approach is invariably supplemented and enhanced by the application of the other method. The range of phenomena measurable during a DTA-run is found to be much larger than in a TGA-run.

It is pertinent to mention here that in the course of TGA many vital processes, for instance : crystallization, crystalline transitions, pure fusion reactions, glass transitions, and solid-state reactions devoid of volatile components might not be indicated as they happen to cause little change in weight of the sample. TGA invariably describes with ample precision the stoichiometry related to chemical changes that are indicated during DTA by an endothermal or exothermal duration from the base-line.

11.3.2. INSTRUMENTATION

A differential thermal analyzer is composed of *five* basic components, namely :

(*i*) Sample holder with built-in thermocouple assembly,

(*ii*) Flow-control system,

(*iii*) Furnace assembly,

(*iv*) Preamplifier and Recorder, and

(*v*) Furnace Power Programmer and Controller.

A typical commercial differential thermal analyzer is schematically illustrated in Figure 11.3.

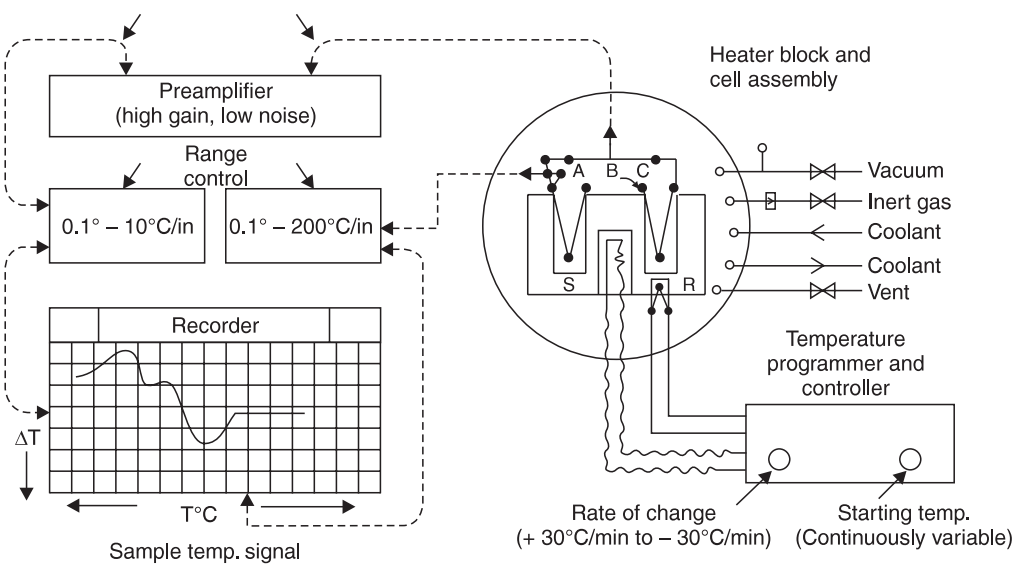

Figure 11.3 : Schematic Diagram of a Differential Thermal Analyzer.
(*Source :* E.I. Du Pont De Nemours, Inc, USA)

(*a*) Thermocouples employed are normally unsheathed Platinum *Vs* Platinum and Sodium *Vs* 10% Rhodium. The said two thermocouples help in measuring the difference in temperature between a sample S and an absolutely inert reference substance R, as both are subjected to heating in a ceramic or metal block inside a furnace being operated by a temperature programmer and controller.

(*b*) The output of the differential thermocouple is amplified adequately through a high gain, low-noise preamplifier and subsequently hooked to the recorder, one axis of which is driven by the block temperature signal and is measured by a third thermocouple.

(*c*) **Heating/Cooling Device :** A sufficient versatility is achieved by the aid of a pressure-vacuum, high-temperature electric furnace. An almost constant heating rate is usually achieved by using a motor-driven variable auto transformer.

Both heating rates and cooling rates may be conveniently adjusted continuously :

(*i*) From 0°-30°C/minute by some instruments, and

(*ii*) From a choice of several commonly employed heating rates *viz*., 2°, 4°, 8° and 16°C/minute.

Usual workable sample temperatures are upto : 500°C. Exceptional maximum temperatures are upto : 1000°C.

(*d*) Relatively small sample volumes help in *two* ways : *first*, they make evacuation easy ; and *secondly*, they minimize thermal gradients.

11.3.3. METHODOLOGY

(*i*) Insert a very thin thermocouple into a disposable sample tube 2 mm in diameter and containing 0.1-10 mg of sample,

(*ii*) Another identical tube is either kept empty or filled with a reference substance, such as quartz, sand, alumina or alundum powder,

(*iii*) The two tubes are simultaneously inserted into the sample block and subsequently heated (or cooled) at a uniform predetermined programmed rate, and

(*iv*) DTA—being a dynamic process, it is extremely important that all aspects of the technique must be thoroughly standardised so as to obtain reproducible results. A few of these aspects vital aspects are :

- Pretreatment of the specimen,
- Particle size and packing of the specimen,
- Dilution of the specimen,
- Nature of the inert diluent,
- Crystalline substances must be powdered, and sieved through 100-mesh sieve,
- For colloidal particles (*e.g.*, clays), micelle-size is very critical, and
- Either to supress an unwanted reaction (*e.g.*, oxidation), or to explore the study of a reaction (*e.g.*, gaseous reaction product)—the atmosphere should be controlled adequately.

Figure 11.4, depicts the differential thermal analysis investigation of calcium acetate monohydrate at a uniform programmed heating rate of 12°C/minute.

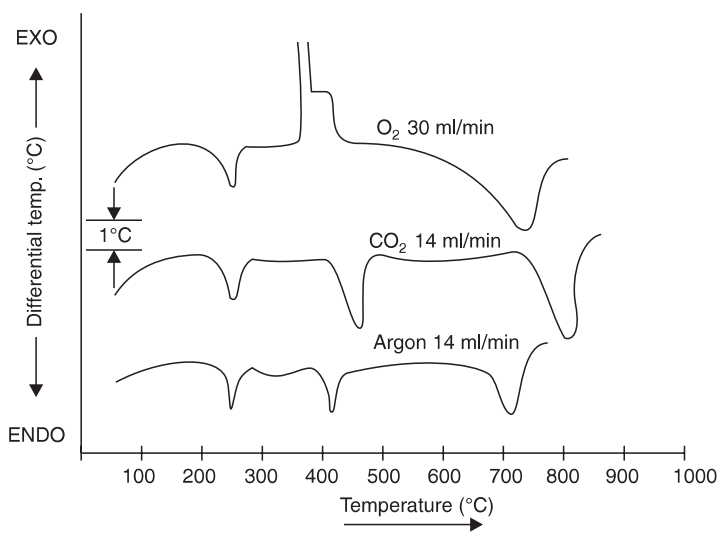

Figure 11.4 : DTA-Investigation of Calcium Acetate Monohydrate, 12°C. min^{-1} (After-American Inst. Co. Inc., USA, Bulletin).

The chemical reactions involved in the differentiated thermal analysis of calcium acetate monohydrate may be expressed as follows :

$$Ca(CH_3COO)_2.H_2O \underset{\text{(Stage-I)}}{\overset{200\text{-}250^\circ C}{\rightleftharpoons}} Ca(CH_3COO)_2 \underset{\text{(Stage-II)}}{\overset{350\text{-}400^\circ C}{\rightleftharpoons}} CaCO_3 \underset{\text{(Stage-III)}}{\overset{800\text{-}950^\circ C}{\rightleftharpoons}} CaO$$

Stage I : The endothermal dehydration of calcium acetate monohydrate occurs giving rise to the anhydrous salt. It is easily noticed by an endothermal band on DTA curve between 200°C and 250°C.

Stage II : The anhydrous salt undergoes endothermal decomposition reaction at 350-400°C resulting into the formation of $CaCO_3$. It has been observed that this decomposition reaction seems to be almost alike in the presence of either CO_2 or Ar.

Stage III : The decomposition of calcium carbonate to calcium oxide, which is a function of the partial pressure of the CO_2 in contact with the sample. The endothermal band for the carbonate decomposition is sharply peaked spread over a relatively narrower temperature range in an atmosphere of CO_2.

11.3.4. APPLICATIONS

The various important applications of DTA are :

(*i*) Rapid identification of the compositions of mixed clays,

(*ii*) Studying the thermal stabilities of inorganic compounds,

(*iii*) Critically examining in a specific reaction whether a new compound is actually formed or the product is nothing but an unreacted original substance, and

(*iv*) DTA offers a wide spectrum of useful investigations related to reaction kinetics, polymerization, solvent retention, phase-transformations, solid-phase reactions and curing or drying properties of a product.

11.4. THERMOMETRIC TITRATIONS (T T)

11.4.1. THEORY

The thermometric titrations (TT) make use of '**heats of reaction**' to obtain titration curves. In usual practice, the temperature of solution is plotted against the volume of titrant. TT is performed by allowing the titrant to flow from a thermostated-burette directly into a solution contained in a thermally-insulated vessel, and subsequently the observed change in temperature of the solution is recorded precisely either during continuous addition of titrant or after every successive incremental addition. The end-point is aptly indicated by a sharp break in the curve.

As the dielectric constant of a solvent exerts little effect on the thermometric titrations, the latter may be employed effectively in most non-aqueous media.

Hence, in a broader-sense TT may be utilized in a number of reactions with greater efficacy, for instance : complexation, precipitation, redox, neutralization. Further, TT can be used to titrate gases against other gases devoid of a liquid-phase ; and to titrate liquid solutions with gaseous reagents.

11.4.2. INSTRUMENTATION

A standard thermometric titration assembly essentially consists of the following *four* vital components, namely :

(*i*) Motor-driven Burette,

(*ii*) Adiabatic Titration Chamber

(*iii*) Thermister Bridge Assembly, and

(*iv*) Recorder.

Fiaure 11.5, represents the schematic thermometric titration assembly complete with a bridge-circuit. To minimise heat transfer losses from the solution by its immediate surroundings, the thermometric titrations are usually carried out in an isolated-beaker tightly closed with a stopper having provision for a burette-tip, a motorized-glass stirrer, and a temperature-monitoring arrangement.

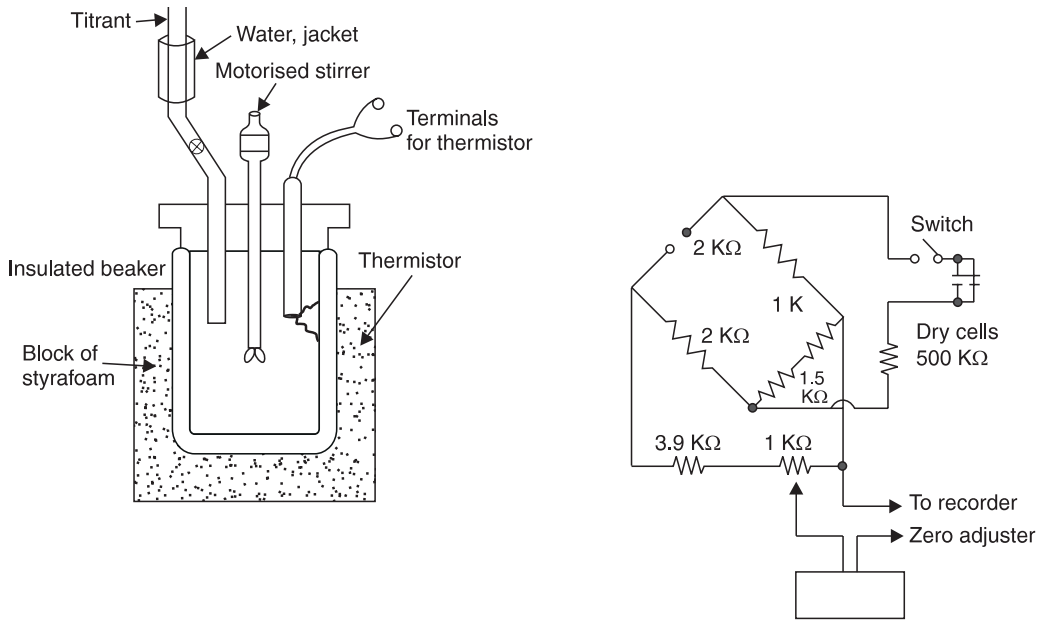

Figure 11.5 : Schematic Thermometric Titration Assembly Complete with a Bridge-Circuit (After Linde *et al.*, Anal Chem. 25, 494, 1953).

Procedure :

(*a*) Introduce the titrant from a burette that is duly mounted in a thermostated-water-jacket to maintain the temperature of the titrant within ± 0.05°C,

(*b*) Experimental parameters are predetermined in such a fashion such that the volume of titrant needed for each titration must lie between 1-3 ml,

(*c*) Automated device delivering reagent at a steady and constant rate of 600 µl per minute usually permits recording,

(*d*) Constant-speed motorized stirrer at 600 rpm is employed to effect uniform mixing of solution,

(*e*) Variations in temperature are measured with the help of a sensitive thermister-sensing-element with fast response, that is sealed completely in glass and immersed in solution,

(*f*) In the course of a thermometric titration, the thermister attached to the insulated-beaker is connected to one arm of the Wheatstone Bridge as displayed in Figure 11.5. The values of the circuit component listed are for a thermister having an approximate resistance of 2 KΩ and a sensitivity of $^{-}$0.04 Ω/Ω/°C in the 25°C temperature range. Hence, an observed change of 1°C ≡ an unbalanced potential of 15.7 mV, and

(*g*) The heat of reaction is either absorbed or generated upon addition of the titrant to the beaker, thereby unbalancing the Wheatstone Bridge caused by simultaneous variations in the resistance (temperature) in the insulated-beaker thermister. Thus, the bridge unbalance potential is promptly plotted by the recorder.

Note : (*i*) **To minimise the temperature variations between the titrant and the solution and also to obviate volume corrections, the concentration of the titrant is invariably maintained 10–100 times higher than that of the reactant, and**

(*ii*) **To obtain optimum results, temperatures of the titrant and the solution must be always within 0.2°C of each other before a titration is commenced.**

11.4.3. METHODOLOGY

Thermometric titration curves usually represent both the entropy and the free energy involved. The titrant is added to the solution at a constant rate in order that the voltage output of the thermister-temperature-transducer changes linearly with time upto the equivalence point.

TT-method affords exact end-point due to :

(*a*) Coloured solutions, and

(*b*) Poisoning of Electrodes.

In usual practice it has been observed that thermometric titrations are mostly feasible with such systems that provide rates of temperature change more than 0.01°C/second.

A few typical examples are cited below :

S.No.	Titrant (M)	Solution (M)	Temp. Change (°C)
1.	NaOH (1 M ; 1 ml)	HCl (0.33 M ; 30 ml)	+ 0.4°C
2.	Na_2-EDTA (1 M)	$MgCl_2$ (0.033 M)	– 0.08°C

Precautions :

(*i*) Lower limit of concentrations which can be titrated effectively is 0.002 M,

(*ii*) No transfer of heat between the titration vessel and its immediate surroundings is allowed, and

(*iii*) During titration temperature fluctuation must not exceed 0.001°C.

11.4.4. APPLICATIONS

Various important applications of thermometric titrations are enumerated below :

(*i*) **Precipitation Reactions :** *e.g.*, Chloride ions (Cl^-) with Ag^+ ions. Besides, phase relations have been studied extensively in precipitation reactions.

(*ii*) **Ion-combination Reactions :** *e.g.*, divalent cations like Ca^{2+}, Mg^{2+} with EDTA (complexometric estimation),

(*iii*) **Conversion of Amides to Amines :** *e.g.*,

An aromatic sulphonic acid amide $\xrightarrow[\substack{\text{Sodium Hypochlorite}\\(\text{Neutral/Alk. Medium})}]{NaClO_3}$ A Monochloramine

(*iv*) **Estimation of H_2O and $(CH_3CO)_2O$ concentrations in a mixture :** The concentration of either of these reactions in the presence of the other may be determined successfully by measuring the rise in temperature taking place during the exothermic reactions of water and acetic anhydride in glacial acid solution along with a trace of perchloric acid ($HClO_4$) acting as a catalyst, and

(*v*) **Benzene in Cyclohexane :** Benzene may be estimated rapidly with fairly good accuracy in cyclohexane by measuring the heat of nitration, whereby a previously prepared standard nitrating acid mixture (benzene and cyclohexane) and the subsequent temperature rise is noted which is a direct function of the quantity of benzene present.

Details involving various experimental parameters for the above estimation are enumerated below :

11.4.4.1. Estimation of Benzene in Cyclohexane

Materials Required : Thermometric titration assembly as per Figure 11.5, minus the burette; a stop-watch or timer ; standard nitrating acid mixture [mix 2 volumes of 70% HNO_3 (d = 1.41) with 1 volume of 95% H_2SO_4 (d = 1.82)] ; Bakelite screw-cap bottle (4 oz. capacity) : 2.

Procedure :

(1) Weigh 50 g of sample in a Bakelite screw-cap bottle and in a similar bottle put the standard nitrating mixture. Place these two bottles in a thermostat maintained at 20°C until the contents have attained an equilibrium temperature,

(2) Transfer 50 ml of the standard nitrating-acid to the insulated vessel and insert the motorised stirrer. Just wait for about 3-5 minutes and then start the motorized stirrer. After exactly 1 minute record the initial temperature,

(3) Stop the motor. Insert the sample into the reaction vessel and start the stirrer. Now, start taking readings of the rise in temperature after each interval 1, 2, 3 and 5 minutes respectively, and

(4) Plot a 'calibration curve' between the observed temperature-rise in a 3 minute interval *Vs* percent benzene present in cyclohexane. Run pure cyclohexane and standards containing 0.5-5.0 percent benzene by weight.

THEORETICAL AND PRACTICAL EXERCISES

1. How does '**thermoanalytical analysis**' give rise to various types of '**thermograms**' that help in characterizing either a single or multicomponent system ?

2. Discuss, the fundamental theory of '**thermogravimetric analysis**', and its instrumentation aspects in an elaborated manner.

3. What are the pharmaceutical applications of TGA ? Explain the interpretation of thermogram obtained by TGA of calcium oxalate monohydrate being heated at the rate of 6°C per minute.

4. Attempt the following aspects of **'differential thermal analysis' :**

(*a*) Theory (*b*) Instrumentation

(*c*) Methodology (*d*) Applications.

5. How would your differentiate '**thermometric titrations**' from TGA and DTA ? Explain.

6. Describe a '**standard thermometric titration**' assembly comprising of the following **FOUR** important components and explain its working :

(*i*) Motor-driven burette (*ii*) Adiabatic titration chamber

(*iii*) Thermister bridge assembly (*iv*) Recorder.

How does it help in **'complexometric titrations'** ? Explain.

RECOMMENDED READINGS

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G. MISCELLANEOUS METHODS

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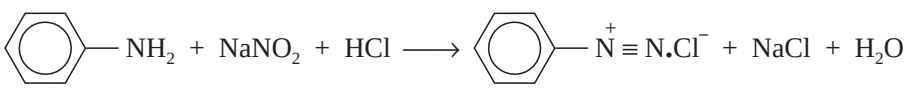
12 DIAZOTIZATION (SODIUM NITRITE TITRATION)

CONTAINS :

12.1. INTRODUCTION

In general, aromatic primary amino moiety (*i.e.*, Ar-NH_2), as present in a host of sulphadrugs *viz.*, succinyl sulphathiazole, sulphamethoxazole, sulphaphenazole and other potent pharmaceutical substances, for instance sodium or calcium aminosalicylate, isocarboxazid, primaquine phosphate, procainamide hydrochloride, procaine hydrochloride and dapsone react with sodium nitrite in an acidic medium to yield the corresponding diazonium salts as expressed below :



Aniline — Phenyl diazonium chloride

It is interesting to observe here that the above reaction is absolutely quantitative under experimental parameters. Therefore, it forms the basis for the estimation of pharmaceutical substances essentially containing a free primary amino function as already illustrated earlier.

12.2. THEORY

Nitrous acid is formed by the interaction of sodium nitrite and hydrochloric acid as follows :

$$NaNO_2 + HCl \longrightarrow NaCl + HNO_2$$

The end-point in the sodium nitrite titration is determined by the liberation of iodine from iodide which may be expressed by the following equations :

$$KI + HCl \longrightarrow HI + KCl$$

$$2HI + 2HNO_2 \longrightarrow I_2 + 2NO + 2H_2O$$

In other words, the small excess of HNO_2 present at the end-point can be detected visually by employing either starch-iodide paper or paste as an external indicator. Thus, the liberated iodine reacts with starch to form a blue green colour which is a very sensitive reaction. Besides, the end-point may also be accomplished electrometrically by adopting the dead-stop end-point technique, using a pair of platinum electrodes immersed in the titration liquid.

12.3. ASSAY METHODS

A number of pharmaceutical substances can be assayed by official methods employing sodium nitrite titrations. A few typical examples are described below to get an indepth knowledge about sodium nitrite titrations.

12.3.1. PREPARATION OF 0.1 M SODIUM NITRITE SOLUTION

Materials Required : Sodium nitrite : 7.5 g.

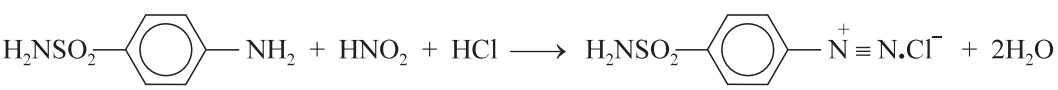
Procedure : Weigh accurately 7.5 g of sodium nitrite and add sufficient DW to produce 1 litre in a 1000 ml volumetric flask.

12.3.2. STANDARDIZATION OF 0.1 M SODIUM NITRITE SOLUTIOIN WITH SULPHANILAMIDE

Materials Required : Sulphanilamide (previously dried at 105°C for 3 hours) : 0.5 g ; hydrochloric acid ($\simeq$ 11.5 N) : 20 ml ; 0.1 M sodium nitrite.

Theory : The nitrous acid, generated on the introduction of sodium nitrite solution into the acidic reaction mixture, reacts with the primary amino group of sulphanilamide quantitatively, resulting into the formation of an unstable nitrite that decomposes ultimately with the formation of a diazonium salt. The diazonium salt thus produced is also unstable, and if the reaction mixture is not maintained between 5-10°C, it shall undergo decomposition thereby forming phenol products which may react further with nitrous acid. The reactions involving the formation of the diazonium salt may be expressed in the following manner :

$$NaNO_2 + HCl \longrightarrow HNO_2 + NaCl$$



Sulphanilamide (172.2)

Diazonium salt

$$C_6H_8N_2O_2S \equiv NaNO_2$$

or $$172.2 \text{ g } C_6H_8N_2O_2S \equiv 1000 \text{ ml M}$$

or $$17.22 \text{ g } C_6H_8N_2O_2S \equiv 1000 \text{ ml } 0.1 \text{ M}$$

or $$0.01722 \text{ g } C_6H_8N_2O_2S \equiv 1 \text{ ml of } 0.1 \text{ M } NaNO_2$$

At the equivalence point a slight excess of HNO_2 is present which must persist for at least 1 minute. This excess HNO_2 may be detected by employing either starch iodide strip or paste and designated by the following equation :

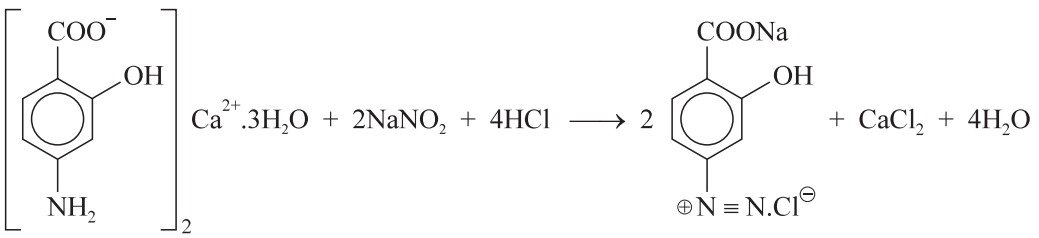
$$2I^- + 2HNO_2 + 2H^+ \longrightarrow I_2 + 2NO + 2H_2O$$

Procedure : Weigh accurately 0.5 g of suphanilamide and transfer to a beaker. Add to it 20 ml of hydrochloric acid and 50 ml of DW, stir until dissolved and cool to 15°C in an ice-bath. Add to it 25 g of crushed ice, and titrate slowly with sodium nitrite solution, stirring vigorously, until the tip of the glass rod dipped into the titrated solution immediately produces a distinct blue ring on being touched to starch-iodide paper. The titration is supposed to be complete when the end-point is deducible after the resulting mixture has been allowed to stand for 1 minute. Each 0.01722 g of sulphanilamide is equivalent to 1 ml of 0.1 N sodium nitrite.

12.3.3. CALCIUM AMINOSALICYLATE

Materials Required : Calcium aminosalicylate : 0.5 g ; hydrochloric acid (≃ 11.5 N) : 10.0 ml ; potassium bromide : 1.0 g ; 0.1 M sodium nitrite ; starch-iodide paper.

Theory : The assay of calcium aminosalicylate is based upon the reaction designated by the following equation :



344.38

Therefore, 344.38 g $C_{14}H_{l2}CaN_2O_6 \equiv 2NaNO_2 \equiv 2000$ ml M

or 172.1 g $C_{14}H_{l2}CaN_2O_6 \equiv 1000$ ml M

or 17.21 g $C_{14}H_{l2}CaN_2O_6 \equiv 1000$ ml 0.1 M

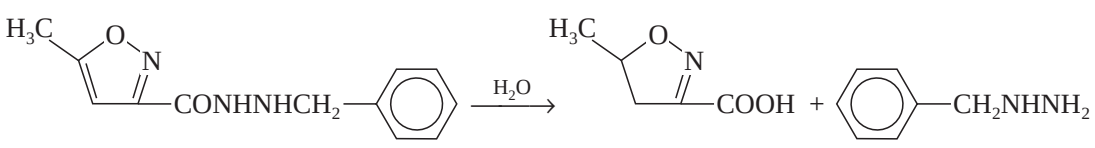
or 0.1722 g $C_{14}H_{l2}CaN_2O_6 \equiv 1$ ml of 0.1 M $NaNO_2$

Procedure : Weigh accurately about 0.5 g of calcium aminosalicylate, into a funnel placed in the mouth of a 250 ml volumetric flask. Wash through with 10 ml of hydrochloric acid and enough DW to dissolve, add 1.0 g potassium bromide and make up the volume upto 250 ml mark. Pipette 50 ml into a conical flask, cool to below 15°C (in ice-bath) and titrate gradually with 0.1 M sodium nitrite solution while shaking the contents of the flask vigorously and continuously until a distinct blue colour is achieved when a drop of the titrated solution is placed on a starch-iodide paper 5 minutes after the last addition of the 0.1 M $NaNO_2$ solution. Care must be taken to add $NaNO_2$ solution at the rate of 0.1 ml near the end of the titration. Each ml of 0.1 M sodium nitrite is equivalent to 0.01722 g of $C_{14}H_{l2}CaN_2O_6$.

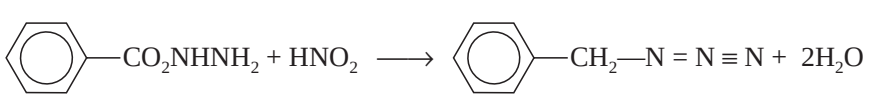
12.3.4. ISOCARBOXAZID

Materials Required : Isocarboxazid : 0.5 g ; glacial acetic acid (99% w/w or 17.5 N) : 20.0 ml ; hydrochloric acid (≃ 11.5 N) : 20.0 ml ; 0.1 M sodium nitrite ; starch-iodide paper.

Theory : The estimation is based on the fact that isocarboxazid undergoes rapid cleavage in acidic medium to produce benzylhydrazine. The latter reacts quantitatively with nitrous acid ($NaNO_2$ and HCl) to give rise to benzylazide. The various reactions involved are expressed as follows :



(231.25) Benzylhydrazine



Benzylazide

Therefore, $C_{12}H_{13}N_3O_2 \equiv NaNO_2 \equiv 1000$ ml M

or 231.25 g $C_{12}H_{13}N_3O_2 \equiv 1000$ ml M

or 23.125 g $C_{12}H_{13}N_3O_2 \equiv 1000$ ml 0.1 M

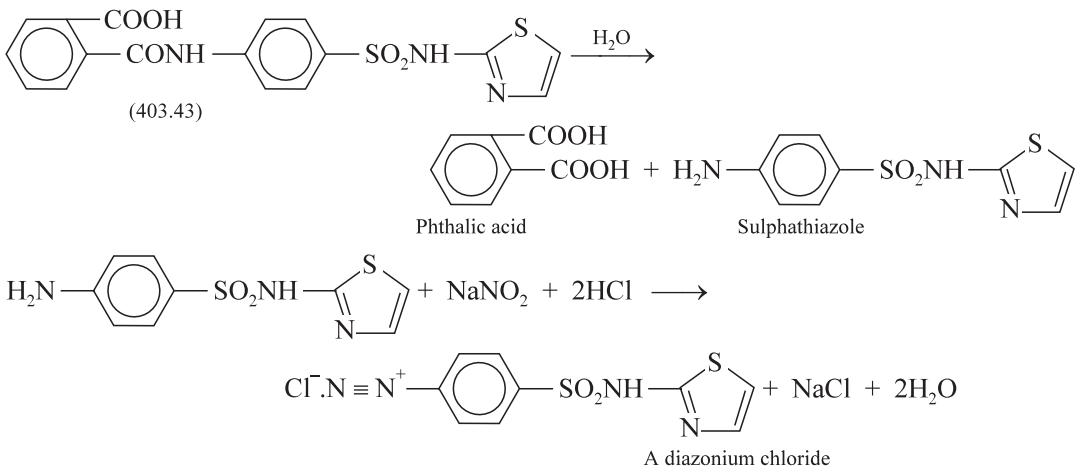
or 0.02313 g $C_{12}H_{13}N_3O_2 \equiv 1$ ml 0.1 M $NaNO_2$

Procedure : Weigh accurately about 0.5 g of isocarboxazid and dissolve it in 20 ml of glacial acetic acid. Add to it 20 ml of hydrochloric acid and 50 ml of DW. Cool to about 15°C in an ice-bath and titrate slowly with 0.1 M $NaNO_2$ while shaking vigorously and continuously until a distinct blue colour is obtained on a starch-iodide paper that lasts for 5 minutes after the final addition of the 0.1 M $NaNO_2$ solution to the titrated solution. Add $NaNO_2$ solution very carefully at the rate of 0.1 ml at a time as the end-point is approached. Each mole of 0.1 M sodium nitrite is equivalent to 0.02313 g of $C_{12}H_{13}N_3O_2$.

12.3.5. PHTHALYLSULPHATHIAZOLE

Materials Required : Phthalylsulphathiazole : 0.5 g ; sodium hydroxide solution (20% w/v in water) : 10.0 ml ; hydrochloric acid (≃ 11.5 N) : 20.0 ml ; 0.1 M sodium nitrite ; starch-iodide paper.

Theory : The assay is based upon the reactions designated by the following equations :



Therefore, $C_{17}H_{13}N_3O_5S_2 \equiv NaNO_2 \equiv 1000$ ml M

or 403.43 g $C_{17}H_{13}N_3O_5S_2 \equiv 1000$ ml M

or 40.343 g $C_{17}H_{13}N_3O_5S_2 \equiv 1000$ ml 0.1 M

or 0.04034 g $C_{17}H_{13}N_3O_5S_2 \equiv 1$ ml 0.1 M $NaNO_2$

Phthalylsulphathiazole undergoes hydrolysis to give phthalic acid and sulphathizole. The latter reacts with nitrous acid to yield the corresponding diazonium salt quantitatively.

Procedure : Weigh accurately about 0.5 g of phthalylsulphathiazole and heat on a water-bath for 2 hours after the addition of 10.0 ml of sodium hydroxide solution. Cool the contents of the flask to 15°C in an ice-bath, add to it 10.0 ml of water and 20.0 ml of hydrochloric acid and carry out the titration slowly with 0.1 M sodium nitrite solution. The contents of the flask are shaken thoroughly and continuously until a distinctly visible blue colour is obtained when a drop of the titrated solution is placed on a starch-iodide paper 5 minutes after the last addition of the 0.1 M $NaNO_2$ solution. Towards the approach of the end-point the addition of $NaNO_2$ solution must be at the rate of 0.1 ml. Each ml of 0.1 M sodium nitrite is equivalent to 0.04034 g of $C_{17}H_{13}N_3O_5S_2$.

12.3.6. COGNATE ASSAYS

A plethora of pharmaceutical substances that can be assayed by the help of sodium nitrite titrations are mentioned in Table 12.1.

Table 12.1 : Substance Assayed by Direct Titrations with Sodium Nitrite

S.No.	Name of Substance	Qty. Prescribed	Indicator Employed	Calculations
1.	Dapsone	0.3 g	Starch-iodide paper/paste	Each ml of 0.1 M $NaNO_2$ ≡ 0.01242 g of $C_{12}H_{12}N_2O_2S$
2.	Primaquine phosphate	1.0 g	-do-	Each ml of 0.1 M $NaNO_2$ ≡ 0.04553 g of $C_{15}H_{21}N_3O$, $2H_3PO_4$
3.	Procainamide hydrochloride	0.5 g	-do-	Each ml of 0.1 M $NaNO_2$ ≡ 0.02718 g of $C_{l3}H_{21}N_3O$, HCl
4.	Procaine hydrochloride	1.0 g	-do-	Each ml of 0.1 M $NaNO_2$ ≡ 0.02728 g of $C_{13}H_{20}N_2O_2$, HCl
5.	Sodium amino-salicylate	2.5 g	- do-	Each ml of 0.1 M $NaNO_2$ ≡ 0.01752 g of $C_7H_6NNaO_3$
6.	Succinylsulpha-thiazole	0.5 g	-do-	Each ml of 0.1 M $NaNO_2$ ≡ 0.03554 g of $C_{13}H_{13}N_3O_5S_2$
7.	Sulphacetamide sodium	0.5 g	-do-	Each ml of 0.1 M $NaNO_2$ ≡ 0.02362 g of $C_8H_9N_2NaO_3S$
8.	Sulphadiazine	0.5 g	-do-	Each ml of 0.1 M $NaNO_2$ ≡ 0.02503 g of $C_{10}H_{10}N_4O_2S$
9.	Sulphadimethoxine	0.5 g	-do-	Each ml of 0.1 M $NaNO_2$ ≡ 0.0313 g of $C_{12}H_{14}N_4O_4S$
10.	Sulphadimidine	0.5 g	-do-	Each ml of 0.1 M $NaNO_2$ ≡ 0.02783 g of $C_{12}H_{14}N_4O_4S$
11.	Sulphadimidine Sodium	0.5 g	-do-	Each ml of 0.1 M $NaNO_2$ ≡ 0.03003 g of $C_{l2}H_{13}N_4NaO_2S$
12.	Sulphalene	0.5 g	-do-	Each ml of 0.1 M $NaNO_2$ ≡ 0.02803 g of $C_{11}H_{12}N_4O_3S$
13.	Sulphamethizole	0.5 g	-do-	Each ml of 0.1 M $NaNO_2$ ≡ 0.02703 g of $C_9H_{10}N_4O_2S_2$
14.	Sulphamethoxazole	0.5 g	-do-	Each ml of 0.1 M $NaNO_2$ ≡ 0.02533 g of $C_{l0}H_{11}N_3O_3S$
15.	Sulphaphenazole	0.5 g	-do-	Each ml of 0.1 M $NaNO_2$ ≡ 0.03144 g of $C_{15}H_{l4}N_4O_2S$

THEORETICAL AND PRACTICAL EXERCISES

1. What is the '**diazotization**' reaction ? How does it help in the assay of drugs ? Explain.

2. Why is it necessary to perform '**sodium nitrite titrations**' invariably in an acidic medium ? Provide a plausible explanation based on chemical reactions.

3. (*a*) How would you prepare 1 L of 0.1 M $NaNO_2$ solution ? How can we standardize the above solution using pure sulphanilamide ?

Explain with various reactions involved, along with the procedural details.

(*b*) Discuss the assay of calcium aminosalicylate.

4. Based on the '**diazotization reaction**' how would you carry out the assay of the following '**drug substances**' :

(*i*) Isocarboxazid (*ii*) Phthalylsulphathiazole

(*iii*) Sulphamelthoxazole (*iv*) Primaquine phosphate.

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7. Beckett, A.H., and J.B. Stenlake, '**Practical Pharmaceutical Chemistry**', 4th ed., Pt-1, New Delhi, CBS-Publishers and Distributors, 1988.
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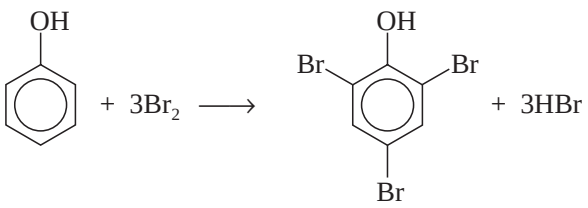
13 ESTIMATION OF PHENOLS AND RELATED COMPOUNDS

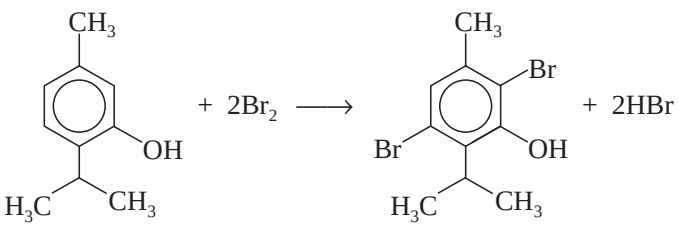
CONTAINS :

13.1. INTRODUCTION

In oxidation-reduction methods bromine is employed as an oxidizing agent in place of iodine, because it is reduced quantitatively be the readily oxidized pharmaceutical organic substances in a reaction which results in either water-insoluble bromine substitution products, for instance :

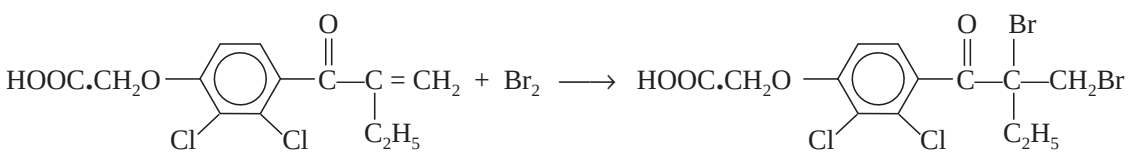


Phenol — 2, 4, 6-Tribromophenol

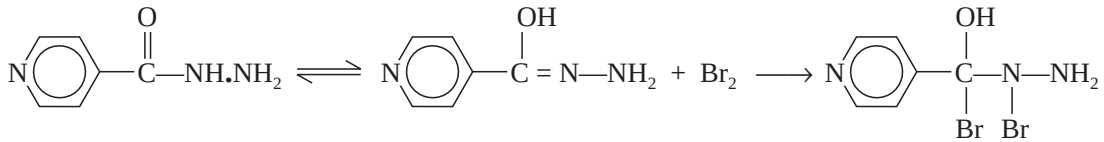


Thymol

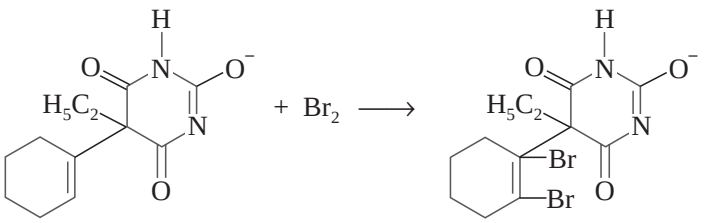
or corresponding water-insoluble bromine-addition products, such as :



Ethacrynic acid — Dibromo derivative



Isoniazid (‘*keto*’-form) Isoniazid (‘*enol*’-form) Dibromo derivative



Cyclobarbitone Dibromo derivative

However, the standard solution used does not have bromine (Br_2) as such but it does contain an equivalent amount of potassium bromate and an excess of potassium bromide and the resulting mixture on subsequent acidification liberates bromine. The reaction may be expressed as follows :

$$5KBr + KBrO_3 + 6HCl \longrightarrow 6KCl + 3Br_2 + 3H_2O$$

The liberated bromine helps in oxidizing iodide to an equivalent amount of iodine as shown below :

$$2KI + Br_2 \longrightarrow 2KBr + I_2$$

The free iodine thus produced is titrated with previously standardized sodium thiosulphate solution as depicted below :

$$I_2 + \underset{\text{Sodium thiosulphate}}{2Na_2S_2O_3} \longrightarrow 2NaI + \underset{\text{Sodium tetrathionate}}{Na_2S_4O_6}$$

13.2. THEORY

In oxidation-reduction assays the use of bromine is judiciously carried out as an oxidizing agent effectively for such specific compounds which ultimately results into the formation of both bromine substitution and bromine additive compounds. These products of reaction are produced quantitatively and are mostly water-insoluble in characteristics ; and more interestingly they take place in an acidic medium.

As it has been discussed earlier, iodine cannot be used directly as an oxidizing agent in such type of assays, whereas the liberated iodine quantitatively produced by the oxidation of iodide with bromine (excess) may be assayed by titrating against sodium thiosulphate solution.

13.3. ASSAY METHODS

Assay methods based on bromine may be classified under the following *three* heads, namely :

(*i*) Titrations with 0.1 N Bromine,

(*ii*) Titrations with Potassium Bromate, and

(*iii*) Titrations with Potassium Iodate.

13.3.1. TITRATIONS WITH 0.1 N BROMINE

This involves the preparation of 0.1 N bromine solution and subsequent standardization with 0.1 N sodium thiosulphate solution. Bromine solution is also known as Koppeschaar’s Solution in some literature.

13.3.1.1. Preparation of 0.1 N Bromine Solution

Materials Required : Potassium bromate : 3.0 g ; potassium bromide : 15 g.

Procedure : Weigh 3 g of potassium bromate and 15 g of potassium bromide in a beaker and dissolve with water. Transfer it quantitatively into a 1 litre volumetric flask and make up the volume with DW.

13.3.1.2. Standardization of 0.1 N Bromine with 0.1 N Sodium Thiosulphate Solution

Materials Required : 0.1 N Bromine solution ; hydrochloric acid ($\simeq$ 11.5 N) : 5 ml ; potassium iodide solution (10% w/v in water) : 5.0 ml ; 0.1 N sodium thiosulphate ; starch solution.

Procedure : Transfer 25 ml of 0.1 N bromine solution with the help of a pipette into a 500 ml iodine flask and dilute it with 120 ml of DW. Add to it 5 ml of hydrochloric acid, moisten the glass-stopper with water and insert the stopper in the flask. Shake the contents gently. Now, add 5 ml of potassium iodide solution, again lace the stopper and allow the resulting mixture to stand for 5 minutes in the dark. Titrate the liberated iodine with previously standardized 0.1 N sodium thiosulphate solution, adding 3 ml of freshly prepared starch solution towards the end-point. Each ml of 0.1 N sodium thiosulphate is equivalent to 0.01598 g of Br_2.

13.3.1.3. Thymol

Materials Required : Thymol : 0.1 g ; N sodium hydroxide : 25.0 ml ; dilute hydrochloric acid (10% v/v of HCl) : 20.0 ml ; 0.1 N bromine ; methyl orange solution (0.1% w/v soln. in 20% alcohol).

Procedure : Weigh accurately about 0.1 g of thymol, transfer to a 250-ml iodine flask and dissolve in 25.0 ml of N sodium hydroxide. Add to it 20.0 ml of dilute hydrochloric acid and immediatelv titrate with 0.1 N bromine to within 1 to 2 ml of the calculated end-point. Warm the solution to about 75°C, add 2 drops of methyl orange solution and continue the titration gradually while swirling the contents of the flask thoroughly after each addition. When the colour of the methyl orange is discharged, add 2 drops of 0.1 N bromine, shake well, add 1 drop of methyl orange solution and shake vigorously. If the colour of the solution is still red, continue the titration dropwise and with constant stirring until the red colour of the indicator is discharged completely. Repeat the alternate addition of 0.1 N bromine and methyl orange solution until the red colour is discharged after the addition of the methyl orange solution. Each ml of 0.1 N bromine is equivalent to 0.003755 g of $C_{10}H_{14}O$.

Calculations :

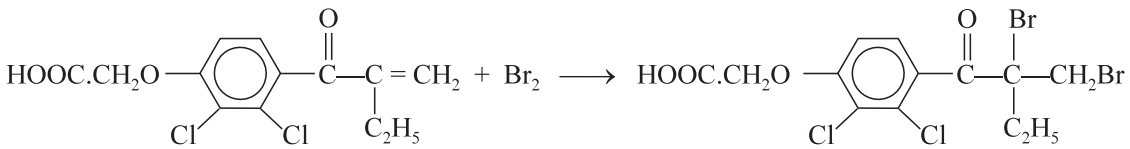
$$\underset{150.22}{C_{10}H_{14}O} + 2Br_2 \longrightarrow C_{10}H_{l2}Br_2O + 2HBr$$

Since, 1 mole of thymol reacts with 2 mol, 4 equivalent of bromine under the conditions of the assay, the equivalent weight of thymol is 37.55 g, 1/4 gramme molecular weight (*i.e.*, 150.22/4 = 37.55). Therefore, each milliliter of 0.1 N bromine consumed in the reaction with thymol is equivalent to 0.1×0.03755 = 0.003755 g or 0.1 meq. of thymol ($C_{10}H_{14}O$).

13.3.1.4. Ethacrynic Acid

Theory : Active bromine is liberated from the standard solution of bromine in an acidic medium (HCl) that subsequently attacks the double bond present in the side chain of the ethacrynic acid molecule thereby resulting into the formation of the corresponding dibromo derivative. This particular reaction takes place quantitatively. Hence, the reactions involved in this assay may be expressed as follows :

$$KBrO_3 + 5KBr + 6HCl \longrightarrow 6Br^{\ominus} + 6KCl + 3H_2O$$



Ethacrynic acid — Dibromo derivative

A blank determination is always performed simultaneously to account for the losses caused by the bromine as well as iodine vapours due to the interaction of excess bromine on potassium iodide.

Materials Required : Ethacrynic acid : 0.2 g ; glacial acetic acid : 40.0 ml ; 0.1 N bromine : 20.0 ml ; hydrochloric acid (≃ 11.5 N) : 3.0 ml ; potassium iodide solution ; (10% w/v in water) : 20 ml ; 0.1 N sodium thiosulphate ; starch solution.

Procedure : Weigh accurately about 0.2 g of ethacrynic acid, dissolve in 40 ml of glacial acetic acid in a 250 ml iodine flask. Add to it 20 ml of 0.1 N bromine and 30.0 ml of hydrochloric acid, immediately place in position the moistened stopper to the ffask, mix the contents vigorously and allow it to stand in a dark place for 60 minutes (to complete the reaction with bromine). Add to it 100 ml of water and 20 ml of KI Solution and titrate immediately with 0.1 sodium thiosulphate, employing freshly prepared starch solution as an indicator towards the end of the titration. Repeat an operation without the pharmaceutical substance (blank titration) ; thus the difference between the titrations represents the amount of bromine required by the ethacrynic acid. Each ml of 0.1 N bromine is equivalent to 0.01516 g of $C_{13}H_{12}Cl_2O_4$.

Calculations : From the above equations, we have :

$$C_{13}H_{12}Cl_2O_4 \equiv Br_2 \equiv 2e$$

or $303.14 \text{ g } C_{13}H_{12}Cl_2O_4 \equiv 2000 \text{ ml N}$

or $151.57 \text{ g } C_{13}H_{12}Cl_2O_4 \equiv 1000 \text{ ml N}$

or $0.01516 \text{ g } C_{13}H_{12}Cl_2O_4 \equiv 1 \text{ ml of 0.1 N Bromine}$

Alternatively, we have :

$$C_{13}H_{12}Cl_2O_4\ \% = \frac{\text{ml difference} \times \text{N} \times (303.14/2000) \times 100}{\text{wt. of sample}}$$

13.3.1.5. Cognate Assays

A number of pharmaceutical substances may be determined quantitatively by titration with bromine as given in Table 13.1.

Table 13.1 : Substances Assayed by Direct Titration with Bromine

S.No.	Name of Substance	Qty. Prescribed	Indicator Employed	Calculations
1.	Compound Benzoic acid Ointment (For Salicylic acid)	2.5 g	Starch solution	Each ml of 0.1 N bromine ≡ 0.002302 g of $C_7H_6O_3$
2.	Cyclobarbitone Tablets	0.5 g	-do-	Each ml of 0.1 N bromine ≡ 0.01277 g of $C_{24}H_{30}CaN_4O_6$
3.	Isoniazid	0.4 g	-do-	Each ml of 0.1 N bromine ≡ 0.003429 g of $C_6H_7N_3O$
4.	Methylparaben	0.1 g	-do-	Each ml of 0.1 N bromine ≡ 0.002536 g of $C_8H_8O_3$
5.	Phenylephrine hydrochloride	0.1 g	-do -	Each ml of 0.1 N bromine ≡ 3.395 g of $C_9H_{13}NO_2$. HCl

13.3.2. TITRATIONS WITH POTASSIUM BROMATE

Potassium bromate can also be employed as an oxidizing agent in the assay of a number of pharmaceutical substances, namely : mephenesin, phenol, and sodium salicylate. This particular method solely depends upon the formation of iodine monobromide (IBr) in relatively higher concentration of hydrochloric acid solution.

13.3.2.1. Preparation of 0.1 N Potassium Bromate

Theory : Potassium bromate can be estimated by the addition of potassium iodide and dilute hydrochloric acid. Thus, we have :

$$KBrO_3 + HI \longrightarrow HIO_3 + KBr$$

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

or $KBrO_3 \equiv IO_3^- \equiv 3I_2 \equiv 6e$

or 167.02 g $KBrO_3 \equiv$ 6000 ml N

or 27.84 g $KBrO_3 \equiv$ 1000 ml N

or 0.002784 g $KBrO_3 \equiv$ 1 ml of 0.1 N Sodium thiosulphate

Materials Required : Potassium bromate : 2.784 g.

Procedure : Weigh accurately 2.784 g of potassium iodide into a beaker and dissolve it in suffcient DW. Transfer the solution quantitatively into a 1 litre volumetric flask and make up the volume to the mark.

13.3.2.2. Standardization of 0.1 N Potassium Bromate Solution with the help of 0.1 N Sodium Thiosulphate

Materials Required : 0.1 N Potassium bromate ; potassium iodide : 3.0 g ; hydrochloric acid ($\simeq$ 11.5 N) : 3.0 ml ; 0.1 N sodium thiosulphate ; starch solution : 3.0 ml.

Procedure : Transfer an accurately measured volume of about 30.0 ml of 0.1 N potassium bromate solution into a 250 ml iodine flask. Add to it 3.0 g potassium iodide, followed by 3.0 ml of potassium iodide, followed by 3.0 ml of hydrochloric acid. Mix the contents thoroughly and allow it to stand for 5 minutes with its stopper in position. Titrate the liberated iodine with previously standardized 0.1 N sodium thiosulphate, using 3.0 ml of freshly prepared starch solution as an indicator at the end-point. Carry out a blank run using the same quantities of the reagents and incorporate the necessary corrections, if any. Each ml of 0.1 N sodium thiosulphate is equivalent to 0.002784 g of $KBrO_3$.

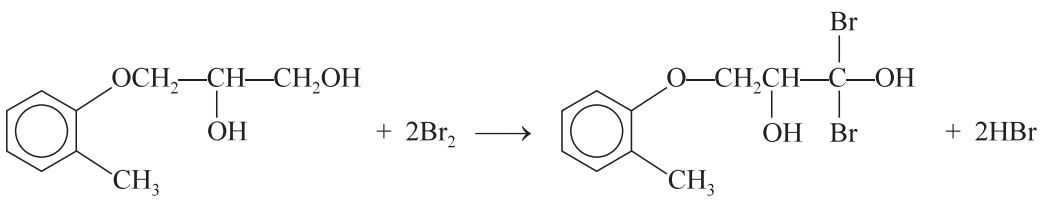
13.3.2.3. Mephenesin

Theory : Mephenesin undergoes oxidation with bromine to yield a dibromo derivative as expressed in the following equation :

$$BrO_3^- + 6e + 6H^+ \longrightarrow Br^- + 3H_2O \quad ...(a)$$

$$2BrO_3^- + 10e + 12H^+ \longrightarrow Br_2 + 6H_2O \quad ...(b)$$

In this instance an excess of potassium bromate is employed. Therefore, any bromide formed [Eq. (*a*)] is oxidized to bromine, and the excess bromate and the bromine are assayed bromometrically. The reduction of bromate to bromine may be designated as in [Eq. (*b*)].



Mephenesin Dibromo derivative

Hence, we have :

$$C_{10}H_{l4}O_3 \equiv Br_2 \equiv 2e$$

or $182.22 \text{ g } C_{10}H_{l4}O_3 \equiv 2000 \text{ ml N}$

or $91.11 \text{ g } C_{10}H_{l4}O_3 \equiv 1000 \text{ ml N}$

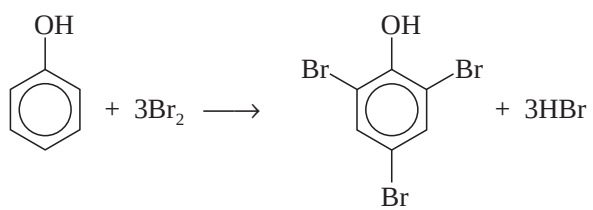
or $0.00911 \text{ g } C_{10}H_{l4}O_3 \equiv 1 \text{ ml of } 0.1 \text{ N } KBrO_3$

Materials Required : Mephenesin : 0.15 g ; 0.1 N potassium bromate : 25.0 ml ; potassium bromide powder : 10.0 g ; hydrochloric acid (25% w/v) : 10.0 ml ; potassium iodide solution (10% w/v in water) : 10.0 ml ; 0.1 N sodium thiosulphate solution ; starch solution.

Procedure : Weigh accurately 0.15 g of mephenesin and dissolve in 50 ml of DW into a 250 ml iodine-flask. Add to it 25.0 ml of 0.1 N potassium bromate solution and 10.0 g of powdered potassium bromide. After the dissolution of KBr, add 10 ml of hydrochloric acid, insert the moistened stopper, and after 10 seconds add 10 ml of potassium iodide solution. Titrate with 0.1 N sodium thiosulphate using starch solution as indicator. Each ml of 0.1 N potassium bromate is equivalent to 0.00911 g of $C_{10}H_{l4}O_3$.

13.3.2.4. Phenol

Theory : Phenol interacts with bromine whereby the former undergoes bromination to yield a water-insoluble 2, 4, 6-tribromophenol. This reaction takes place quantitatively as shown below :



Phenol — 2, 4, 6-Tribromophenol

Thus, we have :

$$C_6H_5\text{—}OH \equiv 3Br_2 \equiv 6e$$

or $94.11 \text{ g } C_6H_5\text{—}OH \equiv 6000 \text{ ml N}$

or $15.685 \text{ g } C_6H_5\text{— } OH \equiv 1000 \text{ ml N}$

or $0.001569 \text{ g } C_6H_5\text{— } OH \equiv 1 \text{ ml } 0.1 \text{ N Potassium Bromate}$

Materials Required : Phenol : 0.5 g ; 0.1 N potassium bromate : 25.0 ml ; potassium iodide (powdered) : 1.0 g ; dilute hydrochloric acid (10% w/w of HCl) : 10.0 ml ; potassium iodide (10% w/v in water) : 10 ml ; chloroform : 10.0 ml ; 0.1 N sodium thiosulphate ; starch solution.

Procedure : Weigh accurately 0.5 g of phenol and dissolve in sufficient water to produce 500 ml in a volumetric flask. Mix 25.0 ml of this solution with 25.0 ml of 0.1 N potassium bromate in a 250 ml iodine flask and add to it 1 g of powdered KI and 10.0 ml of dilute hydrochloric acid. Moisten the glass stopper with a few drops of KI solution and place it in position. Set it aside in a dark place for 20 minutes while shaking the contents frequently in between. Add to it 10 ml of KI solution, shake the contents thoroughly and allow it to stand in the dark for a further duration of 5 minutes. Wash the stopper and neck of the flask carefully with DW, add 10 ml chloroform and titrate with the liberated iodine with 0.1 N sodium thiosulphate using freshly prepared starch as an indicator. Carry out a blank titration simultaneously and incorporate any necessary correction, if required. Each ml of 0.1 N potassium bromate is equivalent to 0.001569 g of C_6H_6O.

13.3.2.5. Cognate Assays

A few other pharmaceutical substances may also be assayed by titrating with 0.1 N potassium bromate as indicated in Table 13.2.

Table 13.2. Substances Assayed by 0.1 N Potassium Bromate

S.No.	Name of Substance	Qty. Prescribed	Indicator Employed	Calculations
1.	Sodium Salicylate	0.1 g	—	Each ml of 0.2 N $KBrO_3 \equiv 0.005336$ g of $C_7H_5NaO_3$
2.	Chlorocresol	0.07 g	Starch solution	Each ml of 0.0167 M $KBrO_3 \equiv 0.003565$ g of C_7H_7ClO

13.3.3. TITRATIONS WITH POTASSIUM IODATE

Potassium iodate is a fairly strong oxidizing agent that may be used in the assay of a number of pharmaceutical substances, for instance : benzalkonium chloride, cetrimide, hydralazine hydrochloride, potassium iodide, phenylhydrazine hydrochloride, semicarbazide hydrochloride and the like. Under appropriate experimental parameters the iodate reacts quantitatively with both iodides and iodine. It is, however, interesting to observe here that the iodate titrations may be carried out effectively in the presence of saturated organic acids, alcohol and a host of other organic substances.

The oxidation-reduction methods with potassium iodate invariably based on the formation of iodine monochloride (ICl) in a medium of strong hydrochloric acid solution.

13.3.3.1. Preparation of 0.05 M Potassium Iodate

Theory : First of all the potassium iodate is dried to a constant weight at 110°C to make it completely free from moisture and then brought to room temperature in a desiccator. It is pertinent to mention here that KIO_3 is a very stable salt and may be obtained in a very pure form. Therefore, it is possible to prepare the standard solutions of KIO_3 by dissolving the calculated weight of the salt in water and diluting the same to an approximate volume.

Since, the normality of iodate solution varies significantly depending on the nature of the reaction, therefore, in usual practice standard iodate solutions of known molarity are used.

The reduction of potassium iodate to iodide is usually not feasible in a direct titrimetric method (unlike the reduction of potassium bromate to bromide) and hence, has no viable application in the official procedures :

$$IO_3^- + 6e + 6H^+ \longrightarrow I^- + 3H_2O \qquad ...(a)$$

In this type of reaction, 1 mol of KIO_3 is 6 equivalent and a 0.05 M solution would be 0.3 N.

In a situation, whereby excess of potassium iodate is employed, any I^- formed [Eq. (*a*)] is readily oxidized to iodine, and subsequently the excess iodate and the iodine are estimated by the iodometric procedure. Thus, the reduction of the iodate to iodine may be expressed as shown below :

$$2IO_3^- + 10e + 12H^+ \longrightarrow I_2 + 6H_2O \qquad ...(b)$$

In such a reaction, 1 mol of iodate is 5 equivalent and a 0.05 M solution would be 0.25 N. This reaction of iodate is never used in the offcial assay methods.

Interestingly, at higher concentrations of hydrochloric acid, both the iodide and iodine obtained as reduction products of iodate [Eqs. (*a*) and (*b*)] are quantitatively converted to I^+. It forms the basis of official procedures for iodate titrations.

The iodine produced intially by the reduction of iodate [Eq. (*b*)] undergoes solvolysis in a polar solvent as expressed in the following reaction :

$$I_2 \rightleftharpoons I^+ + I^-$$

The iodine cation forms iodine monochloride (ICl) in a medium having sufficiently high concentration of HCl and the latter is subsequently stabilized by complex ion formation. Thus, we have :

$$I^+ + HCl \rightleftharpoons ICl + H^+ \quad ...(c)$$

$$ICl + HCl \rightleftharpoons ICl_2^- + H^+ \quad ...(d)$$

Adding Equations (*c*) and (*d*), we may have :

$$I^+ + 2HCl \rightleftharpoons ICl_2^- + 2H^+$$

In actual practice, either carbon tetrachloride or chloroform is usually added so as to make the end-point distinctly visible. Iodine is liberated at the initial stages of the titration which renders the chloroform layer coloured. At that material point when all the reducing agent under estimation has been duly oxidized, the iodate completes the oxidation of iodine and iodide to I^+, and hence the colour from the chloroform layer disappears.

In official methods of analysis *i.e.*, the iodine monochloride method, the reduction of KIO_3 can be expressed as follows :

$$IO_3^- + 4e + 6H^+ \longrightarrow I^+ + 3H_2O \quad ...(e)$$

In Eq. (*e*), 1 mol of KIO_3 is 4 equivalent, and a 0.05 solution would be 0.2 N.

Materials Required : Potassium iodate : 10.7 g.

Procedure : Weigh accurately 10.7 g of pure potassium iodate, previously dried at 110°C to constant weight, in sufficient DW to produce 1 litre in a volumetric flask.

13.3.3.2. Benzalkonium Chloride

Materials Required : Benzalkonium chloride : 4.0 g ; chloroform : 60.0 ml ; 0.1 N sodium hydroxide : 10.0 ml ; potassium iodide (5% w/v in water) : 10.0 ml ; hydrochloric acid ($\simeq$ 11.5 N) : 40.0 ml ; 0.05 M potassium iodate.

Procedure : Weigh accurately benzalkonium chloride 4.0 g and dissolve it in sufficient DW to make 100 ml. Pipette 25.0 ml into a separating funnel, add 25 ml of chloroform, 10 ml of 0.1 N NaOH and 10 ml of potassium iodide solution. Shake the contents thoroughly, allow to separate and collect the chloroform layer in another separating funnel. Treat the aqueous layer with 3 further quantities each of 10 ml of chloroform and discard the chloroform layer. To the aqueous layer add 40 ml of hydrochloric acid, cool and titrate with 0.05 M potassium iodate till the solution becomes pale brown in colour. Add 2 ml of chloroform and continue the titration until the chlorofonn layer becomes colourless. Titrate a mixture of 29 ml of water, 10 ml of KI solution and 40 ml of hydrochloric acid with 0.05 M potassium iodate under identical conditions (Blank Titration). The differences between the titrations represent the amount of 0.05 M potassium iodate required. Each ml of 0.05 M potassium iodate is equivalent to 0.0354 g of $C_{22}H_{40}ClN$.

Calculations :

$$2C_{22}H_{40}ClN \equiv 2KI \equiv KIO_3$$

or $$354 \text{ g } C_{22}H_{40}ClN \equiv 1000 \text{ ml } 0.5 \text{ M } KIO_3$$

or $$35.4 \text{ g } C_{22}H_{40}ClN \equiv 1000 \text{ ml } 0.05 \text{ M } KIO_3$$

or $$0.0354 \text{ g } C_{22}H_{40}ClN \equiv 1 \text{ ml of } 0.05 \text{ M } KIO_3$$

13.3.3.3. Potassium Iodide

Theory : The iodine monochloride method described earlier employing standard potassium iodate is the basis for the official assay of potassium iodide. Vigorous shaking is a prime requirement, as the end-point is approached in this assay, because of the fact that both iodine and iodate in different phases attribute a heterogeneous medium. However, the reaction involving the oxidation of KI by iodate may be designated as shown below :

$$\underset{2(166.0)}{2KI} + KIO_3 + 6HCl \longrightarrow 3ICl + KCI + 3H_2O$$

The reduction of KIO_3 may be expressed as :

$$IO_3^+ + 4e + 6H^+ \longrightarrow I^+ + 3H_2O$$

Hence, from the above equation we have, 1 mol of KIO_3 is 4 equivalent and a 0.05 M solution would be 0.2 N.

Thus, we have :

$$2KI \equiv IO_3^- \equiv 4e$$

or $$166 \text{ g KI} \equiv 2e \equiv 2000 \text{ ml N}$$

or $$83 \text{ g KI} \equiv 1000 \text{ ml N}$$

or $$16.60 \text{ g KI} \equiv 1000 \text{ ml } 0.2 \text{ N} \equiv 1000 \text{ ml } 0.05 \text{ M}$$

or $$0.01660 \text{ g KI} \equiv 1 \text{ ml } 0.05 \text{ M } KIO_3$$

Materials Required : Potassium iodide : 0.5 g ; hydrochloric acid ($\simeq$ 11.5 N) : 35 ml ; chloroform : 5 ml ; 0.05 M potassium iodate.

Procedure : Weigh accurately 0.5 g of potassium iodide and dissolve it in about 10 ml of DW. Add to it 35 ml of hydrochloric acid and 5 ml of chloroform. Titrate with 0.05 M potassium iodate till the purple colour of iodine disappears from the chloroform layer. Add the last portion of the iodate solution carefully and dropwise while shaking the contents of the flask vigorously and continuously. Allow to stand for 5 minutes. In case any colour still develops in the chloroform layer continue the titration. Each ml of 0.05 M potassium iodate is equivalent to 0.0166 g of potassium iodide.

13.3.3.4. Cognate Assays

A host of other pharmaceutical substances, namely : cetrimide, hydralazine hydrochloride, phenylhydrazine hydrochloride may be assayed by titration with potassium iodate as mentioned in Table : 13.3.

Table 13.3 : Substances Assayed by Potassium Iodate

S.No.	Name of Substance	Qty. Prescribed	Calculations
1.	Cetrimide	2.0 g	Each ml of 0.05 M $KIO_3 \equiv$ 0.03364 g of $C_{17}H_{38}BrN$
2.	Hydralazine hydrochloride	0.15 g	Each ml of 0.02 M $KIO_3 \equiv$ 0.03933 g of $C_8H_8N_4$. HCl
3.	Phenylhydrazine hydrochloride	0.2 g	Each ml of 0.05 M $KIO_3 \equiv$ 0.007231 g of $C_6H_5NHNH_2$. HCl

THEORETICAL AND PRACTICAL EXERCISES

1. Why is '**bromine**' preferred to '**iodine**' in redox methods for the assay of pharmaceutical organic substances ? Explain with suitable examples.

2. (*a*) How would your prepare 1 L of 0.1 N Bromine solution and standardize it with $Na_2S_2O_3$ solution ? Explain.

(*b*) Using 0.1 N Br_2 solution how would you carry out the assay of the following '**drugs**' :

(*i*) Thymol (*ii*) Ethacrynic acid

(*iii*) Isoniazid (*iv*) Methylparaben.

3. (*a*) Explain the procedural details of preparing 1 L of 0.1 N $KBrO_3$ solution and its subsequent standardization with 0.1 N $Na_2S_2O_3$ solution.

(*b*) Discuss the assay of the following medicinal compounds :

(*i*) Phenol (*ii*) Mephenesin

(*iii*) Chlorocresol (*iv*) Sodium salicylate.

4. (*a*) Give the sequential procedure which one may adopt to prepare 1 L of 0.05 M KIO_3 solution, and standardize it.

(*b*) How would you carry out the assay of the following '**drugs**' :

(*i*) Cetrimide (*ii*) Hydralazine Hydrochloride

(*iii*) Phenylhydrazine Hydrochloride.

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14 KARL FISCHER METHOD FOR DETERMINATION OF WATER

CONTAINS :

14.1. INTRODUCTION

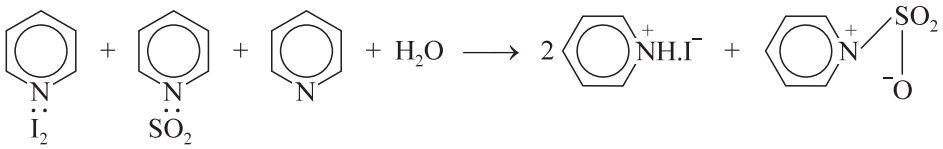
A plethora of chemical compounds for the determination of small amounts of water present in organic solids, pharmaceutical substances and organic solvents have been devised over a length of time. But unquestionably the most important of these is the one proposed by Karl Fischer (1935), which is considered to be relatively specific for water*. It essentially makes use of the Karl Fischer reagent which is composed of iodine, sulphur dioxide, pyridine and methanol.

Note : Both pyridine and methanol should be anhydrous.

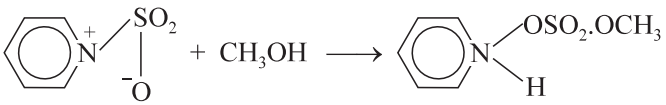
14.2. THEORY

Water present in the analyte reacts with the Karl Fischer reagent in a two-stage process as shown below :

Stage 1 :



Stage 2 :



...(*b*)

From Eq. (*a*) step l, it is obvious that the oxidation of sulphur dioxide takes place by iodine to yield sulphur trioxide and hydrogen iodide thereby consuming one mole of water. In other words, each one molecule

* Mitchell, J., **Anal. Chem 23,** 1069 (1951).

* Mitchell, J., and D.M. Smith, '**Aquametry**', 2nd ed., New York, Interscience, (I977).

of iodine disappears against each molecule of water present in the given sample. It is pertinent to mention here that in the presence of a large excess of pyridine (C_5H_5N), all reactants as well as the resulting products of reaction mostly exist as complexes as evident from Eqs. (*a*) and (*b*).

Stability of the Reagent : The stability of the original Karl Fischer reagent initially prepared with an excess of methanol was found to be fairly poor and hence, evidently needed frequent standardization. However, it was estabtished subsequently that the stability could be improved significantly by replacing the methanol by 2-methoxyethanol.

It has been observed that the titer of the Karl Fischer reagent, which stands at 3.5 mg of water per milliliter of reagent, falls rapidly upon standing with the passage of time. Hence, the following precautions must be observed rigidly using the Karl Fischer reagent, namely :

(*a*) Always prepare the reagent a day or two before it is to be used,

(*b*) Great care must be taken to prevent and check any possible contamination either of the reagent or the sample by atmospheric moisture,

(*c*) All glassware(s) must be thoroughly dried before use,

(*d*) Standard solution should be stored out of contact with air, and

(*e*) Essential to minimise contact between the atmosphere and the solution during the course of titration.

End-point Detection : The end-point of the Karl Fischer titration may be determined quite easily by adopting the electrometric technique employing the dead-stop end-point method. When a small quantum of e.m.f. is applied across two platinum electrodes immersed in the reaction mixture, a current shall tend to flow till free iodine exists, to remove hydrogen and ultimately depolarize the cathode. A situation will soon arise when practically all the traces of iodine have reacted completely thereby setting the current to almost zero or very close to zero or attain the end-point.

Limitations of Karl Fischer Titration : The Karl Fischer titration has a number of serious limitations due to possible interferences tantamount to erroneous results, namely :

(*i*) **Oxidizing agents**, for instance : chromates, Cu(II), Fe(III), $Cr_2O_7^{2-}$, peroxides, salts, higher oxides,

Example :

$$MnO_2 + 4C_5H_5NH^+ + 2I^- \longrightarrow Mn^{2+} + 4C_5H_5N + I_2 + H_2O$$

(*ii*) **Reducing agents**, such as : Sn(II) salts, sulphides, and $S_2O_3^{2-}$, and

(*iii*) Compounds that have a tendency to form water with the ingredients of the Karl Fischer reagent, for instance :

(*a*) **basic oxides :** *e.g.*, ZnO ;

Example : $ZnO + 2C_5H_5NH^+ \longrightarrow Zn^{2+} + C_5H_5N + H_2O$

(*b*) **salts of weak oxy-acids** *e.g.*, $NaHCO_3$;

Example : $NaHCO_3 + C_5H_5NH^+ \longrightarrow Na^+ + H_2O + CO_2 + C_5H_5N$

Note : As H_2CO_3, carbonic acid, is very unstable ; hence it splits up to yield a mole each of water and CO_2.

14.3. INSTRUMENTATION

Figure 14.1 illustrates a simple dead-stop end-point assembly or a Karl Fischer titration apparatus. The titration vessel is fitted with a pair of identical platinum electrodes, a mechanical stirrer with adjustable speed, and a burette. It will be observed that absolutely little or no current may flow unless and until the solution is totally free from any polarizing substances ; this could perhaps be due to the absorbed layers of oxygen and hydrogen on the anode and cathode respectively. However, the current shall flow only when the two electrodes

get depolarized. The Karl Fischer reagent is pumped into the burette by means of hand bellows, the eccess of moisture is usually prevented by employing an appropriate arrangement of desiccant tubes. Alternatively, the stirring may also be accomplished either by using a magnetic stirrer or by means of a suitably dried nitrogen passed gently through the solution during the course of titration.

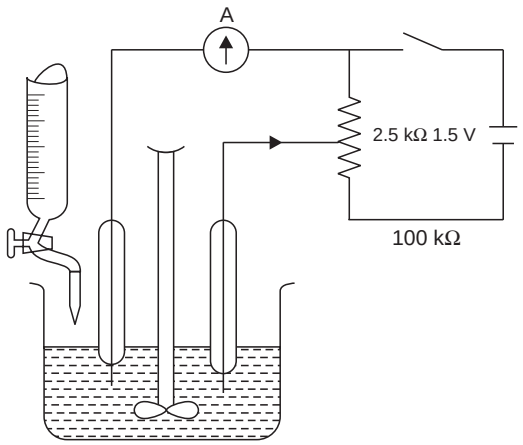


Figure 14.1 : Dead-Stop End-point Assembly.

The end-point is achieved by employing an eiectrical circuit comprising of a microammeter (A), platinum electrodes, together with a 1.5 V to 2.0 V battery connected across a variable resistance of about 2.5 kΩ. First of all the resistance is adjusted in such a manner that an initial current passes through the platinum electrodes in series with a microammeter (A). After each addition of reagent, the pointer of the microammeter gets deflected but quickly returns to its original position. At the end of the reaction a deflection is obtained which persists for 10-15 seconds.

14.3.1. AUTOMATED ELECTROCHEMICAL KARL FISCHER ANALYSIS

Commercially available Modern KF-Titrators are usually equipped with specifically designed titration vessels that are exclusively meant to check and prevent the contact with atmospheric moisture. Quite a few such devices are armed with microprocessors that will perform the requisite operations sequentially in a programmed manner automatically ; and may also dish out a print-out of the desired results including the percentage moisture content. In fact, these Modern KF-Titrators not only afford greater accuracy and precision in results but also offer much ease and convenience in routine analysis as compared to the classical techniques based on either caulometry or controlled current potentiometry using two indicator electrodes.

In this procedure the iodide needed for the reaction with water is normally generated within the titration vessel *caulometrically* as shown below :

$$H_2O + I_2 + SO_2 + 3C_5H_5N + CH_3OH \longrightarrow 2C_5H_5N.HI + C_5H_5NH.SO_4.CH_3$$

Thus, the basis of the analysis rests upon the quantitative relationship existing between charge passed and iodine produced by the reagent according to the above reaction. Therefore, the generation of iodine is automatically stopped when an excess of it is detected by the indicator electrode. It essentially consists of two platinum electrodes across which an AC is applied and subsequently a marked drop in voltage between the electrodes takes place as soon as an excess of iodine is present. Normally such automated instruments make use of *proprietory reagents exclusively*.

The major advantage of this approach to KF-analysis being that no calibration is required as the method is absolute and is entirely based on the stoichiometry of the aforesaid equation. It is noteworthy that one may determine the amounts of water ranging between 10 mcg and 10 mg in solid as well as liquid samples.

14.4. APPLICATIONS OF KARL FISCHER METHOD FOR DETERMINATION OF WATER IN PHARMACEUTICAL ANALYSIS

The Karl Fischer method for the determination of water is used for prednisolone sodium phosphate as described below.

14.4.1. PREDNISOLONE SODIUM PHOSPHATE

Materials Required : Karl Fischer Reagent* : 100 ml ; prednisolone sodium phosphate : 0.2 g ; anhydrous methanol : 20.0 ml.

Procedure : Add about 20 ml of anhydrous methanol to the titration vessel and titrate to the amperometric end-point with the Karl Fischer reagent. Quickly add 0.2 g of prednisolone sodium phosphate sample, stir for 1 minute and again titrate to the amperometric end-point with the Karl Fischer reagent. The difference between the two titrations gives the volume (v) of Karl Fischer reagent consumed by the sample.

The minimum water equivalent is 3.5 mg of water per ml of Karl Fischer reagent. Hence, the percentage of water w/w in the given sample may be calculated by the following expression :

$$\text{Water \% (w/w)} = \frac{v \times 3.5}{\text{wt. of sample (mg)}} \times 100$$

Precautions :

(1) The reagents and solutions used must be kept anhydrous and necessary care should be taken throughout to prevent exposure to atmospheric moisture,

(2) The Karl Fischer reagent should be protected from light and preferably stored in a bottle fitted with an automatic burette, and

(3) The water equivalent of Karl Fischer reagent should always be determined before use.

14.4.2. COGNATE ASSAYS

A number of other *official pharmaceutical substances* may be assayed for their water content by the Karl Fischer method as summarized in the following Table 14.1.

Table 14.1 : Cognate Assays of Pharmaceutical Substances by Karl Fischer Method

S.No.	Name of Substance	Qty. Prescribed	Prescribed Limit of Water % (w/w)
1.	Rifamycin Sodium	0.2 g	12.0-17.0
2.	Sodium Methyl Hydroxybenzoate	1.0 g	NMT** 5.0
3.	Triamcinolone Acetonide	0.2 g	NMT 2.0

* The use of commercially available Karl Fischer Reagent must be validated in order to verify in each case the stoichiometry and the absence of incompatibility between the substance being examined and the reagent.

** NMT = Not More Than.

THEORETICAL AND PRACTICAL EXERCISES

1. How would you explain the presence of water in an '**anlyte**' usually reacts with Karl Fischer reagent in a *two-stage process* ? Give the chemical reactions involved in the above procedure.

2. Give a brief account on the following :

(*a*) Stability of the KF-reagent

(*b*) End-point detection

(*c*) Limitations of Karl Fischer Titration.

3. (*a*) With the help of a neat-labeled-diagramatic sketch explain the working of a '**Dead-Stop End-point**' assembly.

(*b*) Describe a Modern KF-Titrator using a chemical reaction associated with it. Why is it advantageous in comparison to the dead-stop end-point apparatus.

4. How would you assay the following medicinal compounds :

(*i*) Prednisolone sodium phosphate (*ii*) Rifamycin sodium

(*iii*) Sodium methyl hydroxybenzoate (*iv*) Triamcinolone acetonide.

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15 TETRAZOLIUM ASSAY OF STEROIDS

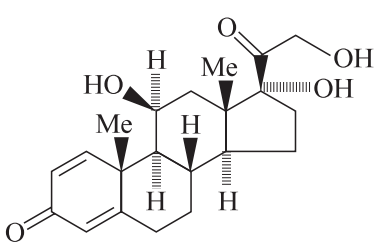
CONTAINS :

15.1. INTRODUCTION

A number of steroids essentially having a α-ketol (21-hydroxy-20 keto) side-chain group, for instance : hydrocortisone, hvdrocortisone acetate, prednisolone, methylprednisolone, methylprednisolone acetate, flucocinolone acetonide, triamcionolone acetonide and the like—are quantitatively reduced by tetrazolium salts to their respective coloured formazan derivatives. Thus, it is possible to carry out the assay of a number of formulations that contain corticosteroids by using triphenyltetrazolium chloride. The said reaction is usually performed in an alkaline medium (tetramethylammonium hydroxide) between a temperature ranging between 30° to 35°C for a duration of 1 to 2 hours. The absorbance of the resulting *formazan derivative* producing a **red product** is usually measured around 484 nm.

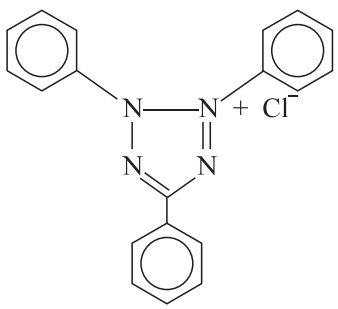
15.2. THEORY

The oxidation of the α-ketol moiety present in the steroid under examination and the subsequent reduction of triphenyltetrazolium chloride to the corresponding triphenylformazan are depicted in the following reaction :

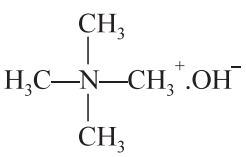


Prednisolone
(C-21 : —CH_2OH)

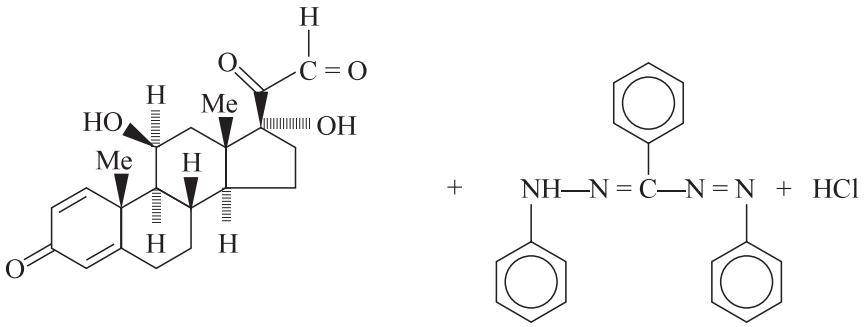
+



Triphenyltetrazolium chloride



Tetramethyl ammonium hydroxide
(30–35° ; 1–2 Hours)



Prednisolone
(C-21 : —CHO)

Triphenylformazan
(Red product)

The triphenyltetrazolium chloride ring undergoes cleavage, as shown by the dotted line, and 2H-atoms are given out by the steroid prednisolone in being converted from C-21, —CH_2OH to C-21, —CHO function ; one of the H-atoms from above is utilized in the formation of the open-chain compound *i.e.*, triphenylformazan derivative ; whereas, the second H-atom abstracts the Cl^- ion as a mole of HCl. The above interaction is of a quantitative nature.

However, it is pertinent to mention here that certain steroids esterified at C-21 position, such as : hydrocortisone acetate, methylprednisolone acetate are duly hydrolyzed in the alkaline medium to give rise to the corresponding free C-21 hydroxy steroids and hence, may also be assayed by adopting the same procedure.

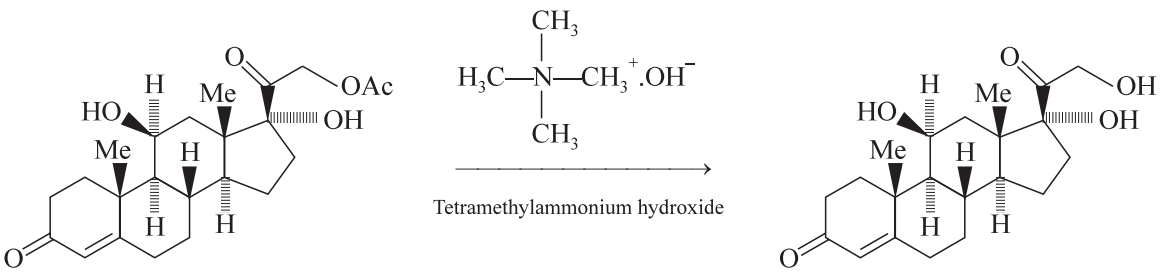
Precautions : All these assays are to be carried out strictly in the absence of light and atmospheric oxygen to get optimum results.

15.3. ASSAY OF PHARMACEUTICAL SUBSTANCES

A number of steroidal pharmaceutical substances listed in the *official compendia* may be assayed by the tetrazolium method of analysis. A few typical examples are described below :

15.3.1. HYDROCORTISONE ACETATE

Theory : Hydrocortisone acetate is first hydrolysed by strong trimethylammonium hydroxide solution to yield the free 21-hydroxysteroid *i.e.*, hydrocortisone as shown below :



Hydrocortisone acetate

Tetramethylammonium hydroxide

Hydrocortosone

The resulting hydrolysed product is then treated with triphenyltetrazolium chloride and the coloured triphenylformazan is measured at 525 nm.

Materials Required : Hydrocortisone acetate : 0.350 g ; aldehyde-free absolute ethanol : 100 ml ; triphenyltetrazolium chloride solution [a 0.5% w/v solution of 2,3,5,-triphenyltetrazolium chloride in aldehyde-free ethanol (96%)] : 10 ml ; dilute tetramethylammonium hydroxide solution [Dilute 10 ml of tetramethylammonium hydroxide solution (10%) to 100 ml with aldehyde-free ethanol (96%). It contains about 1% w/v of $C_4H_{13}NO$. To be prepared immediately before use] : 10 ml.

Procedure : The following steps are to be followed sequentially strictly protected from light :

(1) Dissolve accurately weighed hydrocortisone acetate 300 to 350 mg in 10 ml aldehyde-free absolute ethanol,

(2) Transfer 10 ml to a 25 ml graduated flask, add 2 ml of triphenyltetrazolium chloride solution, displace the air in the flask with oxygen-free nitrogen,

(3) Immediately add 2 ml of dilute tetramethylammonium hydroxide solution and again displace the air with oxygen-free nitrogen,

(4) Stopper the flask, mix the contents by gently swirling and allow to stand in a water-bath maintained at 30°C for 1 hour,

(5) Cool rapidly, add sufficient aldehyde-free absolute ethanol to produce 25 ml,

(6) Mix well and immediately determine the absorbance of the resulting solution in a stoppered cell at the maximum at 485 nm, using in the reference cell a solution prepared at the same time and in the same manner using 10 ml of aidehyde-free absolute ethanol, and

(7) Repeat the operation using the hydrocortisone acetate EPCRS* in place of the substance being examined under the same experimental parameters.

15.3.2. COGNATE ASSAYS

The following pharmaceutical substances may also be assayed by the above method, namely :

(*a*) Methylprednisolone,

(*b*) Hydrocortisone,

(*c*) Prednisolone, and

(*d*) Prednisone.

THEORETICAL AND PRACTICAL EXERCISES

1. What is the underlying principle of '**tetrazolium assay of steroids**' ? Explain with necessary equations involved in it.

2. Describe the assay of the following steroidal drugs :

(*i*) Hydrocortisone acetate (*ii*) Hydrocortisone

(*iii*) Prednisolone (*iv*) Prednisone.

RECOMMENDED READINGS

1. Beckett, A.H., and J.B. Stenlake, '**Practical Pharmaceutical Chemistry**', 4th ed., London, The Athlone Press, 1988.

2. **British Pharmacopoeia,** International Edition, Vols. I & II, HMSO, 1993.

* European Pharmacopoeia Chemical Reference Substances.