Pharmaceutical Analysis: Theory and Concepts

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With warm regards and sincere appreciation,

Zain Ul Bashar Khan
Dr. Manjuladevi Kasirajan
Dr. Narottam Pal
Dr. A. V. Kishore Babu

D E D I C A T I O N

✦ ✦ ✦

To My Loving Parents

Mr. Md. Khizar Hayat Khan

&

Mrs. Sajida Khan

Every step I took, your prayers were beneath my feet,

every word I wrote, your love was the ink.

In Loving Memory of My Grandparents

Mr. Md. Dowlat Khan

&

Mrs. Akhtar Khanam

You left before you could see this page —

but I know, somewhere, you are smiling.

And in Loving Memory of

Mrs. Rafat Sultana Khan

My Beloved Aunt

Heaven gained an angel the day it took you,

but your love never left — it lives within these pages.

✦ ✦ ✦

Whose love, prayers, and blessings

have been the guiding light of my life.

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Pharmaceutical Analysis: Theory and Concepts

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"Science advances one carefully measured experiment at a time."

— ZAIN UL BASHAR KHAN

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Abbreviations List

Abbreviation	Full Form / Meaning
AAS	Atomic Absorption Spectroscopy
API	Active Pharmaceutical Ingredient
AUC	Area Under the Curve
$BaCl_2$	Barium Chloride
BP	British Pharmacopoeia
CDSCO	Central Drugs Standard Control Organisation (India)
CE	Capillary Electrophoresis
CI	Confidence Interval
CV	Coefficient of Variation
EDTA	Ethylenediaminetetraacetic Acid
EMA	European Medicines Agency
FAAS	Flame Atomic Absorption Spectroscopy
FDA	Food and Drug Administration (USA)
FTIR	Fourier-Transform Infrared Spectroscopy
GC	Gas Chromatography
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
GMP	Good Manufacturing Practice
HCL	Hollow Cathode Lamp
HPLC	High Performance Liquid Chromatography
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICP-MS	Inductively Coupled Plasma–Mass Spectrometry
IND	Investigational New Drug (application)
IP	Indian Pharmacopoeia

IPQC	In-Process Quality Control
IPC	Indian Pharmacopoeia Commission
IR	Infrared (Spectroscopy)
LOD	Limit of Detection / Loss on Drying (context-dependent)
LOQ	Limit of Quantification
MHRA	Medicines and Healthcare products Regulatory Agency (UK)
MS	Mass Spectrometry
NIR	Near-Infrared (Spectroscopy)
NMR	Nuclear Magnetic Resonance
ORS	Oral Rehydration Salt(s)
PAT	Process Analytical Technology
Ph.Eur.	European Pharmacopoeia (Pharmacopoeia Europaea)
Ph.Int.	International Pharmacopoeia (Pharmacopoeia Internationalis)
ppm	Parts Per Million
ppb	Parts Per Billion
ppt	Parts Per Trillion
QC	Quality Control
RD	Relative Deviation
Rf	Retardation Factor (chromatography)
ROI	Residue on Ignition
RSD	Relative Standard Deviation
SD	Standard Deviation
TDM	Therapeutic Drug Monitoring
TLC	Thin-Layer Chromatography

UV	Ultraviolet
UV-Vis	Ultraviolet-Visible (Spectrophotometry)
USP	United States Pharmacopoeia
USP-NF	United States Pharmacopoeia–National Formulary
WHO	World Health Organization
λmax	Wavelength of Maximum Absorption
ε	Molar Absorptivity (Extinction Coefficient)

UNIT I
INTRODUCTION TO PHARMACEUTICAL ANALYSIS

This unit lays the essential foundation of pharmaceutical analysis. It covers the scope and significance of analytical science in pharmacy, the major techniques employed, the concepts of analytical errors, accuracy and precision, and the statistical methods used to interpret data. A thorough grasp of these fundamentals is indispensable for every pharmacy student and practitioner.

Chapter 1
Scope and Importance of Pharmaceutical Analysis

1.1 Learning Objectives

After studying this chapter, you will be able to:

- Define pharmaceutical analysis and state its scope within the pharmacy profession.
- Describe the role of pharmaceutical analysis in drug discovery, development, quality control, and regulatory affairs.
- Enumerate the various branches of pharmaceutical analysis and differentiate between them.
- Explain the importance of pharmacopoeial standards and list the major pharmacopoeias.
- Correlate the principles of pharmaceutical analysis with clinical pharmacy and patient safety.

1.2 Introduction

Pharmaceutical Analysis is the branch of science that applies analytical chemistry principles and methods to pharmaceutical substances — from raw drug materials to finished dosage forms. It answers three fundamental questions that are central to every medicine: What is it? How much is present? Is it pure and safe enough for use?

These questions — identification, quantification, and purity assessment — are the pillars upon which the entire edifice of pharmaceutical quality is built. Without reliable analytical methods, we cannot ensure the safety, efficacy, or quality of any medicine. Indeed, every tablet we swallow, every injection administered in a hospital, and every syrup given to a child exists within defined standards of quality — standards verified by pharmaceutical analysis.

The importance of pharmaceutical analysis extends far beyond the laboratory. It underpins drug regulatory approval, pharmacovigilance, pharmacokinetic studies,

quality control manufacturing, and clinical pharmacy practice. The subject is therefore not merely an academic discipline; it is a professional lifeline.

1.3 Definition of Pharmaceutical Analysis

Pharmaceutical Analysis may be formally defined as:

> **Definition**
> The branch of applied chemistry that involves the identification, determination, quantification, separation, and purification of drug substances, pharmaceutical excipients, and dosage forms, using physicochemical, biological, or microbiological methods, to verify that they conform to established standards of identity, purity, potency, safety, and quality.

The key operational components of this definition deserve elaboration:

- Identification: Confirms the identity of a substance (e.g., using spectroscopy, chemical reactions).
- Determination/Quantification: Measures the amount or concentration of a drug substance (e.g., by titration, HPLC).
- Purity Assessment: Detects and quantifies impurities, degradation products, or adulterants.
- Separation: Isolates individual components from mixtures (e.g., chromatography, extraction).
- Purification: Removes contaminants to obtain a substance in its pure form.

1.4 Scope of Pharmaceutical Analysis

The scope of pharmaceutical analysis is vast and multidimensional. It permeates virtually every aspect of the pharmaceutical life cycle — from the discovery of a new molecule to post-market surveillance of an established drug product.

1.4.1 Drug Discovery and Development

In drug discovery, analytical techniques are used to characterize newly synthesized or isolated compounds. Nuclear magnetic resonance (NMR), mass spectrometry (MS), infrared (IR) spectroscopy, and X-ray crystallography provide structural information critical to establishing the identity and three-dimensional conformation of potential drug candidates. High-throughput analytical screening allows rapid evaluation of thousands of compounds for biological activity.

During drug development, analytical methods are employed to study drug stability, degradation pathways, polymorphism, solubility profiles, and drug-excipient compatibility. This data forms the basis of Investigational New Drug (IND) applications submitted to regulatory authorities.

1.4.2 Quality Control of Raw Materials

Every raw material — Active Pharmaceutical Ingredient (API) or excipient — entering a manufacturing facility must be subjected to rigorous analytical testing before use. Tests include identity verification (e.g., IR matching), assay for potency, limit tests for heavy metals and arsenic, loss on drying, and microbial contamination testing. This phase of analysis ensures that only compliant materials enter the production process.

1.4.3 In-Process Quality Control

During the manufacture of pharmaceutical products, in-process quality control (IPQC) tests are conducted at various stages of production. These include blend uniformity testing, granule size distribution, tablet hardness and friability, weight variation, and dissolution testing. Analytical chemistry methods are integral to IPQC and help prevent batch failures.

1.4.4 Finished Product Testing

Before any pharmaceutical product is released to the market, it undergoes comprehensive finished product testing as per pharmacopoeial specifications. This includes assay of the active ingredient, uniformity of dosage units, dissolution, disintegration, sterility (for injectables), endotoxin testing, and particulate matter testing.

1.4.5 Stability Studies

Pharmaceutical analysis is central to stability testing, which determines the shelf life of a drug product. ICH guidelines (Q1A–Q1F) mandate stability studies under various conditions (temperature, humidity, light). Analytical methods must be validated to demonstrate their ability to detect changes in drug content, purity, and physical characteristics over time.

1.4.6 Bioanalytical Applications

In pharmacokinetics and clinical pharmacy, biological samples (blood, urine, plasma, saliva) are analyzed to determine drug concentrations. Techniques such as HPLC-MS/MS, immunoassay, and fluorescence spectroscopy are used. This information is vital for therapeutic drug monitoring (TDM), bioequivalence studies, and drug interaction assessments.

1.4.7 Forensic Pharmaceutical Analysis

Pharmaceutical analysis plays a critical role in forensic investigations involving counterfeit medicines, drug adulteration, and medico-legal cases. Techniques like thin-layer chromatography (TLC), mass spectrometry, and near-infrared (NIR) spectroscopy are used for rapid drug identification in field and laboratory settings.

1.5 Importance of Pharmaceutical Analysis

The importance of pharmaceutical analysis may be appreciated from multiple perspectives:

1.5.1 Ensuring Drug Safety

Adulterated or sub-standard drugs can cause serious harm, including treatment failure, adverse drug reactions, and even death. Pharmaceutical analysis provides the tools to detect impurities, degradation products, and contaminants — both organic and inorganic — that may compromise patient safety. Limit tests for arsenic, lead, mercury, and iron, as specified in the Indian Pharmacopoeia, are classic examples.

1.5.2 Guaranteeing Drug Efficacy

A drug must contain the labelled amount of the active ingredient within an acceptable range (typically 95–105% as per IP/BP). Analytical assay methods ensure that patients receive the correct therapeutic dose. Over- or under-dosage, arising from poor manufacturing or degradation, can have serious clinical consequences.

1.5.3 Regulatory Compliance

Global pharmaceutical regulatory frameworks — CDSCO in India, FDA in the USA, EMA in Europe — mandate that every drug product complies with pharmacopoeial or validated in-house specifications. Pharmaceutical analysis is the backbone of this compliance framework, and analytical data forms a major component of drug registration dossiers.

1.5.4 Pharmacoeconomic Impact

Poor analytical standards lead to drug recalls, batch rejections, and regulatory action — all of which have enormous financial consequences for pharmaceutical companies. Robust analytical quality control, by contrast, reduces wastage, improves productivity, and protects brand reputation. Analysis is therefore an investment in economic efficiency as much as in scientific accuracy.

1.5.5 Support to Clinical Pharmacy

Clinical pharmacists use bioanalytical data to individualize drug therapy, monitor drug toxicity, and assess patient adherence. In critical care settings, therapeutic drug monitoring (TDM) of drugs like aminoglycosides, digoxin, and cyclosporine relies entirely on accurate analytical measurement of serum drug concentrations.

1.6 Branches of Pharmaceutical Analysis

Pharmaceutical analysis encompasses several distinct branches, each serving specific purposes:

Branch	Description	Key Methods
Qualitative Analysis	Identifies the nature of a substance	Color reactions, solubility, spectroscopy
Quantitative Analysis	Determines the amount of a substance	Titrations, gravimetry, HPLC
Titrimetric Analysis	Uses titration reactions for quantification	Acid-base, redox, complexometry
Gravimetric Analysis	Based on weight measurement of a precipitate	Precipitation, ignition, volatilization
Spectroscopic Analysis	Uses interaction of radiation with matter	UV-Vis, IR, NMR, AAS
Chromatographic Analysis	Separates and quantifies mixture components	TLC, HPLC, GC
Electroanalytical Methods	Uses electrical properties of analyte solutions	Potentiometry, polarography
Biological Methods	Uses biological systems for assay	Microbiological assay, bioassay

1.7 Pharmacopoeial Standards in Pharmaceutical Analysis

A pharmacopoeia is an authoritative, legally recognized compendium of standards for drugs, excipients, dosage forms, and analytical methods. Compliance with pharmacopoeial standards is mandatory for pharmaceutical manufacturers.

1.7.1 Indian Pharmacopoeia (IP)

The Indian Pharmacopoeia (IP) is published by the Indian Pharmacopoeia Commission (IPC), Ghaziabad, under the authority of the Ministry of Health and Family Welfare, Government of India. It sets standards for drugs manufactured or sold in India. The IP is legally enforceable under the Drugs and Cosmetics Act, 1940. It is revised periodically to incorporate new drugs and updated methods. The IP also publishes supplements and addenda between editions.

1.7.2 British Pharmacopoeia (BP)

The British Pharmacopoeia (BP) is published annually by the Medicines and Healthcare products Regulatory Agency (MHRA), UK. It is widely referenced in Commonwealth

countries and in international regulatory submissions. The BP is particularly noted for its well-developed biological and microbiological assay methods.

1.7.3 United States Pharmacopoeia (USP)

The United States Pharmacopoeia–National Formulary (USP-NF) is published by the United States Pharmacopoeial Convention. It is the standard reference for drug substances and products marketed in the USA and is extensively cited in regulatory submissions worldwide. The USP is known for its comprehensive dissolution and HPLC methods.

1.7.4 European Pharmacopoeia (Ph.Eur.)

The European Pharmacopoeia is published by the Council of Europe and is recognized in all EU member states. It provides harmonized standards for medicines across Europe and has significant influence on global pharmaceutical standards.

Pharmacopoeia	Abbreviation	Published by	Country/Region
Indian Pharmacopoeia	IP	Indian Pharmacopoeia Commission (IPC)	India
British Pharmacopoeia	BP	MHRA, UK Government	United Kingdom
United States Pharmacopoeia	USP	US Pharmacopoeial Convention	USA
European Pharmacopoeia	Ph.Eur.	Council of Europe	Europe
International Pharmacopoeia	Ph.Int.	World Health Organization (WHO)	International

1.8 Summary

Chapter 1 Summary

Pharmaceutical analysis is the science of identifying, quantifying, and assessing the purity of drugs and pharmaceutical products. Its scope spans drug discovery, quality control, stability studies, bioanalysis, and forensic applications. It is critical for ensuring drug safety, efficacy, and regulatory compliance. Major pharmacopoeias (IP, BP, USP, Ph.Eur.) provide the official standards against which pharmaceutical analysis is performed.

Review Questions

Short Answer Questions

1. Define pharmaceutical analysis. What are its three fundamental objectives?
2. Distinguish between qualitative and quantitative analysis with examples.
3. What is the role of pharmaceutical analysis in quality control of raw materials?
4. List any four pharmacopoeias and state the authority responsible for each.
5. What is therapeutic drug monitoring (TDM)? How does pharmaceutical analysis support it?

Long Answer Questions

1. Describe the scope of pharmaceutical analysis with reference to all stages of the pharmaceutical life cycle. Support your answer with suitable examples.
2. Explain the importance of pharmacopoeial standards in pharmaceutical analysis. Discuss the Indian Pharmacopoeia in detail, including its legal basis and structure.
3. Write an essay on the role of pharmaceutical analysis in ensuring drug safety and patient care.

Multiple Choice Questions

1. Pharmaceutical analysis is primarily concerned with:

(a) Drug synthesis
(b) Identification, quantification, and purity of drugs
(c) Drug delivery systems
(d) Pharmacological screening

✓***Answer: (b) Identification, quantification, and purity of drugs***

2. The Indian Pharmacopoeia is published by:

(a) CDSCO
(b) Indian Pharmacopoeia Commission (IPC)
(c) Ministry of Chemicals
(d) Drug Controller General of India

✓***Answer: (b) Indian Pharmacopoeia Commission (IPC)***

3. Which of the following is an example of bioanalytical pharmaceutical analysis?

(a) Assay of a tablet by titrimetry
(b) Stability testing of a drug product
(c) Measurement of drug concentration in plasma
(d) Limit test for heavy metals

✓***Answer: (c) Measurement of drug concentration in plasma***

4. The acceptable potency range for most pharmacopoeial drug products is:
(a) 80-120%
(b) 95-105%
(c) 90-110%
(d) 98-102%
✓***Answer: (b) 95-105%***

5. Therapeutic Drug Monitoring (TDM) uses pharmaceutical analysis to:
(a) Develop new drugs
(b) Monitor and individualize drug therapy in patients
(c) Detect drug adulteration
(d) Perform stability studies
✓***Answer: (b) Monitor and individualize drug therapy in patients***

Chapter 2
Techniques of Pharmaceutical Analysis

2.1 Learning Objectives

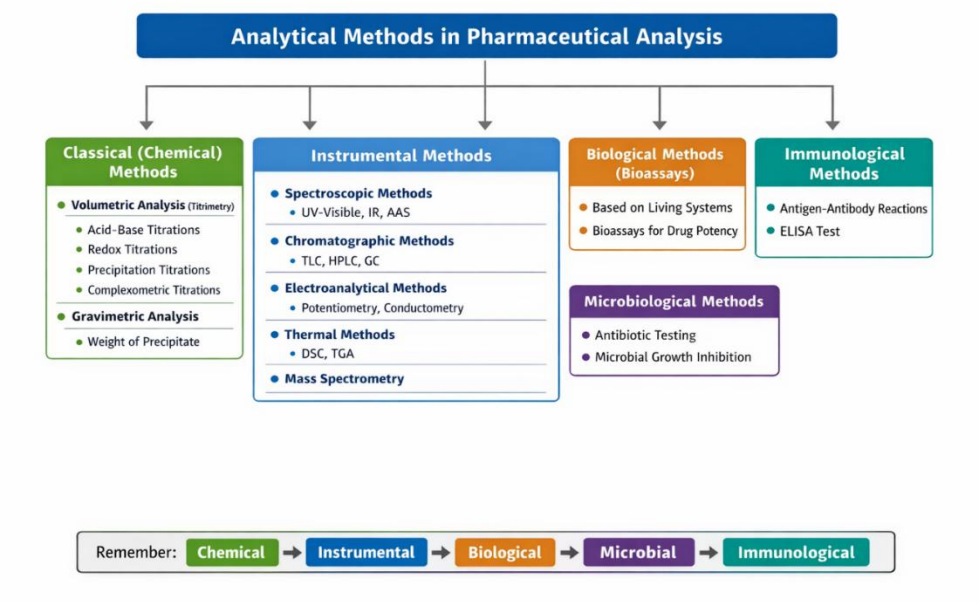
- Classify analytical methods used in pharmaceutical analysis.
- Distinguish between qualitative and quantitative analytical techniques.
- Describe titrimetric, gravimetric, and instrumental methods with examples.
- State the criteria for selecting an appropriate analytical method.

2.2 Introduction

The analytical toolkit available to the pharmaceutical scientist is extraordinarily diverse. From the simple colour reaction performed in a test tube to the sophisticated mass spectrometer interfaced with a chromatograph, pharmaceutical analysis employs a wide spectrum of techniques. Selecting the most appropriate technique for a given analytical task requires an understanding of the available methods, their underlying principles, their sensitivity, specificity, cost, and applicability.

Analytical techniques in pharmacy are broadly classified on the basis of the nature of the measurement, the type of chemical reaction employed, or the physical phenomenon exploited. This chapter provides an overview of the major categories of techniques, which are then explored in depth in subsequent units.

2.3 Classification of Analytical Methods



2.4 Qualitative vs Quantitative Analysis

All analytical procedures can be broadly divided into qualitative and quantitative analysis:

Feature	Qualitative Analysis	Quantitative Analysis
Purpose	Identifies what is present	Determines how much is present
Output	Yes/No; identity confirmed or denied	Numerical value (%, mg, mg/mL)
Examples	Colour tests, solubility, IR identity test	Titration, HPLC assay, UV assay
Sensitivity needed	Lower (presence/absence)	Higher (precise measurement)
Pharmacopoeial use	Identification tests	Assay, limit tests (quantitative)

2.5 Titrimetric (Volumetric) Methods

Titrimetric analysis is a classical quantitative method in which the analyte reacts with a standard solution (titrant) of known concentration. The volume of titrant required to completely react with the analyte (the equivalence point) is used to calculate the amount of the analyte.

Titrimetry is widely used in pharmacopoeial assays because it is simple, inexpensive, rapid, and highly accurate when performed correctly. The IP and BP still rely heavily on titrimetric methods for the assay of many official drug substances.

2.5.1 Types of Titrimetric Methods

- Acid-Base Titrations (Neutralization Titrations): Based on proton transfer reactions. Used for assay of acids, bases, salts, and many drug substances. Example: Assay of Aspirin by NaOH titration.
- Precipitation Titrations: Based on the formation of an insoluble precipitate. Example: Mohr's method for chloride determination using $AgNO_3$.
- Complexometric Titrations: Based on complex formation between the analyte (usually a metal ion) and a complexing agent. Example: EDTA titration for calcium and magnesium.
- Redox Titrations: Based on oxidation-reduction reactions. Example: Permanganate titration of ferrous iron; iodometric assay of Vitamin C.
- Non-aqueous Titrations: Performed in non-aqueous solvents for substances too weakly acidic or basic to be titrated in water. Example: Assay of amine-containing drugs in glacial acetic acid.

2.6 Gravimetric Methods

Gravimetric analysis determines the amount of an analyte by measuring the mass of a pure compound (precipitate, residue, or evolved gas) that is chemically related to the analyte.

It is one of the most accurate methods of classical analysis because mass can be measured with very high precision.

- Precipitation Gravimetry: The analyte is precipitated as a sparingly soluble compound, filtered, dried or ignited, and weighed. Example: Determination of barium as $BaSO_4$.
- Volatilization Gravimetry: The analyte is volatilized and either collected or the loss in mass is measured. Example: Loss on Drying (LOD) test in IP.
- Electrodeposition Gravimetry: The analyte is deposited on an electrode by electrolysis and the electrode mass increase is measured.

2.7 Instrumental Methods — Overview

Instrumental methods exploit the physical properties of matter — such as absorption, emission, or scattering of radiation, electrical conductivity, or migration in an electric field — to characterize and quantify analytes. They generally offer greater sensitivity, speed, and specificity than classical methods, and are essential for trace-level analysis and complex sample matrices.

2.7.1 Spectroscopic Methods

- UV-Visible Spectrophotometry: Based on absorption of ultraviolet or visible radiation. Widely used for quantitative analysis of drugs in solution.
- Infrared (IR) Spectroscopy: Used primarily for identification (fingerprinting) of drug substances. FTIR is the modern variant.
- Fluorescence Spectroscopy: Based on emission of radiation by excited molecules. Very sensitive — useful for trace analysis.
- Atomic Absorption Spectroscopy (AAS): Used for determination of metal ions in pharmaceutical samples.
- Nuclear Magnetic Resonance (NMR): Provides structural information about organic molecules; used in drug discovery and structure elucidation.
- Mass Spectrometry (MS): Identifies compounds based on mass-to-charge ratio of ions; often coupled with GC or HPLC.

2.7.2 Chromatographic Methods

- Thin-Layer Chromatography (TLC): Simple, inexpensive planar chromatography used for identification and purity testing.
- High Performance Liquid Chromatography (HPLC): The most widely used analytical technique in modern pharmaceutical QC. Separates and quantifies drug components in complex mixtures with high precision.

- Gas Chromatography (GC): For volatile compounds and residual solvent analysis.
- Ion-Exchange Chromatography: For separation of ionic species.

2.7.3 Electroanalytical Methods

- Potentiometry: Measures electrode potential. Used in pH measurement, Karl Fischer titrations, and ion-selective electrode assays.
- Conductometry: Measures electrical conductance of solutions. Used for purity testing of water and salt analysis.
- Polarography/Voltammetry: Based on current-voltage relationships in electrochemical cells. Used for trace metal analysis.
- Coulometry: Measures quantity of electricity required to reduce/oxidize an analyte.

2.8 Selection of Analytical Method

The choice of an analytical method for a given pharmaceutical application is guided by multiple factors:

Factor	Considerations
Nature of sample	Pure substance, mixture, biological matrix, solid, liquid, gas
Concentration range	Major component (>1%) — titration/gravimetry; trace (<ppm) — instrumental
Specificity required	Complex mixtures need selective/specific methods (e.g., HPLC)
Accuracy and precision	Gravimetry is most accurate; instrumental methods need validation
Speed	Instrumental methods generally faster than classical methods
Cost and equipment	Classical methods are cheaper; instrumental require capital investment
Sample availability	Destructive vs non-destructive methods
Regulatory requirements	Pharmacopoeial methods preferred for official testing

2.9 Summary

Chapter 2 Summary

Pharmaceutical analysis uses a wide range of methods: classical (titrimetry, gravimetry) and instrumental (spectroscopy, chromatography, electroanalysis). Qualitative methods identify substances while quantitative methods measure amounts. Method selection depends on nature of sample, concentration, specificity, cost, speed, and regulatory requirements. HPLC is the dominant modern technique in pharmaceutical QC.

Review Questions:

Short Answer Questions

1. Differentiate between titrimetric and gravimetric methods of analysis.
2. List five types of titrimetric methods with one example each.
3. What are the advantages of instrumental methods over classical methods?
4. What factors govern the selection of an analytical method?

Long Answer Questions

1. Classify the analytical methods used in pharmaceutical analysis with a neat diagram. Explain each category briefly with examples.
2. Describe the role of HPLC in pharmaceutical analysis. Compare it with TLC.

Multiple Choice Questions

1. Titrimetric analysis is based on:

(a) Measurement of mass
(b) Measurement of volume of standard solution
(c) Spectral absorption
(d) Electrode potential

✓***Answer: (b) Measurement of volume of standard solution***

2. Which method is most suitable for trace metal analysis in pharmaceuticals?

(a) Titrimetry
(b) Gravimetry
(c) Atomic Absorption Spectroscopy (AAS)
(d) Acid-base titration

✓***Answer: (c) Atomic Absorption Spectroscopy (AAS)***

3. HPLC stands for:
(a) High Pressure Liquid Concentrate
(b) High Performance Liquid Chromatography
(c) High Purity Liquid Chemistry
(d) High Potential Liquid Column
✓***Answer: (b) High Performance Liquid Chromatography***

4. Loss on Drying (LOD) test is an example of:
(a) Titrimetric analysis
(b) Volatilization gravimetry
(c) Precipitation gravimetry
(d) Redox titration
✓***Answer: (b) Volatilization gravimetry***

5. Non-aqueous titrations are performed when the analyte is:
(a) A strong acid
(b) A strong base
(c) A very weak acid or base
(d) A reducing agent
✓***Answer: (c) A very weak acid or base***

Chapter 3
Errors in Analysis

3.1 Learning Objectives

- Define the term 'error' in the context of analytical chemistry.
- Classify errors as determinate (systematic) and indeterminate (random).
- Identify sources of each type of error and describe methods to minimize them.
- Calculate absolute error, relative error, and percentage error.
- Understand the propagation of errors in analytical calculations.

3.2 Introduction

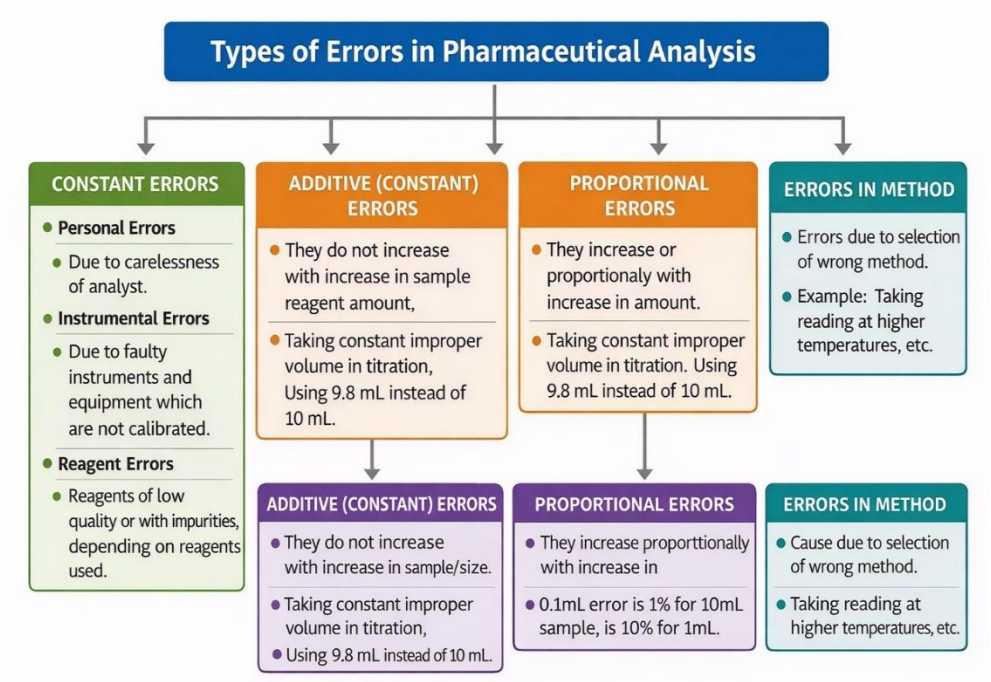
No analytical measurement is perfect. Every quantitative determination is accompanied by some degree of uncertainty — a deviation of the measured value from the true value. This deviation is called an error. Understanding errors is fundamental to interpreting analytical results correctly and to designing analytical procedures that minimize the impact of uncertainty.

The concept of error does not imply incompetence or carelessness (though both can introduce errors). Rather, it reflects the inherent limitations of measuring instruments, the variability of physical and chemical processes, and the finite precision of any analytical method. A competent analyst recognizes potential sources of error, works systematically to minimize them, and applies statistical tools to quantify the remaining uncertainty.

3.3 Basic Terminology

Term	Definition	Formula/Example
True Value	The actual, accepted correct value of the quantity	Reference standard value
Measured Value	The value obtained by the analytical measurement	Experimental result
Absolute Error (E)	Difference between measured and true values	$E = \text{Measured} - \text{True}$
Relative Error (Er)	Absolute error expressed relative to the true value	$Er = E / \text{True} \times 100\%$
Mean ($\bar{x}$)	Arithmetic average of multiple measurements	$\bar{x} = \Sigma x_i / n$
Deviation	Difference between individual value and the mean	$d_i = x_i - \bar{x}$

3.4 Classification of Errors



3.5 Determinate (Systematic) Errors

Determinate errors are errors that have a definite, assignable cause. They are reproducible, and importantly, they can be identified and either corrected or eliminated. They cause the measured result to be consistently higher or consistently lower than the true value — they introduce a bias into the measurement.

3.5.1 Instrumental Errors

These arise from imperfect calibration or malfunction of measuring instruments. Examples include:

- Uncalibrated burette: A burette that is not accurately calibrated may deliver volumes that are consistently larger or smaller than indicated. This introduces a systematic error in every titrimetric determination.
- Faulty balance: A balance that is not properly zeroed, or whose weights are inaccurate, will introduce a constant error in every weighing.
- Uncalibrated volumetric glassware: Flasks, pipettes, and burettes carry a tolerance, and using them at temperatures significantly different from the calibration temperature introduces volumetric errors.
- Spectrophotometer stray light: In UV-Vis spectrophotometry, stray light causes deviations from Beer's Law, resulting in systematic errors in absorbance measurement.

Correction: Regular calibration of instruments against certified reference standards; verification of glassware volumes.

3.5.2 Method Errors

These arise from the chemical or physical principles underlying the analytical method itself. They are often the most difficult to detect and correct. Examples include:

- Co-precipitation: In gravimetric analysis, contaminants may be co-precipitated with the desired precipitate, causing a positive error in the mass of precipitate.
- Incomplete reaction: If the titration reaction does not go to completion, the endpoint does not coincide with the equivalence point, introducing an error.
- Side reactions: Unintended reactions between the titrant and other components in the sample introduce errors in the measured value.
- Indicator error: The endpoint indicated by the indicator may not coincide exactly with the theoretical equivalence point.

Correction: Use of blank corrections, reagent corrections, and careful method development and validation.

3.5.3 Personal (Operator) Errors

These arise from the individual analyst's limitations, habits, or biases. Examples include:

- Parallax error: Reading the meniscus of a burette or pipette from an angle rather than horizontally causes a systematic reading error.
- Colour blindness: Affects the ability to accurately detect indicator colour changes at the endpoint.
- Anticipation of endpoint: Some analysts tend to stop the titration slightly before or after the true endpoint due to expectation of a result.
- Transcription errors: Incorrectly recording observed values.

Correction: Proper training, use of automated titrators, and careful adherence to standardized procedures (SOPs).

3.6 Indeterminate (Random) Errors

Indeterminate errors (also called random errors or accidental errors) are errors that arise from unpredictable, chance variations in the measurement process. Unlike determinate errors, they do not have a single assignable cause, cannot be eliminated, and are equally likely to produce values above or below the true value. Their behavior follows a Gaussian (normal) distribution.

Examples of sources of random error include:

- Slight fluctuations in room temperature affecting a sensitive analytical balance.
- Electrical noise in a detector signal.
- Minute variations in the judgment of an endpoint colour.
- Irreproducibility in sample preparation steps.

The magnitude of random errors can be minimized by increasing the number of replicate measurements and applying statistical treatment of the data (averaging, standard deviation, confidence intervals).

3.7 Absolute and Relative Error — Calculations

Worked Example 3.1
A student determines the content of paracetamol in a tablet. The true value (by reference standard) is 500.0 mg. The student obtains a value of 496.5 mg. Absolute Error (E) = 496.5 − 500.0 = −3.5 mg Relative Error (Er) = (−3.5 / 500.0) × 100 = −0.70% The negative sign indicates the result is lower than the true value (a negative bias).

3.8 Propagation of Errors

In pharmaceutical analysis, the final result is often calculated from several individual measurements, each with its own uncertainty. The propagation of errors describes how individual uncertainties combine to give the overall uncertainty in the final result.

3.8.1 Addition and Subtraction

When quantities are added or subtracted, the absolute errors add:

Rule
If $Z = A + B - C$, then: $E(Z) = \sqrt{[E(A)^2 + E(B)^2 + E(C)^2]}$ (quadrature addition)

3.8.2 Multiplication and Division

When quantities are multiplied or divided, the relative errors add:

Rule
If $Z = (A \times B) / C$, then: $\%E(Z) = \sqrt{[\%E(A)^2 + \%E(B)^2 + \%E(C)^2]}$

3.9 Summary

Chapter 3 Summary
Errors in pharmaceutical analysis are classified as determinate (systematic — assignable cause, correctable) and indeterminate (random — no single cause, minimized by replication). Determinate errors include instrumental, method, and personal errors. Absolute error = measured − true; relative error = (absolute/true) × 100%. Propagation of errors is calculated by quadrature addition of absolute or relative errors depending on the arithmetic operation.

Review Questions

Short Answer Questions

1. Define absolute error and relative error. Give a worked numerical example.
2. What is a parallax error? How can it be avoided?
3. Distinguish between determinate and indeterminate errors.

Long Answer Questions

1. Classify errors in analytical chemistry with a well-labelled diagram. Describe each type with examples relevant to pharmaceutical analysis. How can each type be minimized?
2. In a gravimetric analysis, the following masses were measured: precipitate mass = 0.4852 ± 0.0002 g; crucible mass = 15.3214 ± 0.0001 g. Calculate the propagated error in the mass of precipitate collected.

Multiple Choice Questions

1. Determinate errors differ from indeterminate errors in that they are:

(a) Unpredictable
(b) Due to chance alone
(c) Assignable to a specific cause
(d) Always positive

✓***Answer: (c) Assignable to a specific cause***

2. The parallax error is a type of:

(a) Instrumental error
(b) Personal error
(c) Method error
(d) Indeterminate error

✓***Answer: (b) Personal error***

3. If the true value of a determination is 250 mg and the measured value is 253 mg, the relative error is:

(a) +3 mg
(b) −1.2%
(c) +1.2%
(d) −3 mg

✓***Answer: (c) +1.2%***

4. Random errors in analysis can be minimized by:

(a) Using a better indicator
(b) Performing replicate measurements
(c) Calibrating the burette
(d) Using primary standards

✓***Answer: (b) Performing replicate measurements***

Chapter 4
Accuracy, Precision, and Significant Figures

4.1 Learning Objectives

- Define accuracy and precision in the context of analytical measurements.
- Calculate accuracy using percentage error and percentage recovery.
- Calculate precision using standard deviation and coefficient of variation.
- Explain the relationship and difference between accuracy and precision using the 'target' analogy.
- State and apply the rules for significant figures in analytical calculations.
- Define LOD and LOQ and describe their analytical significance.

4.2 Accuracy

Accuracy is the closeness of a measured value to the true (accepted) value. A measurement is considered accurate if it closely approximates the actual quantity being measured. Accuracy is a measure of systematic error — the lower the systematic error, the higher the accuracy.

4.2.1 Expression of Accuracy

- Percentage Error: % Error = [(Measured − True) / True] × 100
- Percentage Recovery: % Recovery = (Measured / True) × 100. A recovery of 100% indicates perfect accuracy; acceptable range is typically 98–102% for pharmacopoeial assays.

Worked Example 4.1
A USP standard of Paracetamol contains 500.0 mg. An analyst obtains 498.5 mg. % Error = (498.5 − 500.0)/500.0 × 100 = −0.30% % Recovery = (498.5/500.0) × 100 = 99.70% Conclusion: Excellent accuracy; result is within pharmacopoeial limits.

4.3 Precision

Precision is the closeness of agreement among a set of repeated measurements made under the same conditions. It reflects the reproducibility or repeatability of the method. Precision does not require knowledge of the true value; it is assessed entirely from the spread of replicate results.

4.3.1 Types of Precision

- Repeatability: Precision obtained under the same operating conditions over a short time interval (intra-day). Also called within-run precision.
- Intermediate Precision: Precision within the same laboratory under different conditions (different days, different analysts, different equipment). Also called within-laboratory precision or ruggedness.

- Reproducibility: Precision between different laboratories — assessed during collaborative studies or method transfer.

4.3.2 Statistical Measures of Precision

Measure	Formula	Interpretation
Range (R)	R = Maximum − Minimum	Simple; sensitive to outliers
Standard Deviation (SD)	$SD = \sqrt{[\Sigma(xi - \bar{x})^2 / (n-1)]}$	Most widely used precision measure
Variance (s^2)	$s^2 = \Sigma(xi - \bar{x})^2 / (n-1)$	SD squared; used in ANOVA
Coefficient of Variation (CV)	$CV = (SD / \bar{x}) \times 100\%$	Relative precision; allows comparison across methods
Relative Standard Deviation (RSD)	Same as CV	RSD < 2% usually acceptable in pharm. analysis

Worked Example 4.2
Five replicate assays of a tablet gave: 99.1, 98.7, 99.3, 98.9, 99.0 mg. Mean ($\bar{x}$) = (99.1+98.7+99.3+98.9+99.0)/5 = 99.0 mg Deviations: +0.1, −0.3, +0.3, −0.1, 0.0 $\Sigma(di)^2$ = 0.01+0.09+0.09+0.01+0.00 = 0.20 SD = $\sqrt{(0.20/4)} = \sqrt{0.05}$ = 0.224 mg RSD = (0.224/99.0) × 100 = 0.23% ← Excellent precision

4.4 Accuracy vs Precision — The Target Analogy

The target analogy is invaluable for understanding the relationship between accuracy and precision:

- High Accuracy + High Precision: All shots clustered at the bullseye. This is the ideal analytical scenario — the result is both correct and reproducible.
- Low Accuracy + High Precision: Shots tightly clustered, but far from the bullseye. Indicates a systematic error — the method is precise but biased.
- Low Accuracy + Low Precision: Shots scattered and away from the bullseye. Indicates both systematic and random errors.
- High Accuracy + Low Precision: Shots scattered but centred around the bullseye. Average is correct but individual results vary widely — indicates random error without systematic bias.

4.5 Significant Figures

Significant figures (significant digits) are the digits in a measured or calculated number that carry meaningful information about the precision of the measurement. The number of significant figures reported reflects the precision of the measuring instrument.

4.5.1 Rules for Counting Significant Figures

1. All non-zero digits are significant. Example: 4.567 has 4 significant figures.
2. Zeros between non-zero digits are significant. Example: 4.507 has 4 significant figures.
3. Leading zeros (before the first non-zero digit) are NOT significant. Example: 0.0045 has 2 significant figures.
4. Trailing zeros in a decimal number ARE significant. Example: 4.500 has 4 significant figures.
5. Trailing zeros in a whole number without decimal point are AMBIGUOUS — use scientific notation to clarify. Example: 4500 may have 2, 3, or 4 sig. figs.; write 4.500×10^3 for 4 sig. figs.

4.5.2 Rules for Calculations

- Addition/Subtraction: Round result to the least number of decimal places in the inputs. Example: 12.34 + 1.5 + 0.123 = 13.963 → rounded to 14.0 (one decimal place, from 1.5).
- Multiplication/Division: Round result to the least number of significant figures in the inputs. Example: 4.56 × 1.4 = 6.384 → rounded to 6.4 (2 significant figures, from 1.4).

4.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

Two important performance characteristics of an analytical method are the Limit of Detection (LOD) and the Limit of Quantification (LOQ):

Parameter	Definition	ICH Formula	Significance
LOD	Lowest amount of analyte that can be detected but not necessarily quantified	LOD = 3.3σ/S	Determines detectability — confirms presence/absence
LOQ	Lowest amount that can be quantitatively determined with acceptable precision and accuracy	LOQ = 10σ/S	Defines the working range floor for assay methods

Where: σ = standard deviation of response; S = slope of calibration curve

For pharmaceutical impurity testing, LOD and LOQ are critical parameters. The ICH Q2(R1) guideline provides the framework for their determination during method validation.

4.7 Summary

Chapter 4 Summary

Accuracy is closeness to the true value (assessed by % error or % recovery). Precision is the reproducibility of replicate measurements (assessed by SD, RSD, CV). A method can be precise but not accurate (systematic error) or accurate but not precise (random error). Ideal analysis achieves both. Significant figures must be correctly applied in calculations. LOD = 3.3σ/S and LOQ = 10σ/S define the detection and quantification limits of a method.

Review Questions

Short Answer Questions

1. What is % recovery? What is the acceptable % recovery range in pharmacopoeial assays?
2. Define Coefficient of Variation (CV). When is CV preferred over SD?
3. State the rules for significant figures in multiplication and division.
4. Differentiate between LOD and LOQ with formulae.

Long Answer Questions

1. Explain the concepts of accuracy and precision with the help of a target diagram. Describe the statistical parameters used to express precision.
2. Six replicate determinations of chloride in a saline solution gave: 0.896, 0.902, 0.898, 0.905, 0.897, 0.900 g/dL. Calculate the mean, standard deviation (SD), and coefficient of variation (CV). Comment on the precision.

Multiple Choice Questions

1. Precision in analytical measurement refers to:

(a) Closeness to true value
(b) Reproducibility of replicate measurements
(c) Freedom from bias
(d) Correct use of significant figures

✓***Answer: (b) Reproducibility of replicate measurements***

2. A % recovery of 101.5% indicates:

(a) Poor accuracy
(b) Good accuracy
(c) Excellent precision
(d) High random error

✓***Answer: (b) Good accuracy***

3. How many significant figures does the number 0.00452 have?

(a) 5

(b) 6

(c) 3

(d) 2

✓***Answer: (c) 3***

4. According to ICH Q2(R1), LOQ = :

(a) 3.3σ/S

(b) 10σ/S

(c) σ/S

(d) S/σ

✓***Answer: (b) 10σ/S***

Chapter 5
Statistical Treatment of Analytical Data

5.1 Learning Objectives

- Apply statistical concepts (mean, median, mode, range, SD, variance) to analytical data.
- Explain the normal distribution and its significance in analytical chemistry.
- Calculate and interpret confidence intervals.
- Apply the Student's t-test for comparison of analytical results.
- Use Dixon's Q-test to identify and reject outliers.

5.2 Introduction

Pharmaceutical analysis generates numerical data. To extract meaningful information from this data — to answer questions like 'Is this result significantly different from the standard?' or 'Is there an outlier in this data set?' — statistical methods are indispensable. Statistics enables analysts to quantify uncertainty, compare methods and results, and make objective, evidence-based decisions about pharmaceutical quality.

The ICH Q2(R1) guideline on analytical method validation, the USP <1010> chapter on analytical data interpretation, and the IP all require statistical treatment of analytical data. A pharmacy student who understands basic statistics is far better equipped to interpret analytical results, validate methods, and troubleshoot quality control problems.

5.3 Measures of Central Tendency

5.3.1 Mean (Arithmetic Mean)

The arithmetic mean ($\bar{x}$) is the sum of all values divided by the number of values. It is the most widely used measure of central tendency in analytical chemistry.

Formula

$\bar{x} = (x_1 + x_2 + x_3 + ... + x_n) / n = \Sigma x_i / n$ Where n = number of observations

5.3.2 Median

The median is the middle value when data is arranged in ascending or descending order. If n is even, the median is the average of the two middle values. The median is less sensitive to extreme values (outliers) than the mean and is particularly useful when the data set contains suspect values.

5.3.3 Mode

The mode is the value that appears most frequently in a data set. In analytical chemistry, data rarely has a distinct mode and this measure is seldom used in quantitative analysis.

5.4 Measures of Dispersion

5.4.1 Range

The range (R) is the simplest measure of spread: R = Maximum value − Minimum value. While easy to calculate, it is very sensitive to outliers and gives no information about the distribution of values within the range.

5.4.2 Standard Deviation

The standard deviation (SD or s) is the most important measure of dispersion in pharmaceutical analysis. It reflects the average deviation of individual measurements from the mean.

Formula for Sample Standard Deviation

$s = \sqrt{[\ \Sigma(x_i - \bar{x})^2 / (n - 1)\ ]}$ Note: We divide by (n−1) rather than n because we are estimating the population SD from a sample. The quantity (n−1) is called the degrees of freedom.

5.4.3 Variance

Variance (s^2) is the square of the standard deviation. While the SD is expressed in the same units as the original measurements (making it directly interpretable), variance is used extensively in advanced statistical tests such as ANOVA (Analysis of Variance).

5.4.4 Relative Standard Deviation (RSD) / Coefficient of Variation (CV)

The RSD (also known as CV) expresses the standard deviation as a percentage of the mean. It allows comparison of precision between methods or analytes measured on different scales.

Formula

RSD (%) = $(s / \bar{x}) \times 100$ Acceptance criterion: RSD < 2% for most pharmacopoeial assay methods

5.5 Normal (Gaussian) Distribution

When a large number of independent random errors affect a measurement, the distribution of results follows a bell-shaped curve called the normal (Gaussian) distribution. This distribution is symmetric around the mean, with most values clustered near the mean and fewer values farther away.

Normal Distribution Curve (Gaussian Curve)

Key properties of the normal distribution:

- Symmetrical: The distribution is perfectly symmetrical about the mean.
- 68-95-99.7 Rule: 68.27% of values fall within ±1s; 95.45% within ±2s; 99.73% within ±3s of the mean.

- Theoretical basis: The distribution of random errors in analytical measurements is assumed to follow a normal distribution, justifying the use of SD-based statistics.

5.6 Confidence Interval (CI)

A confidence interval is a range of values that is likely to contain the true population mean with a stated probability (confidence level). In pharmaceutical analysis, 95% confidence intervals are most commonly used.

Formula for Confidence Interval

$CI = \bar{x} \pm (t \times s / \sqrt{n})$ Where: $\bar{x}$ = sample mean t = critical t-value from t-distribution table (depends on degrees of freedom and confidence level) s = sample standard deviation n = number of measurements Interpretation: We are 95% confident that the true mean lies within this interval.

Worked Example 5.1

Assay of paracetamol: n=5, $\bar{x}$=499.2 mg, s=1.8 mg. t value for 4 degrees of freedom at 95% CI = 2.776 CI = $499.2 \pm (2.776 \times 1.8 / \sqrt{5})$ $= 499.2 \pm (2.776 \times 0.805)$ $= 499.2 \pm 2.23$ mg Result: 499.2 ± 2.23 mg (95% CI: 496.97 to 501.43 mg) The true mean paracetamol content lies within this range with 95% confidence.

5.7 Student's t-Test

The t-test is a statistical test used to determine whether the difference between two sets of data is statistically significant, or whether it could be due to random variation alone.

5.7.1 Single-Sample t-Test (Comparison with a Known Standard)

Used to compare the mean of a set of measurements with a known reference value (e.g., the labelled potency of a drug):

Formula

$t_{calculated} = |\bar{x} - \mu| / (s / \sqrt{n})$ Where μ = known reference value (true value). If $t_{calc} > t_{critical}$ (from table), the difference is statistically significant.

5.7.2 Two-Sample t-Test (Comparison of Two Methods)

Used to compare the means of two different analytical methods or two different sample groups to determine whether they give significantly different results.

5.8 Dixon's Q-Test for Outliers

Occasionally, one result in a data set appears to be very different from the others. Before discarding such a result, a statistical test must confirm that it is truly an outlier and not just a manifestation of natural variability. Dixon's Q-test is the most commonly used test for this purpose in analytical chemistry.

Dixon's Q-Test Procedure

1. Arrange data in ascending order: x_1, x_2, ..., x_n 2. Calculate Q_experimental: Q_exp = |Suspect value − Nearest neighbour| / Range 3. Compare Q_exp with Q_critical (from Q-table for given n and confidence level) 4. If Q_exp > Q_critical → Reject the outlier If Q_exp ≤ Q_critical → Retain the value (not a statistically confirmed outlier)

Worked Example 5.2

Data (n=5): 98.5, 99.0, 98.8, 99.1, 102.4 mg Arranged: 98.5, 98.8, 99.0, 99.1, 102.4 Suspect value: 102.4 (highest) Nearest neighbour: 99.1 Range: 102.4 − 98.5 = 3.9 Q_exp = (102.4 − 99.1) / 3.9 = 3.3 / 3.9 = 0.846 Q_critical (n=5, 95% confidence) = 0.710 Since Q_exp (0.846) > Q_critical (0.710) → Reject 102.4 as an outlier.

5.9 Summary

Chapter 5 Summary

Statistical treatment of analytical data includes measures of central tendency (mean, median, mode) and dispersion (range, SD, variance, RSD/CV). Analytical errors follow a normal (Gaussian) distribution. Confidence intervals quantify the uncertainty in a measured mean. The t-test compares means statistically. Dixon's Q-test identifies and validates rejection of outliers. These tools are essential for interpreting pharmaceutical analytical data and for method validation.

Review Questions

Short Answer Questions

1. What is a confidence interval? How is it calculated in analytical chemistry?
2. State the 68-95-99.7 rule for normal distribution.
3. When would you prefer to report the median rather than the mean?
4. What is Dixon's Q-test? When is it applied?

Long Answer Questions

1. Describe the statistical parameters used to evaluate analytical data. Include measures of central tendency and dispersion. Provide worked examples for mean, standard deviation, and RSD.
2. Explain the Student's t-test with a worked example. Discuss how it helps in comparing analytical methods.

Multiple Choice Questions

1. The standard deviation of a sample is calculated by dividing $\Sigma(x_i - \bar{x})^2$ by:

(a) n
(b) n+1
(c) n−1
(d) n^2

✓***Answer: (c) n−1***

2. In a normal distribution, what percentage of data falls within ±2 standard deviations?

(a) 68.27%
(b) 95.45%
(c) 99.73%
(d) 50.00%

✓***Answer: (b) 95.45%***

3. Dixon's Q-test is used for:

(a) Comparing two methods
(b) Calculating confidence intervals
(c) Identifying and rejecting outliers
(d) Testing for systematic errors

✓***Answer: (c) Identifying and rejecting outliers***

4. The coefficient of variation (CV) is expressed as:

(a) SD × mean
(b) (SD / mean) × 100%
(c) mean / SD
(d) mean − SD

✓***Answer: (b) (SD / mean) × 100%***

5. A 95% confidence interval means:

(a) 95% of measurements are within the interval
(b) We are 95% certain the true mean lies within the interval
(c) Only 5% of errors are systematic
(d) The SD is less than 5%

✓***Answer: (b) We are 95% certain the true mean lies within the interval***

UNIT II
ACID-BASE TITRATIONS

Acid-base titrations are among the oldest, most reliable, and most widely used quantitative analytical methods in pharmaceutical science. This unit provides a comprehensive treatment of the underlying theory, the role of indicators, the shapes of neutralization curves, and the extensive applications of acid-base titrations in the pharmaceutical industry and pharmacopoeial analysis.

Chapter 6
Theory of Acid-Base Titrations

6.1 Learning Objectives

- Define acids and bases according to Arrhenius, Brønsted-Lowry, and Lewis theories.
- Explain the concept of ionization constant (Ka, Kb) and pKa.
- Calculate pH of strong and weak acid/base solutions.
- Define acidimetry and alkalimetry and identify their applications.
- Distinguish between primary and secondary standard solutions.

6.2 Introduction

An acid-base titration is a quantitative analytical procedure in which a solution of known concentration (the standard solution or titrant) is slowly added to a solution of unknown concentration (the analyte) until the reaction between them is complete. The point at which the reaction is chemically complete is called the equivalence point. The endpoint is the experimentally observed point, usually detected by a colour change of an indicator or an instrumental signal.

Acid-base titrations are the most extensively used titrimetric methods in pharmaceutical analysis. They are employed for the assay of organic acids, organic bases, pharmaceutical salts, amino acids, and many other drug substances. Their popularity stems from their simplicity, precision, speed, low cost, and the availability of certified primary standards.

6.3 Theories of Acids and Bases

6.3.1 Arrhenius Theory

Proposed by Svante Arrhenius in 1884:

- Acid: A substance that produces hydrogen ions (H^+) in aqueous solution. Example: $HCl \rightarrow H^+ + Cl^-$

- Base: A substance that produces hydroxide ions (OH^-) in aqueous solution. Example: $NaOH \rightarrow Na^+ + OH^-$

Limitation: The Arrhenius theory is restricted to aqueous solutions and cannot explain the behaviour of bases that do not contain OH groups (e.g., NH_3) or acid-base reactions in non-aqueous media.

6.3.2 Brønsted-Lowry Theory

Proposed independently by Johannes Brønsted and Thomas Lowry in 1923:

- Acid: A proton donor (any species that can donate H^+).
- Base: A proton acceptor (any species that can accept H^+).
- Conjugate acid-base pair: When an acid donates a proton, it forms its conjugate base. When a base accepts a proton, it forms its conjugate acid.

Significance in pharmacy: The Brønsted-Lowry theory explains why drug salts behave as acids or bases in solution, which is crucial for understanding drug solubility, absorption, and formulation.

6.3.3 Lewis Theory

Proposed by Gilbert Lewis (1923):

- Lewis Acid: An electron pair acceptor.
- Lewis Base: An electron pair donor.

The Lewis theory is the most general acid-base concept. It encompasses all Brønsted-Lowry acid-base reactions and also explains reactions where no proton transfer occurs (e.g., metal complex formation in complexometric titrations). In pharmaceutical analysis, Lewis acid-base concepts underpin the chemistry of metal-ion complexometric titrations with EDTA (Unit III).

Theory	Acid	Base	Medium	Application
Arrhenius	H^+ producer	OH^- producer	Aqueous only	Simple aqueous neutralizations
Brønsted-Lowry	Proton donor	Proton acceptor	Aqueous & non-aqueous	Non-aqueous titrations, drug ionization
Lewis	Electron pair acceptor	Electron pair donor	Any medium	Complex formation, EDTA titrations

6.4 Ionization Constants (Ka and Kb)

The strength of an acid or base is quantified by its ionization constant. For a weak acid HA:

Weak Acid Ionization

$HA \rightleftharpoons H^+ + A^-$ $K_a = [H^+][A^-] / [HA]$ $pK_a = -\log_{10}(K_a)$ Stronger acid → Higher Ka → Lower pKa Weaker acid → Lower Ka → Higher pKa

Weak Base Ionization

$B + H_2O \rightleftharpoons BH^+ + OH^-$ $K_b = [BH^+][OH^-] / [B]$ For a conjugate acid-base pair: $K_a \times K_b = K_w = 1 \times 10^{-14}$ (at 25°C) ∴ $pK_a + pK_b = 14$

Pharmaceutical significance of pKa:

- Predicts the ionization state of a drug at physiological pH (7.4) — important for absorption and distribution.
- Guides selection of appropriate extraction conditions (acid-base partitioning).
- Used in the Henderson-Hasselbalch equation to calculate pH of buffer solutions used in analysis.

6.5 pH — Concept and Significance

pH was introduced by Søren Sørensen in 1909 as a convenient way to express the concentration of hydrogen ions in solution:

pH Definition

$pH = -\log_{10}[H^+]$ At 25°C: pH + pOH = 14 pH < 7 → Acidic; pH = 7 → Neutral; pH > 7 → Basic For a strong acid (HCl): $[H^+] = C$ → $pH = -\log C$ For a weak acid (HA): $[H^+] = \sqrt{(K_a \times C)}$ → $pH = ½(pK_a - \log C)$

6.6 The Henderson-Hasselbalch Equation

The Henderson-Hasselbalch (H-H) equation relates the pH of a buffer solution to the pKa of the weak acid and the ratio of the concentrations of the conjugate base to the acid:

Henderson-Hasselbalch Equation

For a weak acid buffer: $pH = pK_a + \log([A^-]/[HA])$ For a weak base buffer: $pOH = pK_b + \log([BH^+]/[B])$ then pH = 14 − pOH Applications in pharmaceutical analysis: 1. Preparation of buffer solutions for HPLC mobile phases 2. Calculation of indicator transition pH ranges 3. Prediction of drug ionization state in biological fluids

6.7 Acidimetry and Alkalimetry

In pharmaceutical titrimetry, two complementary sub-disciplines are recognized:

- Acidimetry: The analysis of bases (or basic substances) by titration with a standard acid solution. Example: Assay of sodium hydroxide using standard hydrochloric acid; assay of antacid tablets using standard H_2SO_4.

- Alkalimetry: The analysis of acids (or acidic substances) by titration with a standard alkali solution. Example: Assay of aspirin (acetylsalicylic acid) using standard NaOH; determination of acidity in vinegar using NaOH.

6.8 Primary and Secondary Standards

Titrimetric analysis requires solutions of accurately known concentration. The quality of the standard solution is paramount.

6.8.1 Primary Standards

A primary standard is a substance of sufficient purity, stability, and known composition to be used directly in preparing a standard solution by weighing. The concentration of the solution is calculated directly from the mass of primary standard dissolved.

Requirements of a primary standard:

- High purity (≥ 99.9%) — commercially available in certified form.
- Stable to drying conditions and to atmospheric moisture and CO_2.
- High molecular weight — reduces the relative weighing error.
- Reacts stoichiometrically with the analyte.
- Non-hygroscopic and readily soluble in the titration medium.

Primary Standard	Used for Standardizing	Formula Wt.	Reaction
Potassium Hydrogen Phthalate (KHP)	NaOH solutions	204.22	KHP + NaOH → KNaP + H_2O
Anhydrous Sodium Carbonate (Na_2CO_3)	HCl / H_2SO_4 solutions	105.99	Na_2CO_3 + 2HCl → 2NaCl + H_2O + CO_2
Oxalic Acid ($H_2C_2O_4 \cdot 2H_2O$)	NaOH solutions / $KMnO_4$	126.07	2NaOH + $H_2C_2O_4$ → $Na_2C_2O_4$ + 2H_2O
Potassium Dichromate ($K_2Cr_2O_7$)	Sodium thiosulfate solutions	294.18	Redox reaction in acidic medium
Sodium Chloride (NaCl)	$AgNO_3$ solutions	58.44	$AgNO_3$ + NaCl → AgCl↓ + $NaNO_3$

6.8.2 Secondary Standards

A secondary standard is a solution whose concentration has been determined by titrating it against a primary standard. Most working standard solutions in the pharmaceutical laboratory are secondary standards. Examples include: standardized NaOH solution (standardized against KHP), standardized HCl solution (standardized against Na_2CO_3), standardized $KMnO_4$ solution (standardized against oxalic acid).

6.9 Summary

Chapter 6 Summary

Acid-base titrations are based on proton-transfer reactions. The three acid-base theories are Arrhenius (aqueous), Brønsted-Lowry (proton donor-acceptor), and Lewis (electron pair). Ka, Kb, and pKa quantify acid-base strength. pH = $-\log[H^+]$. Acidimetry titrates bases with acid; alkalimetry titrates acids with alkali. Primary standards (KHP, Na_2CO_3) are used to standardize secondary standard solutions. The Henderson-Hasselbalch equation relates pH to pKa and buffer composition.

Review Questions

Short Answer Questions

1. State the Brønsted-Lowry definition of an acid and a base. Give two examples of conjugate acid-base pairs.
2. Write the Henderson-Hasselbalch equation and explain its significance in pharmaceutical analysis.
3. What are the requirements of a primary standard? Give two examples.
4. Calculate the pH of a 0.1 M solution of acetic acid (pKa = 4.74).

Long Answer Questions

1. Compare and contrast the Arrhenius, Brønsted-Lowry, and Lewis theories of acids and bases with examples. Discuss their relevance to pharmaceutical analysis.
2. Explain the concept of pKa and its pharmaceutical significance. How does pKa relate to drug absorption and ionization in biological systems?

Multiple Choice Questions

1. According to Brønsted-Lowry theory, a base is:

(a) An electron pair acceptor
(b) A proton donor
(c) A proton acceptor
(d) A hydroxide ion producer

✓***Answer: (c) A proton acceptor***

2. The pH of a 0.01 M HCl solution is:

(a) 1
(b) 2
(c) 12
(d) 7

✓***Answer: (b) 2***

3. Potassium Hydrogen Phthalate (KHP) is used as a primary standard for:

(a) HCl solutions

(b) NaOH solutions

(c) $AgNO_3$ solutions

(d) $KMnO_4$ solutions

✓***Answer: (b) NaOH solutions***

4. The Henderson-Hasselbalch equation is: pH = pKa + log:

(a) $[HA]/[A^-]$

(b) $[A^-]/[HA]$

(c) $[H^+]/[A^-]$

(d) Ka × Kb

✓***Answer: (b) $[A^-]/[HA]$***

5. Alkalimetry involves:

(a) Titration of bases with standard acid

(b) Titration of acids with standard alkali

(c) Titration using non-aqueous solvents

(d) Titration of metal ions with EDTA

✓***Answer: (b) Titration of acids with standard alkali***

Chapter 7
Indicators in Acid-Base Titrations

7.1 Learning Objectives

- Explain the theory of acid-base indicators according to Ostwald's ionic theory and the quinonoid theory.
- Define the transition interval (pH range) and transition point of an indicator.
- List common acid-base indicators with their pH ranges and colour changes.
- Explain the concept of mixed indicators and their advantage.
- Describe the criteria for selecting an appropriate indicator for a given titration.

7.2 Theory of Indicators

An acid-base indicator is a substance that changes colour in response to changes in the pH of the solution. It is usually a weak acid or weak base whose ionized and un-ionized forms differ markedly in colour. Only a few drops (or 1–3 drops of a dilute solution) of indicator are used in a titration so that the indicator itself does not significantly affect the pH of the solution being titrated.

7.2.1 Ostwald's Ionic Theory

Wilhelm Ostwald proposed that the colour change of an indicator is due to a shift in the ionization equilibrium:

Ostwald's Theory

For an acid indicator (HIn): $HIn \rightleftharpoons H^+ + In^-$ (Colour 1) (Colour 2) In acidic solution: Equilibrium shifts left → Colour 1 dominates (acid form) In alkaline solution: H^+ is removed by OH^- → Equilibrium shifts right → Colour 2 dominates (base form) $K_{In} = [H^+][In^-] / [HIn]$ pH at transition = pK_{In} (indicator constant)

7.2.2 Quinonoid Theory (Chromophore Theory)

The quinonoid theory explains the colour change in terms of structural transformation of the indicator molecule:

- The un-ionized form of an indicator has a benzenoid structure (typically colourless or one colour).
- The ionized form undergoes a structural rearrangement to a quinonoid structure (typically a different, more intense colour).
- Phenolphthalein is the classic example: In acidic solution, it exists as a lactone (colourless benzenoid structure). In alkaline solution, the lactone ring opens to give a quinonoid carboxylate anion (pink-magenta colour).

7.3 pH Range (Transition Interval) of Indicators

The transition interval of an indicator is the pH range over which the colour change is visible to the human eye. This range spans approximately 2 pH units, centred around the pKIn of the indicator:

Transition Interval Formula

Transition Interval = pKIn ± 1 (i.e., pKIn − 1 to pKIn + 1) At the lower pH limit: $[HIn]/[In^-] = 10:1$ → Colour of HIn visible At the upper pH limit: $[HIn]/[In^-] = 1:10$ → Colour of In^- visible At pKIn: $[HIn] = [In^-]$ → Intermediate (mixed) colour

7.4 Common Acid-Base Indicators

Indicator	pKIn	pH Range	Colour Change (Acid→Base)	Used for
Methyl Orange	3.46	3.1 – 4.4	Red → Orange-Yellow	Strong acid vs strong base; weak base titrations
Methyl Red	5.00	4.2 – 6.3	Red → Yellow	Strong acid vs weak base (pKb < 5)
Litmus	6.5	5.0 – 8.0	Red → Blue	Educational use; not sharp enough for titrations
Bromothymol Blue	7.10	6.0 – 7.6	Yellow → Blue	Near-neutral titrations
Phenol Red	7.90	6.8 – 8.4	Yellow → Red	Near-neutral titrations
Phenolphthalein	9.10	8.3 – 10.0	Colourless → Pink	Strong acid vs strong base; weak acid vs NaOH
Thymolphthalein	9.90	9.3 – 10.5	Colourless → Blue	Strongly alkaline titrations

7.5 Mixed Indicators

Mixed indicators are prepared by combining two indicators, or an indicator with an inert dye, to obtain a sharper colour change at the endpoint. The components of the mixture are chosen so that one absorbs the complement of the other, producing a more dramatic visual transition over a narrower pH range.

Example of a mixed indicator:

- Methyl Red + Bromocresol Green: Changes from red-orange to green at pH 5.1 — very sharp and clear transition used in the assay of ammonium salts.
- Phenolphthalein + Thymol Blue: Changes from yellow to violet at pH 9.0.
- Screened methyl orange (Methyl orange + Indigo carmine): Changes from violet to green at pH 4.0 — sharper than methyl orange alone.

7.6 Criteria for Selecting an Indicator

The correct choice of indicator is critical to the accuracy of an acid-base titration. The following criteria should be applied:

1. The pH transition interval of the indicator must lie within the steep portion of the neutralization curve (the pH jump at the equivalence point). This ensures that the colour change coincides with the equivalence point.
2. For a strong acid-strong base titration: the steep region spans approximately pH 4–10, so either methyl orange, phenolphthalein, or bromothymol blue is suitable.
3. For a weak acid-strong base titration: the equivalence point is at pH > 7 (basic). Use phenolphthalein (pH 8.3–10.0).
4. For a strong acid-weak base titration: the equivalence point is at pH < 7 (acidic). Use methyl orange (pH 3.1–4.4) or methyl red (pH 4.2–6.3).
5. For a weak acid-weak base titration: The pH change at equivalence is too gradual for any indicator to give a sharp endpoint — potentiometric methods are preferred.

7.7 Summary

Chapter 7 Summary

Acid-base indicators are weak acids/bases that change colour as pH changes. Ostwald's ionic theory explains colour change as a shift in equilibrium between HIn (acid colour) and In^- (base colour). The quinonoid theory explains it via structural (benzenoid to quinonoid) transformation. Transition interval ≈ pKIn ± 1. Common indicators: methyl orange (3.1–4.4), methyl red (4.2–6.3), phenolphthalein (8.3–10.0). Indicator choice depends on equivalence point pH: weak acid/strong base → phenolphthalein; strong acid/weak base → methyl orange.

Review Questions **Short Answer Questions**

1. What is an acid-base indicator? Describe Ostwald's ionic theory of indicators.
2. Define transition interval. How is it calculated from pKIn?

3. Why is phenolphthalein used for weak acid vs strong base titrations?

Long Answer Questions

1. Describe the quinonoid theory of indicators with the example of phenolphthalein. How does this explain its colour change?
2. What are mixed indicators? Describe the preparation and advantage of mixed methyl red-bromocresol green indicator.

Multiple Choice Questions

1. The colour change of phenolphthalein in alkaline solution is explained by:

(a) Arrhenius theory
(b) Ostwald's ionic theory
(c) Quinonoid (structural) theory
(d) Lewis electron theory

✓***Answer: (c) Quinonoid (structural) theory***

2. The pH transition interval of methyl orange is:

(a) 6.0 – 7.6
(b) 3.1 – 4.4
(c) 8.3 – 10.0
(d) 4.2 – 6.3

✓***Answer: (b) 3.1 – 4.4***

3. For a strong acid – weak base titration, the recommended indicator is:

(a) Phenolphthalein
(b) Litmus
(c) Methyl Orange or Methyl Red
(d) Thymolphthalein

✓***Answer: (c) Methyl Orange or Methyl Red***

4. An indicator is used in small quantities in a titration because:

(a) It is expensive
(b) It must not significantly alter the pH of the solution
(c) It reacts with the titrant
(d) It has low solubility

✓***Answer: (b) It must not significantly alter the pH of the solution***

5. In Ostwald's theory, at pH = pKIn:

(a) The solution is neutral

(b) [HIn] = [In^-] and an intermediate colour is observed

(c) Only the base form exists

(d) The indicator is inactive

✓Answer: (b) [HIn] = [In^-] and an intermediate colour is observed

Chapter 8
Neutralization Curves

8.1 Learning Objectives

- Define a neutralization (titration) curve and explain its pharmaceutical significance.
- Describe and calculate the pH at different stages of titration for: strong acid-strong base, weak acid-strong base, strong acid-weak base, and weak acid-weak base systems.
- Interpret the shape of neutralization curves and identify the equivalence point and buffer region.
- Explain the titration of polyprotic acids and its significance.

8.2 Introduction

A neutralization curve (titration curve) is a graph of the pH of the solution being titrated (plotted on the y-axis) against the volume of titrant added (plotted on the x-axis). Neutralization curves are fundamental to understanding acid-base titrations because they reveal the pH at every stage of the titration — including the equivalence point — and they guide the selection of an appropriate indicator.

The shape of the neutralization curve is determined by the strength of the acid and the strength of the base being titrated. The critical feature of every curve is the steep, near-vertical inflection (the 'pH jump') that occurs at or around the equivalence point. An indicator that changes colour within this steep region will give an accurate endpoint.

8.3 Strong Acid vs Strong Base (e.g., HCl vs NaOH)

This is the simplest and most straightforward case. Consider the titration of 50 mL of 0.1 M HCl with 0.1 M NaOH.

8.3.1 pH Before Adding NaOH

[HCl] = 0.1 M; HCl is fully dissociated. $[H^+]$ = 0.1 M. pH = −log(0.1) = 1.00

8.3.2 pH Before Equivalence Point (e.g., after adding 25 mL NaOH)

Calculation

Moles HCl initial = 0.1 × 50 = 5 mmol Moles NaOH added = 0.1 × 25 = 2.5 mmol Excess HCl = 5 − 2.5 = 2.5 mmol Total volume = 75 mL $[H^+]$ = 2.5/75 = 0.0333 M pH = −log(0.0333) = 1.48

8.3.3 pH at Equivalence Point (after adding 50 mL NaOH)

All HCl has been neutralized. The solution contains only NaCl (a salt of strong acid and strong base) — neutral. pH = 7.00 (at 25°C).

8.3.4 pH After Equivalence Point (e.g., after adding 55 mL NaOH)

Calculation
Moles NaOH added = 0.1 × 55 = 5.5 mmol; Moles HCl = 5 mmol Excess NaOH = 0.5 mmol; Total volume = 105 mL $[OH^-]$ = 0.5/105 = 4.76×10^{-3} M pOH = −log(4.76×10^{-3}) = 2.32 pH = 14 − 2.32 = 11.68

Neutralization Curve: Strong Acid (HCl) vs Strong Base (NaOH)
Key features of the strong acid-strong base curve:

- Initial pH is low (strongly acidic).
- A large, steep pH jump occurs at the equivalence point (from ~pH 4 to ~pH 10), spanning ~6 pH units.
- Equivalence point is at exactly pH 7.00 (neutral salt, NaCl, formed).
- Any indicator with a transition interval within pH 4–10 is suitable: methyl orange, phenolphthalein, or bromothymol blue.

8.4 Weak Acid vs Strong Base (e.g., CH_3COOH vs NaOH)

Consider the titration of 50 mL of 0.1 M acetic acid (pKa = 4.74) with 0.1 M NaOH.

8.4.1 pH Before Adding NaOH

Initial pH
$CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$; Ka = 1.8×10^{-5} $[H^+] = \sqrt{(Ka \times C)} = \sqrt{(1.8 \times 10^{-5} \times 0.1)} = \sqrt{(1.8 \times 10^{-6})} = 1.34 \times 10^{-3}$ M pH = −log(1.34×10^{-3}) = 2.87

8.4.2 Buffer Region (Before Equivalence)

As NaOH is added, acetic acid is partially neutralized, creating a buffer of CH_3COOH/CH_3COO^-. The pH in this region is described by the Henderson-Hasselbalch equation:

H-H Equation in Buffer Region
pH = pKa + log($[CH_3COO^-]/[CH_3COOH]$) At half-equivalence point (25 mL NaOH added): $[CH_3COO^-] = [CH_3COOH]$ ∴ pH = pKa = 4.74 ← Half-equivalence pH = pKa

8.4.3 pH at Equivalence Point

At the equivalence point, all acetic acid has been converted to sodium acetate (CH_3COONa). Sodium acetate is the salt of a weak acid and a strong base — it hydrolyzes to give a basic solution:

Equivalence Point pH
$CH_3COO^- + H_2O \rightleftharpoons CH_3COOH + OH^-$ (basic hydrolysis) Kb = Kw / Ka = $(1 \times 10^{-14}) / (1.8 \times 10^{-5}) = 5.56 \times 10^{-10}$ $[OH^-] = \sqrt{(Kb \times C)} = \sqrt{(5.56 \times 10^{-10} \times 0.05)} = 5.27 \times 10^{-6}$ M pOH = 5.28; pH = 14 − 5.28 = 8.72 ← Equivalence point is BASIC

Key features:

- Initial pH higher than for HCl (partial dissociation of weak acid).
- Buffer region between initial point and equivalence — characteristic flat slope.
- At half-equivalence: pH = pKa (a useful experimental way to determine pKa).
- Equivalence point is basic (pH ~8.72) — the salt (sodium acetate) hydrolyzes.
- Steep pH jump is smaller (~7 to ~11) and shifted to alkaline side.
- Indicator: Phenolphthalein (pH 8.3–10.0) is appropriate; methyl orange would give a gross error.

8.5 Strong Acid vs Weak Base (e.g., HCl vs NH_3)

Consider titration of 50 mL of 0.1 M ammonia (pKb = 4.74, therefore pKa of NH_4^+ = 14 – 4.74 = 9.26) with 0.1 M HCl.

8.5.1 Initial pH

Initial pH

$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$; Kb = 1.8×10^{-5} $[OH^-] = \sqrt{(Kb \times C)} = \sqrt{(1.8 \times 10^{-5} \times 0.1)}$ = 1.34×10^{-3} M pOH = 2.87; pH = 14 – 2.87 = 11.13

8.5.2 Equivalence Point

At equivalence, all NH_3 is converted to NH_4Cl. NH_4^+ is the conjugate acid of a weak base — it hydrolyzes to give an acidic solution:

Equivalence Point

$NH_4^+ + H_2O \rightleftharpoons NH_3 + H_3O^+$ (acidic hydrolysis) Equivalence point pH ≈ 4 – 6 (acidic) → Indicator: Methyl Red (pH 4.2–6.3) is appropriate.

8.6 Weak Acid vs Weak Base

When both the acid and the base are weak, the neutralization curve shows no well-defined steep inflection at the equivalence point. The pH change occurs gradually throughout the titration, making it impossible to use a colour-changing indicator with confidence. In practice, weak acid vs weak base titrations are performed using potentiometric methods (pH meter with automatic endpoint detection) rather than indicator-based methods.

8.7 Polyprotic Acid Titrations

Some acids are polyprotic — they can donate more than one proton per molecule. Common examples include:

- Diprotic acids: H_2SO_4 ($pKa_1 \approx -3$, $pKa_2 \approx 1.99$), H_2CO_3 ($pKa_1 = 6.35$, $pKa_2 = 10.33$), $H_2C_2O_4$ (oxalic acid).
- Triprotic acids: H_3PO_4 ($pKa_1 = 2.15$, $pKa_2 = 7.20$, $pKa_3 = 12.35$), H_3Cit (citric acid).

When a polyprotic acid is titrated with a strong base, multiple equivalence points are observed — one for each ionization step. The number of visible inflections in the titration curve depends on how far apart the successive pKa values are.

Rule for Distinguishable Equivalence Points
If $\Delta pKa = pKa_2 - pKa_1 \geq 3$, the two equivalence points are distinguishable and separate inflections appear in the titration curve. If $\Delta pKa < 3$, the equivalence points overlap and may not be individually resolved.

Pharmaceutical example: Phosphoric acid (H_3PO_4) titrated with NaOH shows two clearly distinguishable equivalence points ($pKa_1 = 2.15$ and $pKa_2 = 7.20$, $\Delta pKa = 5.05$). The third equivalence point is not detectable in water.

8.8 Summary

Chapter 8 Summary
Neutralization curves plot pH vs volume of titrant added and show a steep pH jump at the equivalence point. Strong acid-strong base: equivalence at pH 7, large jump (~4–10). Weak acid-strong base: equivalence is basic (pH 8–10), use phenolphthalein. Strong acid-weak base: equivalence is acidic (pH 4–6), use methyl orange/red. Weak acid-weak base: no sharp endpoint, use potentiometry. Polyprotic acids show multiple equivalence points; resolved if $\Delta pKa \geq 3$.

Review Questions

Short Answer Questions

1. Why is the equivalence point of a weak acid-strong base titration at a pH greater than 7?
2. What is the significance of the half-equivalence point in a weak acid-strong base titration?
3. Why cannot colour indicators be used in weak acid vs weak base titrations?
4. What are polyprotic acids? Give two pharmaceutical examples.

Long Answer Questions

1. Describe the neutralization curve for the titration of 50 mL of 0.1 M acetic acid with 0.1 M NaOH. Calculate the pH at the initial point, at 25 mL of NaOH addition (half-equivalence), and at the equivalence point. Draw the curve and select an appropriate indicator.
2. Compare the neutralization curves for strong acid-strong base and strong acid-weak base titrations. How does the position of the equivalence point determine indicator selection?

Multiple Choice Questions

1. The equivalence point in a strong acid – strong base titration at 25°C is at:

(a) pH < 7

(b) pH = 7

(c) pH > 7

(d) Depends on concentration

✓***Answer: (b) pH = 7***

2. At the half-equivalence point in a weak acid-strong base titration:

(a) pH = 14 − pKa

(b) pH = pKa

(c) pH = 7

(d) pH = pKb

✓***Answer: (b) pH = pKa***

3. In the titration of NH_3 (weak base) with HCl, the equivalence point pH is:

(a) Greater than 7

(b) Equal to 7

(c) Less than 7

(d) Equal to pKb

✓***Answer: (c) Less than 7***

4. Which indicator is BEST suited for the titration of sodium acetate solution with HCl?

(a) Phenolphthalein

(b) Bromothymol Blue

(c) Methyl Orange

(d) Litmus

✓***Answer: (c) Methyl Orange***

Chapter 9
Applications of Acid-Base Titrations in Pharmaceutical Analysis

9.1 Learning Objectives

- Describe the pharmacopoeial assay procedures for specific drug substances using acid-base titrations.
- Explain the principle and procedure of non-aqueous titrations.
- Calculate drug content from titration data.
- Discuss the advantages and limitations of acid-base titrations in pharmaceutical quality control.

9.2 Introduction

Acid-base titrations are among the most important tools in pharmacopoeial drug analysis. The Indian Pharmacopoeia (IP), British Pharmacopoeia (BP), and United States Pharmacopoeia (USP) all specify titrimetric assays for a large number of drug substances and pharmaceutical preparations. These methods offer simplicity, speed, precision, and low cost — making them ideal for routine quality control.

This chapter focuses on selected pharmacopoeial applications of acid-base titrations, with detailed procedures and calculations. Both aqueous (direct neutralization) and non-aqueous titrations are discussed.

9.3 Assay of Sodium Hydroxide (NaOH) — IP Method

Principle

NaOH is a strong base. It is assayed by direct titration with a standard solution of hydrochloric acid using methyl orange as indicator.

Reaction

$NaOH + HCl \rightarrow NaCl + H_2O$

Procedure (IP)

1. Accurately weigh approximately 1.5 g of NaOH into a conical flask.
2. Dissolve in 20 mL of CO_2-free water.
3. Add 2 drops of methyl orange indicator solution.
4. Titrate with 1 M hydrochloric acid (standardized) until the colour changes from yellow to orange.
5. Record the volume of HCl consumed (V mL).

Calculation

Calculation
Each mL of 1 M HCl ≡ 40.00 mg of NaOH Content of NaOH (%) = (V × M_HCl × 40.00 × 100) / (weight in mg) Example: If 1.52 g NaOH consumed 37.6 mL of 1 M HCl: NaOH content = (37.6 × 1 × 40.00 × 100) / 1520 = 99.0%

9.4 Assay of Aspirin (Acetylsalicylic Acid) — IP Method

Principle
Aspirin is a weak acid (pKa = 3.49) and contains a carboxylic acid group (-COOH). It is assayed by direct titration with standard NaOH using phenolphthalein as indicator.

Reaction
$C_9H_8O_4$ (Aspirin) + NaOH → $C_9H_7O_4Na$ + H_2O

Procedure
1. Accurately weigh approximately 0.5 g of aspirin.
2. Dissolve in 10 mL of neutralized alcohol.
3. Add 2 drops of phenolphthalein indicator.
4. Titrate with 0.1 M NaOH until the colour changes from colourless to pink (stable for 30 seconds).
5. Record the volume of NaOH consumed (V mL).

Calculation

Calculation
MW of Aspirin = 180.16 g/mol; Equivalent weight = 180.16 g/eq (monoprotic acid) Each mL of 0.1 M NaOH ≡ 18.016 mg of aspirin Content (%) = (V × 0.1 × 180.16 × 100) / (Weight of sample in mg × 1000) IP limit: 99.5–100.5% (on dried basis)

9.5 Assay of Sodium Bicarbonate ($NaHCO_3$) — IP Method

Principle
Sodium bicarbonate reacts with hydrochloric acid to form carbon dioxide, water, and sodium chloride:

Reaction
$NaHCO_3$ + HCl → NaCl + H_2O + CO_2↑ Methyl Orange indicator; endpoint: yellow to orange

Procedure

1. Weigh accurately about 0.5 g of $NaHCO_3$.
2. Dissolve in CO_2-free water (20 mL).
3. Add methyl orange indicator (3 drops).
4. Titrate with 0.5 M HCl. Boil near the endpoint to expel CO_2, cool, and complete the titration.
5. Record volume consumed (V mL).

Calculation

MW $NaHCO_3$ = 84.01; Each mL of 0.5 M HCl ≡ 42.00 mg $NaHCO_3$ Content (%) = (V × 0.5 × 84.01 × 100) / (Sample weight in mg × 1000)

9.6 Non-Aqueous Titrations

9.6.1 Introduction

Many pharmaceutical substances are weak bases or weak acids that cannot be satisfactorily titrated in aqueous solution because their equilibrium constants are too small to give a detectable endpoint. Non-aqueous titrations, performed in organic solvents, resolve this problem by providing a medium in which the acid-base reaction goes essentially to completion.

9.6.2 Principle

In non-aqueous titrations:

- Weak bases (most amine-containing drugs, alkaloids, antihistamines) are titrated with perchloric acid ($HClO_4$) in glacial acetic acid (AcOH) as solvent. Glacial acetic acid is a less basic solvent than water, so it enhances the basicity of the weak base relative to water. The drug base is protonated by $HClO_4$.
- Weak acids are titrated with tetrabutylammonium hydroxide in solvents like DMF or pyridine.

Reaction: Non-aqueous Titration of a Weak Base Drug

$B + HClO_4 \rightarrow BH^+ClO_4^-$ (in glacial acetic acid) (Weak base) (perchloric acid) (drug perchlorate salt) Examples: Atropine + $HClO_4$ → Atropine perchlorate Ephedrine + $HClO_4$ → Ephedrine perchlorate

9.6.3 Endpoint Detection

Crystal Violet indicator (transition: violet → blue-green in glacial acetic acid) is commonly used. Alternatively, a potentiometric endpoint may be used (more precise and free from colour interference).

9.6.4 Standardization of Perchloric Acid

The perchloric acid ($HClO_4$) titrant is standardized against potassium hydrogen phthalate (KHP) in glacial acetic acid using crystal violet indicator or potentiometry.

9.6.5 Pharmaceutical Applications of Non-Aqueous Titrations

Drug	Titrant	Solvent	Indicator
Atropine Sulfate	0.1 M $HClO_4$	Glacial acetic acid	Crystal Violet
Ephedrine Hydrochloride	0.1 M $HClO_4$	Glacial acetic acid	Crystal Violet
Chlorphenamine Maleate	0.1 M $HClO_4$	Glacial acetic acid	Crystal Violet
Codeine Phosphate	0.1 M $HClO_4$	Glacial acetic acid	Crystal Violet / Potentiometric
Quinine Sulphate	0.1 M $HClO_4$	Glacial acetic acid	Crystal Violet
Phenobarbitone (Phenobarbital)	0.1 M KOH in ethanol	Pyridine	Thymolphthalein

9.7 Kjeldahl Method for Nitrogen Determination

The Kjeldahl method is a widely used acid-base titrimetric method for the determination of nitrogen content in organic pharmaceutical substances, proteins, and nitrogen-containing drug compounds. It was developed by Johan Kjeldahl in 1883 and is still extensively used because of its accuracy and universality.

Principle and Steps

1. DIGESTION: The sample is heated with concentrated H_2SO_4 in the presence of a catalyst ($CuSO_4$ + K_2SO_4) at high temperature. Organic nitrogen is converted to ammonium sulfate: N → $(NH_4)_2SO_4$
2. DISTILLATION: The digest is made strongly alkaline with excess NaOH, releasing NH_3: $(NH_4)_2SO_4$ + 2NaOH → Na_2SO_4 + $2NH_3$ + $2H_2O$. NH_3 is distilled into a receiver containing a measured excess of standard HCl (or boric acid solution).
3. TITRATION: The excess HCl is back-titrated with standard NaOH using methyl red indicator. Alternatively, if boric acid is used as receiver, NH_3 converts it to ammonium borate, which is then titrated directly with standard HCl.

Calculation

% Nitrogen = [(V_acid − V_NaOH) × M × 14.01 × 100] / (Sample weight in mg) Where V = volumes of standard solutions; M = molarity; 14.01 = atomic weight of N

9.8 Summary

Chapter 9 Summary

Acid-base titrations have extensive pharmacopoeial applications including assay of NaOH (with HCl, methyl orange), aspirin (with NaOH, phenolphthalein), and sodium bicarbonate (with HCl, methyl orange). Non-aqueous titrations use $HClO_4$ in glacial acetic acid for weak bases (alkaloids, amines) with crystal violet indicator. The Kjeldahl method determines nitrogen in organic drug substances through a three-step process: digestion, distillation, and back-titration.

Review Questions

Short Answer Questions

1. Why is phenolphthalein used in the assay of aspirin by NaOH titration?
2. What is the role of glacial acetic acid in non-aqueous titrations?
3. State the three steps in the Kjeldahl method for nitrogen determination.
4. How is sodium bicarbonate assayed by IP? Why must CO_2 be expelled during titration?

Long Answer Questions

1. Describe the principle, procedure, and calculation for the assay of aspirin by direct titration using NaOH as per the Indian Pharmacopoeia.
2. Write a detailed note on non-aqueous titration. Describe its principle, solvent selection, titrant, indicator, and applications in the assay of pharmaceutical substances.

Multiple Choice Questions

1. The titrant used in non-aqueous titration of weak organic bases is:

(a) NaOH in ethanol

(b) $HClO_4$ in glacial acetic acid

(c) HCl in methanol

(d) H_2SO_4 in acetic acid

✓***Answer: (b) $HClO_4$ in glacial acetic acid***

2. The equivalent weight of aspirin (MW = 180.16) in acid-base titration is:

(a) 90.08

(b) 360.32

(c) 180.16

(d) 60.05

✓***Answer: (c) 180.16***

3. In the Kjeldahl method, the digestion step converts organic nitrogen to:
(a) Nitrogen gas (N_2)
(b) Ammonium sulfate $(NH_4)_2SO_4$
(c) Nitric acid (HNO_3)
(d) Ammonia (NH_3)
✓Answer: (b) Ammonium sulfate $(NH_4)_2SO_4$

4. Crystal Violet indicator is used in non-aqueous titration. Its colour changes from:
(a) Colourless to pink
(b) Red to yellow
(c) Violet to blue-green
(d) Yellow to blue
✓Answer: (c) Violet to blue-green

5. In the assay of $NaHCO_3$, CO_2 must be expelled near the endpoint to:
(a) Prevent back-titration
(b) Remove the buffer effect of CO_2/HCO_3^- system and get a sharper endpoint
(c) Increase the rate of reaction
(d) Stabilize the indicator
✓Answer: (b) Remove the buffer effect of CO_2/HCO_3^- system and get a sharper endpoint

UNIT III
PRECIPITATION AND COMPLEXOMETRIC TITRATIONS

This unit explores two major categories of titrimetric methods — precipitation titrations and complexometric titrations. Precipitation titrtions exploit the formation of sparingly soluble salts (notably silver salts) for quantification, while complexometric titrations use chelating agents such as EDTA to quantify metal ions. Both categories find extensive application in pharmacopoeial analysis, water quality testing, and pharmaceutical formulation analysis.

Chapter 10
Precipitation Titrations — Principles and Theory

10.1 Learning Objectives

- Define precipitation titration and state the conditions required for a successful precipitation titration.
- Explain the concept of solubility product (Ksp) and its role in precipitation analysis.
- Describe the general procedure of argentometric titrations.
- Identify common end-point detection methods used in precipitation titrations.
- Compare the Mohr, Volhard, and Fajans methods of argentometric titration.

10.2 Introduction to Precipitation Titrations

A precipitation titration is a volumetric method in which the analyte reacts with a standard titrant to form a precipitate of definite, reproducible composition. The endpoint is detected when essentially all of the analyte has been precipitated — indicated by a change in colour of an indicator, appearance of a new precipitate, or an instrumental signal.

The most important and widely used precipitation titrations in pharmaceutical analysis are argentometric titrations — those using silver nitrate ($AgNO_3$) as the titrant. These methods are used to assay halide-containing drugs (chlorides, bromides, iodides) and thiocyanate salts — a class that includes many important pharmaceutical compounds such as sodium chloride, potassium chloride, ammonium chloride, ephedrine hydrochloride, and many other hydrochloride drug salts.

Argentometry is named after Argentum — the Latin name for silver — and the three classical methods (Mohr, Volhard, and Fajans) remain in active use in current pharmacopoeial analysis.

10.3 Solubility Product (Ksp) — The Theoretical Basis

The driving force and the theoretical underpinning of all precipitation titrations is the solubility product constant (Ksp). When a sparingly soluble salt AB dissolves in water, an equilibrium is established between the dissolved ions and the undissolved solid:

Solubility Product

$AB(s) \rightleftharpoons A^+(aq) + B^-(aq)$ $Ksp = [A^+][B^-]$ The lower the Ksp, the more insoluble the salt, and the more complete the precipitation reaction. Relevant Ksp values: AgCl: $Ksp = 1.8 \times 10^{-10}$ AgBr: $Ksp = 5.0 \times 10^{-13}$ AgI: $Ksp = 8.5 \times 10^{-17}$ Ag_2CrO_4: $Ksp = 1.2 \times 10^{-12}$ AgSCN: $Ksp = 1.1 \times 10^{-12}$

The order of solubility (AgCl > AgBr > AgI) has direct analytical implications: in the Fajans method, eosin indicator (used for bromide and iodide) cannot be used for chloride because the chloride precipitate would be too soluble. Similarly, in the Volhard back-titration of chloride, the AgCl precipitate must be filtered off (or coated with nitrobenzene) before back-titrating with KSCN, because AgCl would otherwise redissolve (Ksp of AgSCN > Ksp of AgCl is FALSE — AgSCN is less soluble than AgCl, so the AgCl would convert to AgSCN).

10.4 Conditions for a Successful Precipitation Titration

Not every precipitation reaction is suitable for titrimetry. The following conditions must be met:

Condition	Requirement	Explanation
Low Ksp	$Ksp < 10^{-8}$	Complete reaction; < 0.1% of analyte remains in solution at EP
Rapid precipitation	Fast kinetics	Slow precipitation causes 'lag' and a poorly defined endpoint
Stoichiometric reaction	Defined 1:1 or simple ratio	Enables accurate calculation from titre volume
Sharp endpoint	Detectable colour/property change	Indicator or instrumental method must clearly signal EP
No interfering reactions	Selectivity required	No co-precipitation or side reactions with matrix components

10.5 End-Point Detection in Precipitation Titrations

Four methods are used to detect the endpoint in precipitation titrations:

- Chemical Indicator Method: A chemical indicator forms a coloured precipitate or a soluble coloured complex at the endpoint. Used in Mohr (K_2CrO_4) and Volhard ($Fe^{3+}/FeSCN^{2+}$) methods.
- Adsorption Indicator Method: An adsorption indicator changes colour upon adsorption onto the precipitate surface at the endpoint. Used in the Fajans method (fluorescein, dichlorofluorescein, eosin).
- Potentiometric Method: A silver electrode monitors the potential of the Ag^+/Ag couple as $AgNO_3$ is added. The endpoint corresponds to a sharp inflection in the potential-volume plot. Highly accurate; used in pharmacopoeial referee assays.
- Turbidimetric Method: Endpoint detected by the change in turbidity of the solution. Used in automated analysers.

Chapter 11
Mohr, Volhard, and Fajans Methods

11.1 The Mohr Method

11.1.1 Principle

The Mohr method is a direct argentometric titration in which chloride (or bromide) is titrated with standard silver nitrate in the presence of potassium chromate (K_2CrO_4) as the indicator. The endpoint is signalled by the formation of a brick-red precipitate of silver chromate (Ag_2CrO_4).

Reactions in the Mohr Method

Primary Reaction (during titration): $Ag^+ + Cl^- \rightarrow AgCl\downarrow$ (white, $K_{sp} = 1.8 \times 10^{-10}$)

Endpoint Reaction (after all Cl^- is precipitated): $2Ag^+ + CrO_4^{2-} \rightarrow Ag_2CrO_4\downarrow$ (brick-red, $K_{sp} = 1.2 \times 10^{-12}$)

Because $K_{sp}(AgCl) < K_{sp}(Ag_2CrO_4)$, AgCl precipitates preferentially until $[Cl^-]$ is very low, after which Ag_2CrO_4 appears.

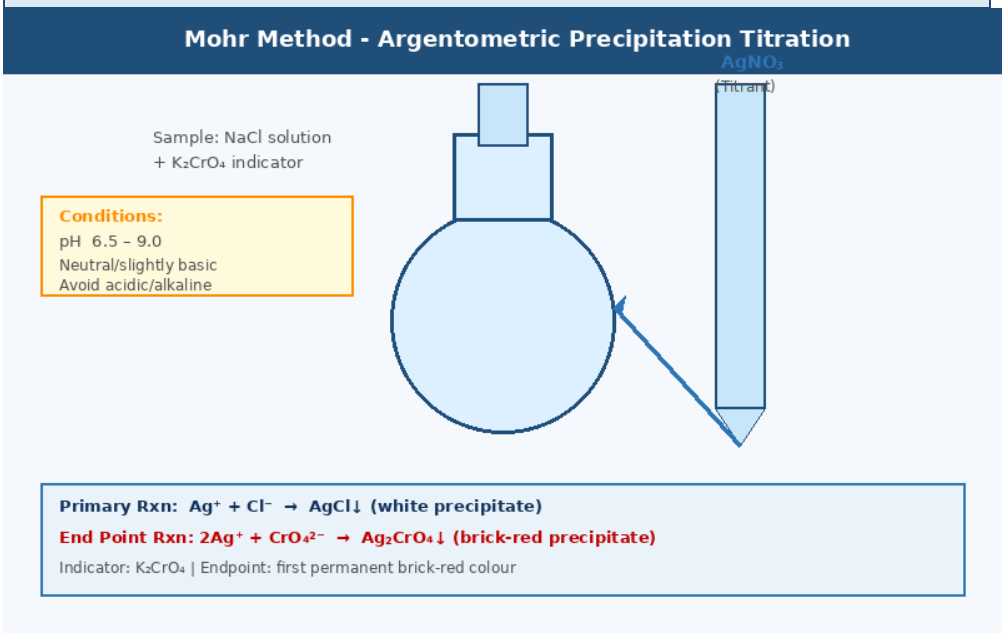


Figure: Mohr Method — Argentometric Titration Principle and Endpoint

11.1.2 Conditions for the Mohr Method

- pH must be between 6.5 and 9.0 (neutral to slightly basic). In acidic solution, CrO_4^{2-} is converted to $Cr_2O_7^{2-}$ (dichromate) which does not precipitate Ag^+. In strongly alkaline conditions, Ag_2O (brown) precipitates.

- Concentration of K_2CrO_4 indicator: The optimal indicator concentration is 2–5 × 10^{-3} M. Too much chromate shifts the endpoint before the true equivalence; too little delays it.
- Temperature: Room temperature preferred; elevated temperatures increase Ksp and may cause errors.
- Interfering ions: Ions that precipitate with Ag^+ (PO_4^{3-}, SO_4^{2-}, AsO_4^{3-}, oxalate) or with CrO_4^{2-} (Ba^{2+}, Pb^{2+}) must be absent.

11.1.3 Procedure (Mohr Method — Chloride Determination)

1. Pipette the sample solution (containing Cl^-) into a conical flask.
2. Neutralize to pH 7–9 with dilute $NaHCO_3$ or borax buffer if required.
3. Add 1 mL of 5% K_2CrO_4 indicator solution.
4. Titrate with 0.1 M $AgNO_3$ (standardized) with continuous swirling.
5. The endpoint is the first permanent brick-red colour that persists for at least 30 seconds.
6. Record the volume of $AgNO_3$ consumed (V mL).

Calculation

Each mL of 0.1 M $AgNO_3$ ≡ 3.545 mg of Cl^- (Mol. wt. of Cl = 35.45; 35.45 × 0.1 / 1000 × 1000 = 3.545 mg/mL) % Cl^- = (V × M_$AgNO_3$ × 35.45 × 100) / (Sample wt in mg × 1000)

11.1.4 Pharmaceutical Applications of the Mohr Method

- Assay of Sodium Chloride (IP): Direct Mohr titration in neutral aqueous solution.
- Assay of Potassium Chloride: Direct titration with $AgNO_3$.
- Assay of Sodium Chloride Injection (0.9% w/v): After appropriate dilution.
- Chloride content in dialysis fluids, oral rehydration salts, and electrolyte preparations.
- Testing chloride content in pharmaceutical-grade water (limit test).

11.1.5 Limitations of the Mohr Method

- Cannot be used in strongly acidic or alkaline solutions (pH must be 6.5–9.0).
- Not applicable for iodide (AgI is too insoluble for the chromate indicator to function) or thiocyanate determination.
- Coloured solutions may mask the brick-red endpoint.
- Large amounts of barium, lead, or other chromate-precipitating ions interfere.

11.2 The Volhard Method

11.2.1 Principle

The Volhard method is an indirect (back-titration) method for the determination of silver ion, or halides by indirect determination. A known excess of silver nitrate is added to the halide sample; the excess unreacted Ag^+ is back-titrated with a standard

thiocyanate solution (KSCN or NH_4SCN) using ferric ammonium sulfate [$FeNH_4(SO_4)_2$] as the indicator.

Reactions in the Volhard Method

Step 1 — Add excess $AgNO_3$: Ag^+(excess) + Cl^- → AgCl↓ (white precipitate) Step 2 — Back-titrate excess Ag^+ with KSCN: Ag^+(remaining) + SCN^- → AgSCN↓ (white precipitate) Endpoint Reaction: Fe^{3+} + SCN^- → $FeSCN^{2+}$ (blood-red complex, Ksp indicator) Note: For chloride determination, the AgCl precipitate MUST be filtered or treated with nitrobenzene before back-titration, because AgCl (Ksp = 1.8×10^{-10}) is more soluble than AgSCN (Ksp = 1.1×10^{-12}). Without this step, the KSCN titrant would convert AgCl to AgSCN, consuming additional KSCN and giving a falsely high Cl^- result.

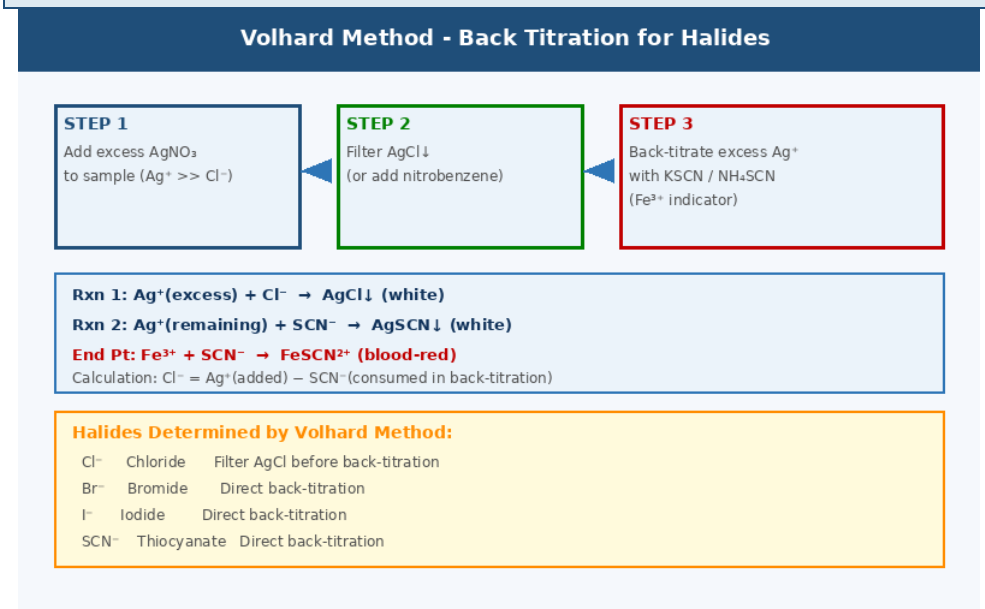


Figure: Volhard Method — Back Titration Steps and Reactions

11.2.2 Procedure (Volhard Method)

1. Add a measured, excess volume of standard 0.1 M $AgNO_3$ to the sample in a conical flask (acidify with dilute HNO_3 to prevent interference from phosphates and carbonates).
2. For chloride: Filter off AgCl precipitate, or add 1–2 mL of nitrobenzene and shake to coat the precipitate.
3. Add 2 mL of ferric alum indicator solution (saturated $FeNH_4(SO_4)_2$ in dilute HNO_3).
4. Back-titrate with 0.1 M KSCN until a permanent blood-red colour ($FeSCN^{2+}$) appears.

Calculation
Moles Cl^- = Moles Ag^+ added – Moles Ag^+ back-titrated with KSCN % Cl^- = [(V_$AgNO_3$ × M_$AgNO_3$ – V_KSCN × M_KSCN) × 35.45 × 100] / (Sample wt in mg × 1000)

11.2.3 Advantages of the Volhard Method

- Can be used in acidic (HNO_3) medium — phosphates and carbonates do not interfere (unlike the Mohr method).
- More versatile: can determine Cl^-, Br^-, I^-, SCN^-, and also silver salts directly.
- Applicable to coloured solutions — blood-red endpoint is dramatic and easily seen.
- Can be used for determination of Ag^+ directly without a back-titration step.

11.2.4 Pharmaceutical Applications of the Volhard Method

- Assay of Ephedrine Hydrochloride: Back-titration after precipitating chloride with excess $AgNO_3$.
- Assay of Homatropine Hydrobromide: Bromide determination by Volhard method.
- Assay of Silver-containing preparations (e.g., Silver Sulfadiazine): Direct titration of Ag^+ with KSCN.
- Thiocyanate determination in biological samples and chemical formulations.

11.3 The Fajans Method — Adsorption Indicators

11.3.1 Principle

The Fajans method uses adsorption indicators — anionic dyes that signal the endpoint by changing colour when adsorbed onto the surface of the precipitate. Kazimierz Fajans introduced this elegant method in 1923, exploiting the charge reversal that occurs on the precipitate surface at the equivalence point.

Mechanism of the Fajans Method
BEFORE Equivalence Point: Excess Cl^- present → AgCl particles adsorb Cl^- → surface is negatively charged (AgCl·Cl^-) Fluorescein anion (Fl^-) is REPELLED from the negatively charged surface → stays in solution → YELLOW-GREEN colour AT Equivalence Point: All Cl^- precipitated → slight excess Ag^+ → AgCl particles now adsorb Ag^+ → surface becomes positively charged (AgCl·Ag^+) Fluorescein anion (Fl^-) is NOW ATTRACTED to positive surface → ADSORBS → structural change → PINK-RED colour ENDPOINT: Yellow-green → Pink-red

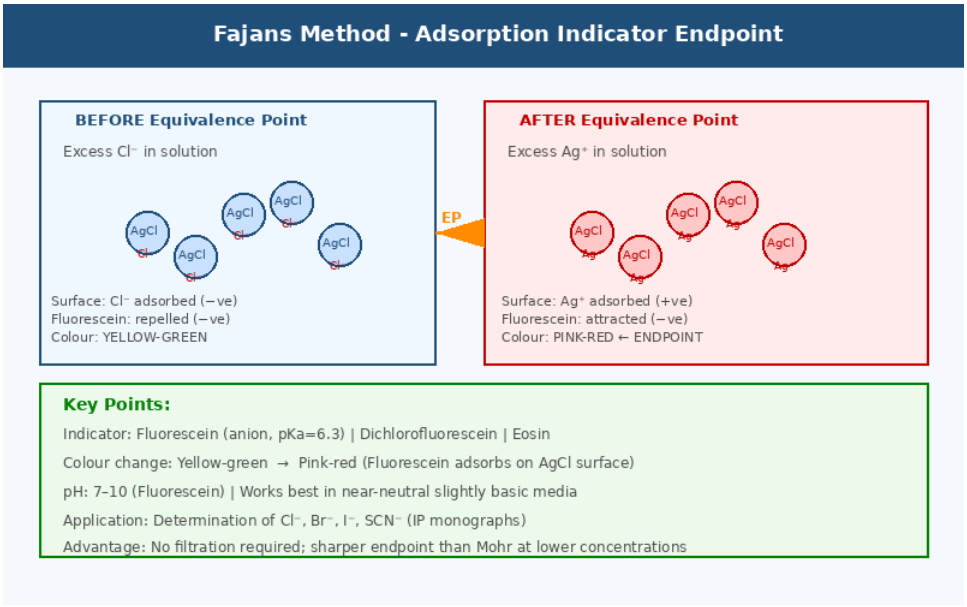


Figure: Fajans Method — Adsorption Indicator Mechanism at Endpoint

11.3.2 Common Adsorption Indicators

Indicator	Analyte Determined	pH Range	Colour Change	Notes
Fluorescein	Cl^-	7–10	Yellow-green → Pink-red	Most common; cannot use in acid
Dichlorofluorescein	Cl^-, Br^-	4–9	Yellow-green → Pink	Wider pH range; preferred in IP
Eosin (Tetrabromofluorescein)	Br^-, I^-, SCN^-	1–7	Orange → Pink-red	Cannot use for Cl^- (too soluble)
Rhodamine 6G	Ag^+ in acidic soln.	Acidic	Orange → Red-violet	Used in reverse titration
Bromophenol Blue	Br^-, I^-	Neutral	Yellow → Blue-violet	Less common; educational use

11.3.3 Procedure (Fajans Method — Chloride Determination)

1. Pipette sample solution into a conical flask; adjust pH to 7–9 with $NaHCO_3$.
2. Add 10 drops of 0.1% dichlorofluorescein indicator.

3. Add a small amount of dextrin solution (to prevent coagulation of the precipitate).
4. Titrate with 0.1 M $AgNO_3$ with swirling. The suspension is yellowish-green.
5. At the endpoint: the white AgCl suspension suddenly turns pink-red. Stop immediately.

Calculation
Each mL of 0.1 M $AgNO_3 \equiv 3.545$ mg Cl^- (same as Mohr calculation)

11.3.4 Advantages and Limitations of Fajans Method

- Advantages: Simple procedure; no filtration needed; applicable to dilute solutions; sharper endpoint at lower concentrations than Mohr method.
- Limitation: pH-dependent; cannot use in strongly acidic or basic media. Eosin cannot be used for chloride. Fluorescein cannot be used in acidic solutions. Solution must not be too dilute.

11.4 Comparison of the Three Argentometric Methods

Feature	Mohr	Volhard	Fajans
Titrant	$AgNO_3$	KSCN (back-titration)	$AgNO_3$
Indicator	K_2CrO_4 (chromate)	Fe^{3+} / $FeSCN^{2+}$	Adsorption indicator (fluorescein)
Endpoint signal	Brick-red Ag_2CrO_4 ppt	Blood-red $FeSCN^{2+}$ complex	Pink-red colour on ppt surface
Medium	Neutral (pH 6.5–9)	Acidic (HNO_3 medium)	Near-neutral to neutral
Method type	Direct	Indirect (back-titration)	Direct
Analytes	Cl^-, Br^-	Cl^-, Br^-, I^-, SCN^-, Ag^+	Cl^-, Br^-, I^-, SCN^-
Interference	Basic/acidic pH, Ba^{2+}, Pb^{2+}	Bromide, iodide give errors if not filtered	Strongly coloured solutions

11.5 Summary

Chapter 11 Summary

Three argentometric methods are used in pharmaceutical analysis: (1) Mohr method — direct $AgNO_3$ titration with K_2CrO_4 indicator, pH 6.5–9, endpoint is brick-red Ag_2CrO_4; used for Cl^- and Br^-. (2) Volhard method — back-titration with KSCN, Fe^{3+} indicator, acidic medium; versatile for Cl^-, Br^-, I^-, SCN^-, Ag^+; filter AgCl before back-titration for chloride. (3) Fajans method — adsorption indicator (fluorescein, dichlorofluorescein, eosin); endpoint by charge reversal on precipitate surface; no filtration needed.

Review Questions

Short Answer Questions

1. Why must the AgCl precipitate be filtered off before back-titration in the Volhard method for chloride?
2. Explain the mechanism of endpoint detection in the Fajans method with fluorescein indicator.
3. State the pH requirements and reasons for the Mohr method.
4. Why cannot fluorescein be used as an adsorption indicator for iodide determination?

Long Answer Questions

1. Describe the Mohr method of argentometric titration with a neat diagram. Include the principle, conditions, procedure, and calculations for assay of sodium chloride.
2. Compare the Mohr, Volhard, and Fajans methods of argentometric precipitation titration with respect to: principle, indicator, medium, analytes determined, and limitations.

Multiple Choice Questions

1. In the Mohr method, the endpoint is indicated by:

(a) Formation of blood-red $FeSCN^{2+}$

(b) Formation of brick-red Ag_2CrO_4 precipitate

(c) Adsorption of fluorescein on AgCl

(d) Colour change of starch indicator

✓***Answer: (b) Formation of brick-red Ag_2CrO_4 precipitate***

2. The Volhard method uses _____ as the indicator:

(a) K_2CrO_4

(b) Fluorescein

(c) Ferric alum (Fe^{3+})

(d) Starch

✓***Answer: (c) Ferric alum (Fe^{3+})***

3. The Fajans method endpoint for chloride with fluorescein is:
(a) Colourless to pink
(b) Yellow-green to pink-red
(c) Red to yellow
(d) Blue to colourless
✓***Answer: (b) Yellow-green to pink-red***

4. Eosin indicator in the Fajans method is used for:
(a) Chloride only
(b) Bromide and iodide
(c) Phosphate
(d) Silver ion only
✓***Answer: (b) Bromide and iodide***

5. The Ksp of AgCl at 25°C is approximately:
(a) 1.8×10^{-5}
(b) 1.8×10^{-10}
(c) 1.0×10^{-14}
(d) 5.0×10^{-6}
✓***Answer: (b)*** 1.8×10^{-10}

Chapter 12
Complexometric Titrations — EDTA

12.1 Learning Objectives

- Explain the chemistry of EDTA and describe its structure and chelate-forming ability.
- Define stability constant and explain its significance in EDTA titrations.
- Describe the role of pH and buffers in EDTA titrations.
- Explain the function of metallochromic indicators with specific examples.
- Describe the types of EDTA titrations and their pharmaceutical applications.

12.2 Introduction

Complexometric titrations are a class of titrimetric methods in which the analyte (usually a metal ion) reacts with a complexing agent (ligand) to form a stable, soluble complex. The most important complexing agent in pharmaceutical analysis is EDTA (Ethylenediaminetetraacetic acid), which forms extremely stable, 1:1 chelate complexes with nearly all metal ions of analytical importance.

Complexometric titrations with EDTA are widely used in the IP, BP, and USP for the assay of:

- Calcium (in calcium gluconate, calcium chloride, calcium lactate injections)
- Magnesium (in magnesium sulfate, antacid preparations)
- Zinc (in zinc sulfate eye drops, zinc oxide preparations)
- Bismuth (in bismuth salts used as antacids and in H. pylori therapy)
- Hardness of pharmaceutical-grade water (total Ca^{2+} + Mg^{2+})

12.3 EDTA — Structure and Chemistry

EDTA (H_4Y) is a polyprotic weak acid with four carboxylic acid groups (pKa_1=2.0, pKa_2=2.67, pKa_3=6.16, pKa_4=10.26) and two amine nitrogen atoms. This gives it six potential donor atoms (4 × $-COO^-$ oxygen and 2 × −N:), making it a hexadentate ligand — it simultaneously occupies all six coordination positions of a metal ion, forming an extremely stable, water-soluble, octahedral chelate complex.

For practical use, the disodium salt ($Na_2H_2Y{\cdot}2H_2O$, MW 372.24) is preferred because it is readily soluble in water, available in high purity, and can be dried and weighed as a primary standard.

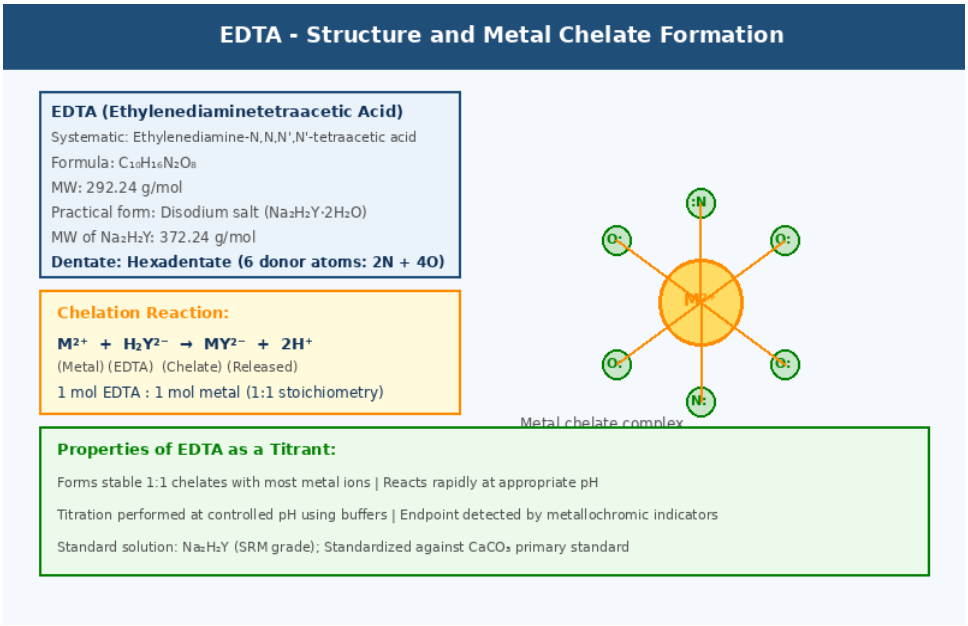


Figure: EDTA Structure, Hexadentate Chelation, and Metal Complex Formation

EDTA Ionisation States

$H_4Y \rightarrow H_3Y^- \rightarrow H_2Y^{2-} \rightarrow HY^{3-} \rightarrow Y^{4-}$ pKa_1=2.0 pKa_2=2.67 pKa_3=6.16 pKa_4=10.26 The fully deprotonated form (Y^{4-}) reacts with metal ions: $M^{n+} + Y^{4-} \rightarrow [MY]^{n-4}$ At lower pH, Y^{4-} is protonated (less available), so titrations are performed at controlled pH using buffers. For Ca^{2+} and Mg^{2+}: pH 10 buffer (NH_3/NH_4Cl) is used.

12.4 Stability Constants of EDTA Chelates

The stability constant (formation constant, Kf) quantifies the stability of the metal-EDTA chelate:

Stability Constant

$M^{n+} + Y^{4-} \rightleftharpoons [MY]^{n-4}$ $Kf = [[MY]^{n-4}] / ([M^{n+}][Y^{4-}])$ Higher Kf (or log Kf) → more stable chelate → more complete reaction → better titration

Metal Ion	log Kf (MY)	Titration pH	Application
Ca^{2+}	10.70	pH 12 (NaOH)	Calcium assay in IP preparations
Mg^{2+}	8.70	pH 10 (NH_3/NH_4Cl)	Magnesium salts, water hardness
Zn^{2+}	16.50	pH 5–6 (acetate)	Zinc sulfate eye drops

Cu^{2+}	18.80	pH 4–6 (acetate)	Copper sulfate preparations
Fe^{3+}	25.10	pH 1–2 (acidic)	Iron(III) analysis
Bi^{3+}	27.90	Acidic medium	Bismuth preparations (IP)
Al^{3+}	16.13	pH 4–5	Aluminium-containing antacids

12.5 The Role of pH in EDTA Titrations

pH profoundly affects EDTA titrations because the equilibrium form of EDTA and the solubility of metal hydroxides are both pH-dependent. The concept of the conditional stability constant (K'f) accounts for the effect of pH:

Conditional Stability Constant

K'f = Kf × αY(H) where αY(H) = fraction of EDTA in the Y^{4-} form at a given pH. At pH 10: αY(H) ≈ 0.36 → K'f(Ca) = 10^10.70 × 0.36 ≈ 1.8 × 10^{10} (adequate for titration) At pH 6: αY(H) ≈ 10^{-5} → K'f(Ca) becomes too small for a sharp endpoint Rule: Minimum pH for an accurate EDTA titration can be estimated from: Minimum pH = 14 − log K'f(minimum) − pKa correction

In practice, pH buffers are always used to maintain pH during EDTA titrations because the titration reaction itself releases protons ($M^{n+} + H_2Y^{2-} \rightarrow MY + 2H^+$), which would lower the pH and reduce the conditional stability constant.

12.6 Metallochromic Indicators

A metallochromic indicator is a metal-complexing dye that changes colour depending on whether it is free in solution or bound to a metal ion. The indicator must form a less stable complex with the metal than EDTA does, so that EDTA can displace the indicator from the metal at the endpoint.

Indicator Behaviour at Endpoint

Before Endpoint: M^{2+} + Ind → M-Ind complex (Colour A — indicator bound to metal) At Endpoint: EDTA displaces indicator: M-Ind + EDTA → M-EDTA + Ind (free indicator, Colour B) Requirement: Kf(M-EDTA) >> Kf(M-Ind) (EDTA complex more stable than indicator complex)

Indicator	Abbreviation	Colour (Free)	Colour (Metal-bound)	Used For	pH
Eriochrome Black T	EBT	Blue	Wine-red	Ca^{2+}, Mg^{2+}, Zn^{2+}	10 (NH_3)
Murexide (Ammonium Purpurate)	—	Purple	Orange-red	Ca^{2+} (selective)	12 (NaOH)
Calcon (Solochrome Black)	—	Blue	Red	Ca^{2+}, Mg^{2+}	10 (NH_3)
Xylenol Orange	XO	Yellow	Red-violet	Bi^{3+}, Pb^{2+}, Zn^{2+}	Acidic (1–5)
Pyrocatechol Violet	PV	Yellow	Blue	Al^{3+}, Bi^{3+}	Acidic
Calmagite	—	Blue	Red	Ca^{2+}, Mg^{2+}	10 (NH_3)

12.6.1 Eriochrome Black T (EBT) — In Detail

EBT (Calconcarboxylic acid) is the most widely used metallochromic indicator in pharmaceutical analysis. It is used in the assay of calcium, magnesium, zinc, and for water hardness determination at pH 10 (ammoniacal buffer).

- Free EBT in aqueous solution at pH 10: BLUE (azure blue colour)
- EBT complexed with Ca^{2+} or Mg^{2+}: WINE-RED colour
- Endpoint: Colour changes from wine-red to clear blue as EDTA displaces EBT from the metal complex
- Important: EBT slowly oxidises and decomposes. It should be freshly prepared or stored as a solid mixture with NaCl or KNO_3 (1% solution stable for only 1–2 weeks).

12.7 Types of EDTA Titrations

12.7.1 Direct Titration

The sample solution containing the metal ion is directly titrated with standard EDTA solution at appropriate pH with the metallochromic indicator. This is the simplest and most common type.

Example — Assay of Zinc Sulfate (IP):

1. Dissolve ~0.5 g of $ZnSO_4$ in water; adjust pH to 10 with ammonia buffer.
2. Add 50 mg of EBT indicator mixture.

3. Titrate with 0.05 M EDTA until colour changes from wine-red to blue.

Calculation
Each mL of 0.05 M EDTA ≡ 8.095 mg $ZnSO_4 \cdot 7H_2O$ (MW = 287.54) $ZnSO_4$ (%) = (V × 0.05 × 287.54 × 100) / (Sample wt in mg × 1000)

12.7.2 Back Titration

Used when: (a) the direct titration is too slow; (b) there is no suitable indicator; or (c) the metal ion forms a very insoluble hydroxide at the titration pH.
Procedure: Add a known excess of standard EDTA to the sample. Adjust to appropriate pH. Back-titrate the excess EDTA with a standard metal salt solution (e.g., $ZnSO_4$ or $MgSO_4$) using EBT indicator.
Pharmaceutical example: Assay of aluminium hydroxide gel — excess EDTA added at pH 4.5, back-titrated with standard $ZnSO_4$.

12.7.3 Indirect (Substitution) Titration

Used for metal ions that do not respond well to direct EDTA titration (e.g., Ca^{2+} determination using the murexide indicator involves an indirect approach where Mg^{2+} is displaced).
Example: Assay of calcium (IP) — at pH 12, Mg^{2+} precipitates as $Mg(OH)_2$, allowing selective titration of Ca^{2+} with EDTA using murexide indicator (orange → purple).

12.7.4 Masking and Demasking

When a sample contains a mixture of metal ions, a masking agent is used to prevent certain metals from reacting with EDTA, allowing selective titration of the analyte ion.

Masking Agent	Metal Ions Masked	Purpose
Cyanide (CN^-)	Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Fe^{3+}	Allows selective titration of Ca^{2+} and Mg^{2+}
Triethanolamine (TEA)	Al^{3+}, Fe^{3+}, Mn^{2+}	Prevents interference in alkaline EDTA titrations
Ascorbic Acid	Fe^{3+} (reduces to Fe^{2+})	Prevents Fe^{3+} blocking of EBT indicator
Fluoride (F^-)	Al^{3+}, Fe^{3+}	Forms stable fluoride complexes; allows other metals to be titrated

12.8 EDTA Titration Curve

The EDTA titration curve plots pM (= $-\log[M^{2+}]$) against volume of EDTA added. The shape is analogous to an acid-base titration curve, with a sharp inflection (large jump in pM) at the equivalence point.

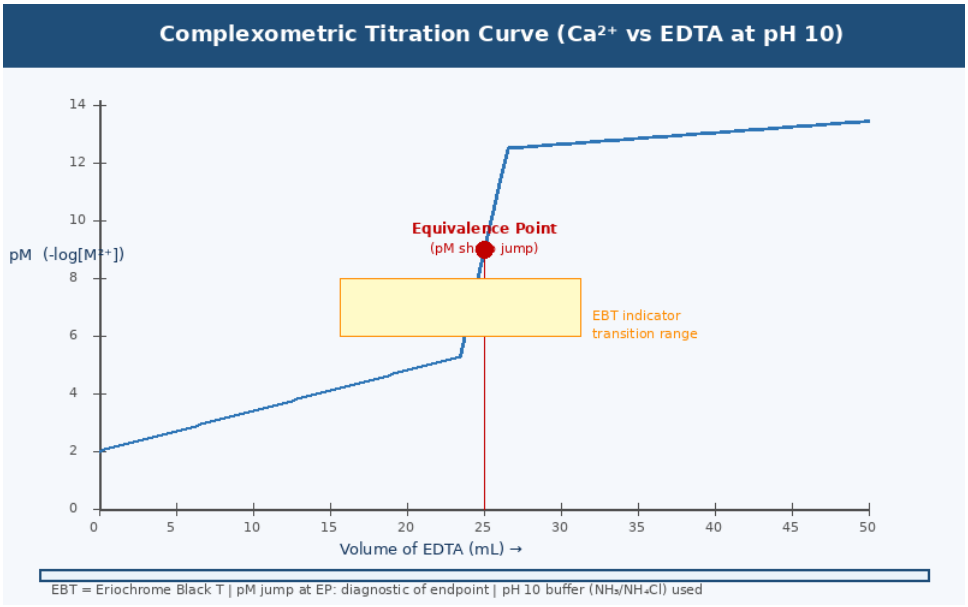


Figure: Complexometric Titration Curve — pM vs Volume of EDTA (Ca^{2+}, pH 10)

Key features of the EDTA titration curve:

- Before equivalence point: pM is low (high $[M^{2+}]$) — slowly rising curve.
- At equivalence point: Sharp jump in pM — the steeper the jump, the more stable the chelate (higher Kf).
- After equivalence point: pM levels off — determined by the excess EDTA added.
- The metallochromic indicator must change colour within the steep pM jump region for an accurate endpoint.

12.9 Standardization of EDTA Solution

EDTA solution (typically 0.1 M or 0.05 M) is standardized against a primary standard. Calcium carbonate ($CaCO_3$) is the preferred primary standard for EDTA:

1. Dry $CaCO_3$ at 110°C for 2 hours; cool in desiccator.
2. Accurately weigh ~0.10 g of $CaCO_3$; dissolve in minimum dilute HCl.
3. Neutralize with dilute NaOH; add pH 10 ammonia buffer; add EBT indicator.
4. Titrate with EDTA solution until colour changes from wine-red to blue.

Calculation

Molarity of EDTA = (Mass $CaCO_3$ in g) / (Volume EDTA in mL × 0.001 × 100.09) [MW $CaCO_3$ = 100.09 g/mol]

12.10 Summary

Chapter 12 Summary

EDTA is a hexadentate chelating agent that forms stable 1:1 chelates with metal ions. Stability of chelate (Kf) governs the sharpness of the titration endpoint. pH control (using buffers) is critical — it determines the fraction of EDTA in the reactive Y^{4-} form. EBT (wine-red to blue), murexide (orange to purple), and xylenol orange are key metallochromic indicators. Types of EDTA titrations: direct, back, indirect, and masking/demasking. Standard EDTA is prepared from Na_2H_2Y and standardized against $CaCO_3$.

Review Questions

Short Answer Questions

1. Why is EDTA described as a hexadentate ligand? How many donor atoms does it possess?
2. What is a conditional stability constant? Why is it important in EDTA titrations?
3. Describe the colour change of EBT indicator at the endpoint of a calcium titration.
4. What is the purpose of masking agents in EDTA titrations? Give two examples.

Long Answer Questions

1. Describe the EDTA titration for assay of zinc sulfate as per IP. Include principle, indicator, buffer, procedure, endpoint detection, and calculation.
2. Explain the different types of EDTA titrations (direct, back, indirect) with appropriate pharmaceutical examples for each.

Multiple Choice Questions

1. EDTA is a hexadentate ligand because it has:

(a) 6 nitrogen donor atoms

(b) 4 carboxylate + 2 amine donor atoms (total 6)

(c) 4 nitrogen donor atoms

(d) 2 donor atoms only

✓***Answer: (b) 4 carboxylate + 2 amine donor atoms (total 6)***

2. The pH buffer used for Ca^{2+} and Mg^{2+} EDTA titration is:

(a) Acetate buffer (pH 4–5)

(b) Phosphate buffer (pH 7)

(c) Ammonia buffer (pH 10)

(d) Borate buffer (pH 8)

✓***Answer: (c) Ammonia buffer (pH 10)***

3. The colour change of EBT at the endpoint of an EDTA titration is:

(a) Colourless to pink

(b) Wine-red to blue

(c) Yellow to violet

(d) Orange to red

✓***Answer: (b) Wine-red to blue***

4. Murexide indicator is used selectively for:

(a) Mg^{2+}

(b) Zn^{2+}

(c) Ca^{2+}

(d) Fe^{3+}

✓***Answer: (c)*** Ca^{2+}

5. The primary standard used to standardize EDTA solution is:

(a) Sodium chloride

(b) Potassium dichromate

(c) Calcium carbonate ($CaCO_3$)

(d) Oxalic acid

✓***Answer: (c) Calcium carbonate*** ($CaCO_3$)

Chapter 13
Pharmaceutical Applications of Precipitation and Complexometric Titrations

13.1 Introduction

Precipitation and complexometric titrations are indispensable tools in pharmacopoeial pharmaceutical analysis. This chapter presents detailed pharmacopoeial assay procedures for specific drug substances and preparations, with full reaction schemes and calculations. These assays represent the direct application of the theoretical principles developed in the preceding chapters.

13.2 Assay of Sodium Chloride (IP) — Mohr Method

Materials

- Sample: Sodium Chloride (NaCl) tablets/powder
- Titrant: 0.1 M Silver Nitrate ($AgNO_3$) — standardized
- Indicator: K_2CrO_4 solution (5% w/v)
- Buffer: Dilute $NaHCO_3$ solution (to adjust pH 7–9)

Reaction

Reaction

$Ag^+ + Cl^- \rightarrow AgCl\downarrow$ (white) $2Ag^+ + CrO_4^{2-} \rightarrow Ag_2CrO_4\downarrow$ (brick-red, endpoint)

Procedure

1. Accurately weigh ~0.25 g of NaCl; dissolve in 50 mL water.
2. Adjust pH to 7–9 with $NaHCO_3$ solution.
3. Add 1 mL K_2CrO_4 indicator.
4. Titrate with 0.1 M $AgNO_3$ to the first permanent brick-red endpoint.

Calculation

Wt NaCl = V(mL) × M × 58.44 mg/mmol % NaCl = (V × 0.1 × 58.44 × 100) / (Sample wt in mg × 1000) IP limit: 99.0–100.5%

13.3 Assay of Calcium Gluconate (IP) — EDTA Method

Calcium gluconate (MW 448.39) is widely used as a calcium supplement and in emergency treatment of hypocalcaemia. It is assayed complexometrically with EDTA.

Reaction

$Ca^{2+} + H_2Y^{2-} \rightarrow CaY^{2-} + 2H^+$ (Calcium) (EDTA) (Calcium-EDTA chelate)

Procedure

1. Dissolve ~0.50 g calcium gluconate in hot water (60°C).
2. Cool; add 10 mL pH 10 ammonia-ammonium chloride buffer.
3. Add 50 mg EBT-NaCl indicator mixture.
4. Titrate with 0.05 M EDTA to the change from wine-red to blue.

Calculation

Each mL of 0.05 M EDTA ≡ 22.42 mg Ca-gluconate ($C_{12}H_{22}CaO_{14}$, MW 448.39) % $C_{12}H_{22}CaO_{14}$ = (V × 0.05 × 448.39 × 100) / (Sample wt in mg × 1000) IP limit: 99.0–101.0%

13.4 Assay of Zinc Sulfate Eye Drops (IP) — EDTA Method

Zinc sulfate ($ZnSO_4 \cdot 7H_2O$, MW 287.54) is used in eye drops (0.25% w/v) as an astringent and mild antiseptic. Its assay is by direct EDTA complexometry.

Procedure

1. Take 20 mL of zinc sulfate eye drops into a flask.
2. Add 10 mL ammonia buffer (pH 10) and 0.1 mL EBT indicator.
3. Titrate with 0.05 M EDTA until wine-red changes to blue.

Calculation

$ZnSO_4 \cdot 7H_2O$ (mg/mL) = (V × M_EDTA × 287.54) / volume of sample taken

13.5 Determination of Water Hardness (Total Hardness — EDTA Method)

Water hardness is caused by dissolved Ca^{2+} and Mg^{2+} salts. Hard water interferes with pharmaceutical manufacturing (precipitation of drug salts, poor solubilization). Hardness is determined by EDTA titration.

- Total Hardness: Determined at pH 10 (ammonia buffer) using EBT indicator — measures Ca^{2+} + Mg^{2+} combined.
- Calcium Hardness: Determined separately at pH 12 (NaOH) using murexide indicator — measures Ca^{2+} only. Mg^{2+} precipitates as $Mg(OH)_2$ at pH 12 and does not react.
- Magnesium Hardness: Calculated by difference (Total Hardness – Calcium Hardness).

Expression of Water Hardness

Hardness is expressed in mg/L (ppm) of $CaCO_3$ equivalent: Total Hardness (ppm $CaCO_3$) = (V_EDTA × M_EDTA × 100.09 × 1000) / Volume of water sample (mL) Acceptable hardness for Purified Water (IP): < 5 ppm $CaCO_3$

13.6 Summary

Chapter 13 Summary

Pharmacopoeial applications of precipitation titrations include assay of NaCl (Mohr method), KCl, and halide-containing drug salts. Complexometric EDTA titrations are used for calcium gluconate, zinc sulfate, magnesium sulfate, and water hardness determination. The choice of indicator and buffer pH is critical: EBT at pH 10 for Ca^{2+}/Mg^{2+}, murexide at pH 12 for Ca^{2+} alone, xylenol orange at pH 5–6 for Zn^{2+} and Bi^{3+}. Calculations follow the general formula using molarity and equivalent weight.

Review Questions

Short Answer Questions

1. Write the calculation formula for the assay of calcium gluconate by EDTA titration.
2. How is calcium hardness distinguished from total hardness in water analysis?
3. What is the role of ammonia buffer in the EDTA assay of zinc?

Long Answer Questions

1. Describe with a complete procedure the EDTA complexometric assay of calcium gluconate as per IP. Include reaction, conditions, indicator, buffer, and calculation.
2. Explain the determination of total water hardness by EDTA titration. Distinguish between total hardness, calcium hardness, and magnesium hardness.

Multiple Choice Questions

1. In the EDTA assay of calcium gluconate, the indicator used is:

(a) Murexide
(b) EBT (Eriochrome Black T)
(c) Xylenol orange
(d) Phenolphthalein

✓***Answer: (b) EBT (Eriochrome Black T)***

2. Total water hardness is determined at pH:

(a) 4–5
(b) 7
(c) 10
(d) 12

✓***Answer: (c) 10***

3. For selective determination of Ca^{2+} in the presence of Mg^{2+}, the pH used is:
(a) 4
(b) 7
(c) 10
(d) 12 (Mg^{2+} precipitates as $Mg(OH)_2$)
✓Answer: (d) 12 (Mg^{2+} precipitates as $Mg(OH)_2$)

4. Water hardness is expressed in units of:
(a) mEq/L
(b) mg/L as $CaCO_3$
(c) % w/v
(d) µg/mL
✓Answer: (b) mg/L as $CaCO_3$

5. The Volhard method is used for the assay of:
(a) Calcium gluconate
(b) Zinc sulfate
(c) Ephedrine hydrochloride (Cl^- determination)
(d) Magnesium sulfate
✓Answer: (c) Ephedrine hydrochloride (Cl^- determination)

UNIT IV
REDOX TITRATIONS

Redox (oxidation-reduction) titrations are based on the transfer of electrons between the analyte and the titrant. They include some of the most powerful and widely applicable titrimetric methods in pharmaceutical analysis, covering the assay of reducing agents, oxidizing agents, and many organic drug substances. This unit covers the fundamental principles of redox chemistry, iodimetry and iodometry, and the permanganate and dichromate titration methods.

Chapter 14
Principles of Oxidation-Reduction

14.1 Learning Objectives

- Define oxidation and reduction in terms of electron transfer and changes in oxidation state.
- Balance redox reactions using the ion-electron (half-reaction) method.
- Explain the concept of standard electrode potential (E°) and the electrochemical series.
- Apply the Nernst equation to calculate cell potentials under non-standard conditions.
- Calculate equilibrium constants from cell potentials.
- Describe the types of indicators used in redox titrations.

14.2 Introduction

Oxidation-reduction (redox) reactions involve the transfer of electrons from one chemical species (the reducing agent, or reductant) to another (the oxidizing agent, or oxidant). Redox titrations exploit these reactions for the quantitative analysis of pharmaceutical substances. The reducing agent loses electrons (is oxidized) while the oxidizing agent gains electrons (is reduced) — a relationship elegantly captured in the mnemonic 'OIL RIG': Oxidation Is Loss; Reduction Is Gain.

Redox titrations are among the most versatile in pharmaceutical analysis because oxidation-reduction reactions are thermodynamically driven and quantitative. They can be used to assay substances as chemically diverse as iron salts, vitamin C (ascorbic acid), vitamin K_1 (phytomenadione), hydrogen peroxide, and reducing sugars.

14.3 Key Definitions

Term	Definition	Example
Oxidation	Loss of electrons (increase in oxidation state)	$Fe^{2+} \rightarrow Fe^{3+} + e^-$
Reduction	Gain of electrons (decrease in oxidation state)	$MnO_4^- + 5e^- \rightarrow Mn^{2+}$
Oxidising agent	Electron acceptor; itself gets reduced	$KMnO_4$, $K_2Cr_2O_7$, I_2
Reducing agent	Electron donor; itself gets oxidised	Fe^{2+}, $H_2C_2O_4$, $Na_2S_2O_3$, Vitamin C
Oxidation number	Charge on atom if bonds were fully ionic	Mn in MnO_4^- = +7; Mn in Mn^{2+} = +2
Half-reaction	Equation showing only oxidation or only reduction	$MnO_4^- + 8H^+ + 5e^- \rightarrow Mn^{2+} + 4H_2O$

14.4 Balancing Redox Reactions — Ion-Electron Method

The ion-electron (half-reaction) method is the standard approach to balancing complex redox equations:

1. Separate the reaction into oxidation and reduction half-reactions.
2. Balance atoms other than O and H.
3. Balance O by adding H_2O.
4. Balance H by adding H^+ (in acid) or OH^- (in base).
5. Balance charge by adding electrons (e^-).
6. Multiply each half-reaction by appropriate integer so electrons cancel.
7. Add the two half-reactions; simplify.

Worked Example: Balance $KMnO_4 + FeSO_4$ in Acidic Solution

Reduction: $MnO_4^- + 8H^+ + 5e^- \rightarrow Mn^{2+} + 4H_2O$ ×1 Oxidation: $Fe^{2+} \rightarrow Fe^{3+} + e^-$ ×5 Adding: $MnO_4^- + 8H^+ + 5Fe^{2+} \rightarrow Mn^{2+} + 5Fe^{3+} + 4H_2O$ Full ionic: $2KMnO_4 + 10FeSO_4 + 8H_2SO_4 \rightarrow 2MnSO_4 + 5Fe_2(SO_4)_3 + K_2SO_4 + 8H_2O$

14.5 Standard Electrode Potentials (E°)

The tendency of a half-reaction to proceed as a reduction is quantified by its standard reduction potential (E°), measured relative to the standard hydrogen electrode (SHE, E° = 0.00 V by definition) under standard conditions (25°C, 1 atm, all concentrations = 1 M).

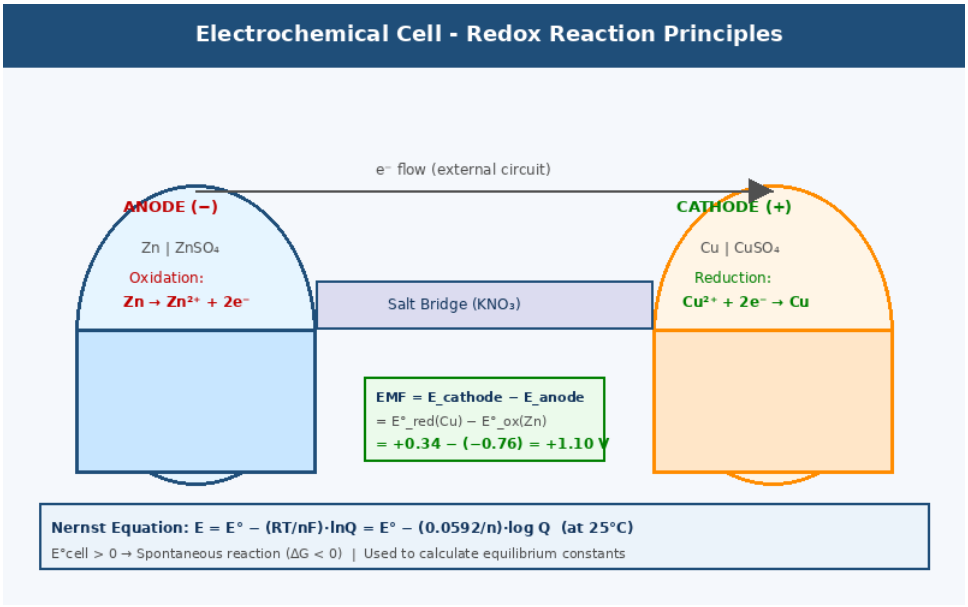


Figure: Galvanic Cell Illustrating Redox Principles and Electrode Potentials

Half-Reaction (Reduction)	E° (V)	Significance
$F_2 + 2e^- \rightarrow 2F^-$	+2.87	Strongest oxidising agent
$MnO_4^- + 8H^+ + 5e^- \rightarrow Mn^{2+} + 4H_2O$	+1.51	$KMnO_4$ — powerful oxidant in analysis
$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O$	+1.33	$K_2Cr_2O_7$ — milder; more selective
$I_2 + 2e^- \rightarrow 2I^-$	+0.54	I_2 — mild oxidant; used in iodimetry
$2H^+ + 2e^- \rightarrow H_2$	0.00	Standard Hydrogen Electrode reference
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	+0.77	Ferric-ferrous couple; used in redox titrations
$S_4O_6^{2-} + 2e^- \rightarrow 2S_2O_3^{2-}$	+0.08	Thiosulfate — used in iodometry

The cell potential for a spontaneous redox reaction is calculated from:

Cell Potential Formula
E°cell = E°cathode (reduction) − E°anode (reduction) If E°cell > 0 → Reaction is spontaneous (ΔG < 0) Relationship: ΔG° = −nFE°cell log K = nE°cell / 0.0592 (at 25°C)

14.6 The Nernst Equation

Standard potentials apply only under standard conditions. For non-standard conditions (concentrations ≠ 1 M), the Nernst equation gives the actual electrode potential:

Nernst Equation

$E = E° - (RT/nF) \times \ln Q$ At 25°C: $E = E° - (0.0592/n) \times \log Q$ Where: E = actual electrode potential (V) E° = standard electrode potential (V) n = number of electrons transferred Q = reaction quotient R = 8.314 J/mol·K; F = 96485 C/mol Significance in pharmaceutical analysis: The Nernst equation explains why redox potentials change during a titration (as concentrations of oxidant/reductant change), and forms the basis of potentiometric endpoint detection in redox titrations.

14.7 Indicators for Redox Titrations

Four types of indicators are used to signal the endpoint in redox titrations:

14.7.1 Self-Indicating Titrants

Some titrants are intensely coloured in one oxidation state but colourless in another. They serve as their own indicator. The classic example is potassium permanganate ($KMnO_4$):

- $KMnO_4$ (purple/violet) is reduced to Mn^{2+} (colourless/pale pink) in acid.
- Endpoint: The first drop of excess $KMnO_4$ imparts a faint, permanent pink colour — no external indicator is needed.

14.7.2 Specific Redox Indicators — Starch

Starch solution forms an intensely blue-black complex with iodine (I_2) and triiodide (I_3^-). This complex dissociates (blue-black → colourless) when I_2 is consumed. Starch is the specific indicator for iodimetric and iodometric titrations:

- Iodimetry endpoint: Colourless solution becomes blue-black (when I_2 appears or is added).
- Iodometry endpoint: Blue-black complex becomes colourless (when all I_2 is consumed by $Na_2S_2O_3$).
- Important: In iodometry, starch is added only near the endpoint (when the iodine colour has faded to pale yellow), not at the beginning. At high $[I_2]$, the starch-iodine complex is too stable and the endpoint is delayed.

14.7.3 Redox Indicators

Redox indicators (also called oxidation-reduction indicators) are compounds that change colour depending on the redox potential of the solution. The indicator has two forms — oxidized (In_ox) and reduced (In_red) — with different colours:

Redox Indicator Theory
In_ox + ne⁻ ⇌ In_red (Colour A) (Colour B) E_In = E°_In − (0.0592/n) × log([In_red]/[In_ox]) Transition interval: E°_In ± (0.0592/n) V The indicator chosen should have E°_In within the steep potential jump at the equivalence point.

Redox Indicator	E° (V)	Colour (Oxidised)	Colour (Reduced)	Used With
Ferroin (Fe-Phenanthroline)	1.06	Pale blue	Red	Ce^{4+} titrations (Cerimetry)
Diphenylamine	0.76	Violet	Colourless	$K_2Cr_2O_7$ titrations
Diphenylamine sulfonate	0.85	Red-violet	Colourless	$K_2Cr_2O_7$ titrations (IP preferred)
Methylene Blue	0.53	Blue	Colourless	Mild reducing agents

14.7.4 Potentiometric Endpoint Detection

A platinum electrode immersed in the titration solution detects the abrupt change in redox potential at the equivalence point. The potential is plotted against volume of titrant; the inflection point gives the equivalence point. This is the most accurate endpoint method for redox titrations and is used in pharmacopoeial referee assays.

14.8 Summary

Chapter 14 Summary
Redox titrations are based on electron transfer. Oxidation = loss of electrons; Reduction = gain. Half-reactions are balanced by the ion-electron method. E°cell = E°cathode − E°anode; spontaneous if E°cell > 0. The Nernst equation gives E at non-standard conditions. Indicators: $KMnO_4$ is self-indicating; starch is specific for I_2/iodometry; redox indicators (ferroin, diphenylamine) and potentiometric detection complete the toolkit.

Review Questions

Short Answer Questions

1. Define oxidising agent and reducing agent with one example each.
2. What is the standard hydrogen electrode (SHE)? What is its assigned E° value?
3. State the Nernst equation and define each symbol.
4. Why is starch indicator added only near the endpoint in iodometric titrations?

Long Answer Questions

1. Balance the following redox equation by the ion-electron method in acidic medium: $KMnO_4 + H_2C_2O_4 \rightarrow MnSO_4 + CO_2$. Calculate E°cell from tabulated E° values.
2. Explain the types of indicators used in redox titrations with examples. Discuss the theory of redox indicators and the criteria for selecting one.

Multiple Choice Questions

1. In a redox reaction, the reducing agent:

(a) Gains electrons
(b) Loses electrons
(c) Accepts protons
(d) Forms a precipitate

✓***Answer: (b) Loses electrons***

2. The standard electrode potential of $KMnO_4/Mn^{2+}$ couple is:

(a) 0.00 V
(b) +0.54 V
(c) +1.33 V
(d) +1.51 V

✓***Answer: (d) +1.51 V***

3. Ferroin indicator is used in:

(a) Iodometric titrations
(b) Cerimetric titrations
(c) EDTA titrations
(d) Acid-base titrations

✓***Answer: (b) Cerimetric titrations***

4. The Nernst equation at 25°C is:

(a) $E = E° + (0.0592/n) \log Q$
(b) $E = E° - (0.0592/n) \log Q$
(c) $E = RT/nF$
(d) $E = \Delta G/nF$

✓***Answer: (b) $E = E° - (0.0592/n) \log Q$***

5. $KMnO_4$ acts as a self-indicator because:

(a) It is a primary standard

(b) It is colourless in both oxidation states

(c) It is intensely purple and its reduced form (Mn^{2+}) is colourless/pale

(d) It changes pH at the endpoint

✓***Answer: (c) It is intensely purple and its reduced form (Mn^{2+}) is colourless/pale***

Chapter 15
Iodimetry and Iodometry

15.1 Learning Objectives

- Define iodimetry and iodometry and distinguish between the two.
- Explain the chemistry of iodine as a titrant and iodide as a source of iodine.
- Describe the role and mechanism of the starch indicator.
- Describe the preparation and standardization of standard iodine and sodium thiosulfate solutions.
- Apply iodimetric and iodometric principles to pharmacopoeial assays.

15.2 Iodimetry — Direct Iodine Titration

15.2.1 Definition and Principle

Iodimetry is a titrimetric method in which a standard solution of iodine (I_2) is used as the titrant to directly oxidize reducing agents. The analyte (reducing agent) is titrated with I_2 solution, and the endpoint is detected by the starch indicator (colourless to blue-black — the first excess I_2 forms the blue-black starch complex).

Iodimetric Half-Reactions

Reduction of I_2 (I_2 acts as oxidant): $I_2 + 2e^- \rightarrow 2I^-$ ($E° = +0.54$ V) Oxidation of reducing agent (e.g., Vitamin C): $C_6H_8O_6 \rightarrow C_6H_6O_6 + 2H^+ + 2e^-$ Overall: $C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2HI$

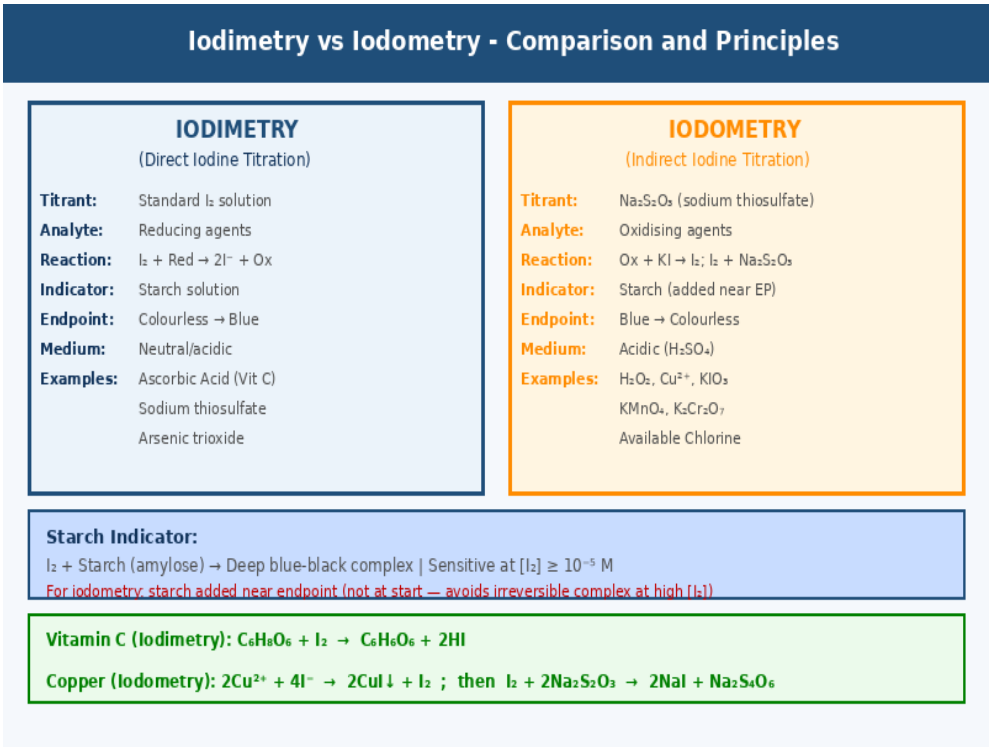


Figure: Iodimetry vs Iodometry — Principles, Indicators, and Reactions

15.2.2 Standard Iodine Solution

Iodine is not a primary standard because:

- Pure iodine is difficult to obtain and weigh accurately (sublimes and is hygroscopic).
- Aqueous iodine solution is unstable (I_2 slowly oxidizes with atmospheric O_2).

Therefore, iodine solution is prepared approximately and standardized against arsenic trioxide (As_2O_3) primary standard, or against sodium thiosulfate which itself has been standardized. Iodine is dissolved in concentrated KI solution (to form KI_3 complex — triiodide) to increase solubility.

Standardization of I_2 against As_2O_3

$As_2O_3 + 2I_2 + 2H_2O \rightarrow As_2O_5 + 4HI$ (or: $AsO_3^{3-} + I_2 + H_2O \rightarrow AsO_4^{3-} + 2H^+ + 2I^-$)

Reaction performed in neutral solution ($NaHCO_3$ buffer, pH ~8) Endpoint: starch indicator (colourless → blue-black)

15.2.3 Pharmacopoeial Assays — Iodimetry

Drug Assayed	Reaction	Starch Endpoint
Ascorbic Acid (Vitamin C)	$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2HI$	Colourless → blue-black
Sodium Thiosulfate	$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI$	Colourless → blue-black
Arsenic Trioxide (As_2O_3)	$As_2O_3 + 2I_2 + 2H_2O \rightarrow As_2O_5 + 4HI$	Colourless → blue-black
Sodium Arsenite	$AsO_2^- + I_2 + 2H_2O \rightarrow AsO_4^{3-} + 2I^- + 4H^+$	Colourless → blue-black
Morphine Sulfate	Morphine (reducing) → Oxidised product + 2HI	Colourless → blue-black

15.2.4 Assay of Ascorbic Acid (Vitamin C) by Iodimetry — IP Method

1. Dissolve ~0.15 g of ascorbic acid in 20 mL of recently boiled and cooled water containing 5 mL dilute H_2SO_4.
2. Add 1 mL of starch indicator solution.
3. Titrate with 0.05 M iodine solution until a persistent blue-black colour appears.

Reaction and Calculation

$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2HI$ (MW Ascorbic Acid = 176.12; n=2; Eq. wt. = 88.06) Each mL of 0.05 M $I_2 \equiv 8.806$ mg Ascorbic Acid % Ascorbic Acid = (V × 0.05 × 176.12 × 100) / (Sample wt in mg × 1000) IP limit: 99.0–100.5%

15.3 Iodometry — Indirect Iodine Titration

15.3.1 Definition and Principle

Iodometry is an indirect titrimetric method in which an oxidising agent liberates iodine (I_2) from potassium iodide (KI) in acidic solution. The liberated I_2 is then titrated with a standard sodium thiosulfate ($Na_2S_2O_3$) solution using starch as the indicator (blue-black to colourless).

Two Steps in Iodometry

STEP 1 — Oxidant liberates I_2: Oxidant (e.g., $KMnO_4$) + KI + H_2SO_4 → I_2 + reduced product STEP 2 — I_2 titrated with thiosulfate: $I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$ (I_2 is the intermediate; thiosulfate reduces it to iodide) Endpoint: Blue-black (I_2-starch complex) → Colourless (all I_2 consumed by thiosulfate)

15.3.2 Sodium Thiosulfate ($Na_2S_2O_3$) — Titrant

Sodium thiosulfate is the standard reducing titrant used in all iodometric titrations. It is not a primary standard and must be standardized against a primary standard oxidant.

Standardization of $Na_2S_2O_3$ against Potassium Dichromate ($K_2Cr_2O_7$):

1. Dissolve accurate weight of $K_2Cr_2O_7$ (primary standard) in water + dilute H_2SO_4.
2. Add excess KI; stopper flask and allow to react in dark for 5 minutes.
3. Dilute; titrate liberated I_2 with $Na_2S_2O_3$ solution.
4. Add starch near endpoint; titrate to colourless.

Reactions

$Cr_2O_7^{2-} + 6I^- + 14H^+ \rightarrow 2Cr^{3+} + 3I_2 + 7H_2O$ $3I_2 + 6Na_2S_2O_3 \rightarrow 6NaI + 3Na_2S_4O_6$

Molarity $Na_2S_2O_3$ = (Wt $K_2Cr_2O_7$ × 6) / (V_thiosulfate × MW_$K_2Cr_2O_7$) [MW $K_2Cr_2O_7$ = 294.18; each mole $K_2Cr_2O_7$ liberates 3 moles $I_2 \equiv$ 6 moles thiosulfate]

15.3.3 Precautions in Iodometric Titrations

- Use excess KI (at least 10-fold): Ensures rapid and complete liberation of I_2; prevents atmospheric oxidation of I^-.
- Acidic medium (H_2SO_4 or HCl): Required for the oxidant to react with KI; do NOT use HNO_3 (oxidising acid interferes).
- React in dark: Prevents photocatalytic oxidation of I^- by atmospheric O_2, which would consume thiosulfate and give high results.
- Add starch near the endpoint: When iodine colour has faded to pale yellow. At high [I_2], the starch-iodine complex is very stable and the endpoint is sluggish.
- Freshly boiled water: Removes dissolved O_2 which would oxidise thiosulfate.
- Titrate promptly: Avoid prolonged exposure to air after adding KI.

15.3.4 Pharmacopoeial Applications — Iodometry

Oxidant Determined	KI Reaction (Step 1)	Pharmaceutical Application
Hydrogen Peroxide (H_2O_2)	$H_2O_2 + 2KI + H_2SO_4 \rightarrow I_2 + K_2SO_4 + 2H_2O$	Assay of 3% H_2O_2 solution (IP)
Copper Sulfate ($CuSO_4$)	$2Cu^{2+} + 4I^- \rightarrow 2CuI\downarrow + I_2$	Assay of Copper Sulfate (IP)
Potassium Iodate (KIO_3)	$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O$	Assay of KIO_3 tablets
Available Chlorine	$Cl_2 + 2KI \rightarrow 2KCl + I_2$	Testing bleaching powder, chlorinated water
Potassium Dichromate	$Cr_2O_7^{2-} + 6I^- + 14H^+ \rightarrow 2Cr^{3+} + 3I_2$	Standardization of $Na_2S_2O_3$; Assay Cr preparations

15.3.5 Assay of Hydrogen Peroxide (IP) by Iodometry

1. Pipette 10 mL of H_2O_2 sample into a 250 mL conical flask.
2. Add 50 mL water, 10 mL dilute H_2SO_4, and 2 g KI crystals.
3. Stopper; swirl; keep in dark for 10 minutes.
4. Titrate liberated I_2 with 0.1 M $Na_2S_2O_3$ solution.
5. Add starch near endpoint (when solution is pale yellow); titrate to colourless.

Calculation

$H_2O_2 + 2KI + H_2SO_4 \rightarrow I_2 + K_2SO_4 + 2H_2O$ $I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$ Each mL of 0.1 M $Na_2S_2O_3 \equiv 1.701$ mg H_2O_2 (MW $H_2O_2 = 34.01$; n=2 electrons; each mL 0.1 M $Na_2S_2O_3 \equiv 0.5$ mmol $H_2O_2 \times 34.01 \times 0.1 = 1.701$ mg) % w/v $H_2O_2 = (V \times 0.1 \times 34.01 \times 100) / (\text{Volume of sample} \times 1000 \times 2)$

15.4 Summary

Chapter 15 Summary

Iodimetry uses standard I_2 as titrant for direct oxidation of reducing agents (ascorbic acid, thiosulfate, As_2O_3); endpoint: colourless → blue-black (starch). Iodometry uses excess KI with an oxidant to liberate I_2, which is back-titrated with $Na_2S_2O_3$; endpoint: blue-black → colourless (starch). Precautions: acidic medium, excess KI, dark conditions, add starch near endpoint, freshly boiled water. $Na_2S_2O_3$ is standardized against $K_2Cr_2O_7$. Pharmaceutical applications: Vitamin C, H_2O_2, $CuSO_4$, KIO_3, available chlorine.

Review Questions

Short Answer Questions

1. What is the difference between iodimetry and iodometry? Give one pharmaceutical example of each.
2. State four precautions to be observed during iodometric titrations.

Long Answer Questions

1. Describe the iodimetric assay of ascorbic acid (Vitamin C) as per IP. Include principle, reagents, procedure, endpoint, and calculation.
2. Explain the principle, procedure, and calculations for the iodometric assay of hydrogen peroxide solution.

Multiple Choice Questions

1. In iodimetry, the titrant used is:

(a) KI solution

(b) $Na_2S_2O_3$ solution

(c) Standard I_2 solution

(d) KIO_3 solution

✓***Answer: (c) Standard I_2 solution***

2. The endpoint in iodometric titrations is:

(a) Blue-black to colourless

(b) Colourless to blue-black

(c) Red to yellow

(d) Pink to colourless

✓***Answer: (a) Blue-black to colourless***

3. Sodium thiosulfate is standardized against:

(a) $KMnO_4$

(b) Oxalic acid

(c) Potassium dichromate ($K_2Cr_2O_7$)

(d) As_2O_3

✓***Answer: (c) Potassium dichromate ($K_2Cr_2O_7$)***

4. In iodometric titrations, starch indicator is added:

(a) At the beginning

(b) When half the titrant is added

(c) Near the endpoint when I_2 colour is pale yellow

(d) After adding all $Na_2S_2O_3$

✓***Answer: (c) Near the endpoint when I_2 colour is pale yellow***

5. The equivalent weight of ascorbic acid in iodimetric assay is:

(a) 176.12

(b) 88.06

(c) 44.03

(d) 352.24

✓***Answer: (b) 88.06***

Chapter 16
Permanganate and Dichromate Titrations

16.1 Learning Objectives

- Describe the chemistry of potassium permanganate ($KMnO_4$) as an oxidizing titrant.
- Explain the self-indicating property of $KMnO_4$ and its reactions in acidic, neutral, and alkaline media.
- Describe the chemistry of potassium dichromate ($K_2Cr_2O_7$) as an oxidizing titrant and compare it with $KMnO_4$.
- Discuss the preparation, standardization, and pharmaceutical applications of $KMnO_4$ and $K_2Cr_2O_7$.
- Describe cerimetry as an alternative to permanganometry.

16.2 Permanganometry — Potassium Permanganate Titrations

16.2.1 Properties of $KMnO_4$ as a Titrant

Potassium permanganate ($KMnO_4$) is one of the most powerful oxidizing agents used in titrimetric analysis. Its intense purple-violet colour is its most distinctive feature — and its greatest analytical advantage: $KMnO_4$ is self-indicating, meaning no external indicator is needed for endpoint detection.

Property	Details
Colour	Intensely purple-violet (MnO_4^-) → Colourless (Mn^{2+}) in acidic medium
Oxidising power	E° = +1.51 V in acidic medium (very powerful oxidant)
Medium of reaction	Acidic (H_2SO_4): Mn(+7) → Mn(+2); Neutral/basic: Mn(+7) → Mn(+4) MnO_2
Self-indicator	Yes — first excess $KMnO_4$ gives faint pink persisting for 30 sec
Primary standard?	No — contains Mn_2O_7 impurities; must be standardized
Standardized against	Oxalic acid (primary std), sodium oxalate, or As_2O_3
Storage	Dark glass bottle; away from light (photocatalytic decomp.)

16.2.2 Reactions of $KMnO_4$ in Different Media

Reactions in Different Media

ACIDIC MEDIUM (H_2SO_4) — most common in titrations: $MnO_4^- + 8H^+ + 5e^- \rightarrow Mn^{2+} + 4H_2O$ ($E° = +1.51$ V) Colour: Purple → Colourless (Mn^{2+}) NEUTRAL/WEAKLY ACIDIC MEDIUM: $MnO_4^- + 2H_2O + 3e^- \rightarrow MnO_2\downarrow + 4OH^-$ ($E° = +0.59$ V) Colour: Purple → Brown precipitate (MnO_2) ALKALINE MEDIUM: $MnO_4^- + e^- \rightarrow MnO_4^{2-}$ (Manganate) ($E° = +0.56$ V) Colour: Purple → Dark green (MnO_4^{2-}) Note: HCl should NOT be used as the acid medium — $KMnO_4$ oxidizes Cl^- to Cl_2 (Gudermann reaction), consuming extra oxidant.

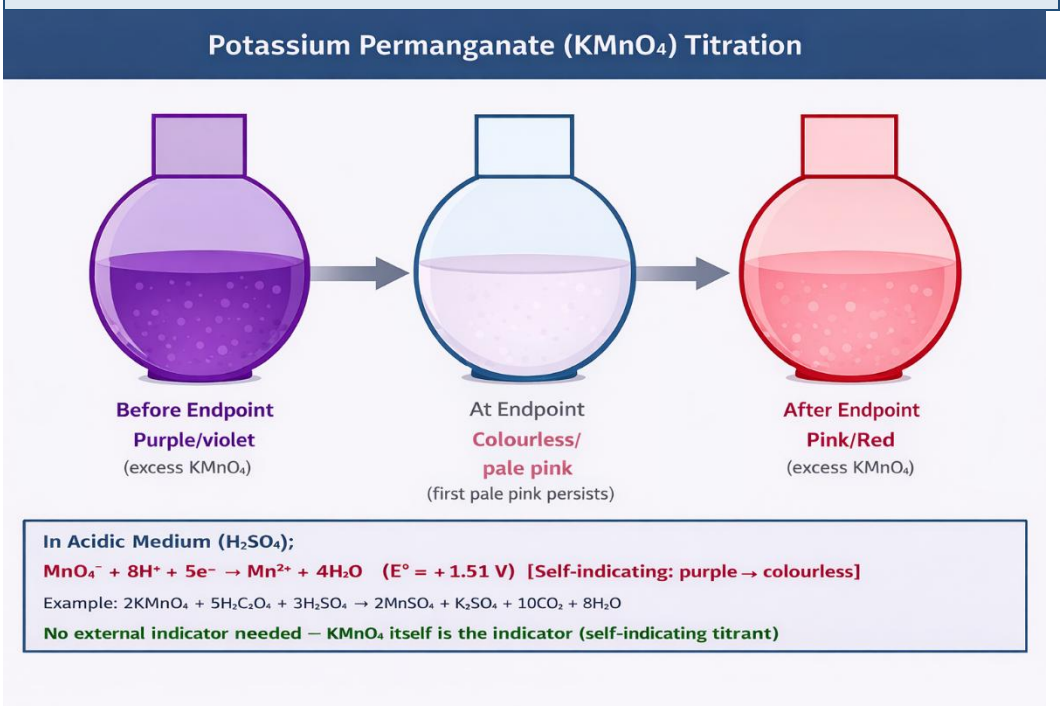


Figure: $KMnO_4$ Titration — Self-Indicating Colour Change at Endpoint

16.2.3 Standardization of $KMnO_4$ Against Sodium Oxalate (Primary Standard)

The most common primary standard for $KMnO_4$ is sodium oxalate ($Na_2C_2O_4$, MW 134.00) or anhydrous oxalic acid ($H_2C_2O_4$, MW 90.03):

Reaction

$2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 \rightarrow 2MnSO_4 + K_2SO_4 + 10CO_2 + 8H_2O$ Or ionically: $2MnO_4^- + 5C_2O_4^{2-} + 16H^+ \rightarrow 2Mn^{2+} + 10CO_2 + 8H_2O$ Conditions: • Temperature: 60–70°C (reaction is slow at room temperature; heat accelerates it) • Acidic medium: 1–2 M H_2SO_4 • Self-indicating endpoint: first permanent faint pink • Mn^{2+} produced acts as autocatalyst (reaction starts slowly, then accelerates)

1. Dissolve accurately weighed $Na_2C_2O_4$ (~0.10 g) in dilute H_2SO_4.
2. Heat to 60–70°C (do not boil).
3. Titrate with $KMnO_4$ solution slowly at first (Mn^{2+} autocatalyst absent initially).
4. Endpoint: First permanent faint pink colour for 30 seconds.

Calculation
Molarity $KMnO_4$ = (5 × Wt $Na_2C_2O_4$ in g) / (2 × V_$KMnO_4$ in L × 134.00)

16.2.4 Pharmacopoeial Applications of $KMnO_4$ Titrations

Drug/Substance	Reaction Type	Notes
Ferrous Sulfate ($FeSO_4 \cdot 7H_2O$)	Direct permanganometry	Fe^{2+} + MnO_4^- in H_2SO_4; self-indicating
Hydrogen Peroxide (H_2O_2)	Direct permanganometry	H_2O_2 reduces $KMnO_4$ in acidic medium
Calcium Gluconate (Ca content)	Via oxalate precipitation	$Ca^{2+} \rightarrow CaC_2O_4\downarrow \rightarrow$ Dissolve → Titrate with $KMnO_4$
Potassium Bromide	Via oxidation to Br_2	$KBrO_3$ + KBr → Br_2; back-titrated iodometrically
Iron preparations	Fe^{2+} assay	Ferrous fumarate, ferrous gluconate, ferrous sulfate

16.2.5 Assay of Ferrous Sulfate (IP) by $KMnO_4$

1. Dissolve ~0.5 g of $FeSO_4 \cdot 7H_2O$ in 25 mL of dilute H_2SO_4 (freshly prepared).
2. Titrate immediately with 0.02 M $KMnO_4$ until a faint pink colour persists for 30 seconds.

Reaction and Calculation
$MnO_4^- + 5Fe^{2+} + 8H^+ \rightarrow Mn^{2+} + 5Fe^{3+} + 4H_2O$ Each mL of 0.02 M $KMnO_4 \equiv 5 \times 0.02 \times 278.01 / 1000 \times 1000 = 27.80$ mg $FeSO_4 \cdot 7H_2O$ % $FeSO_4 \cdot 7H_2O$ = (V × 0.02 × 5 × 278.01 × 100) / (Sample wt in mg × 1000) IP limit: 99.0–100.5%

16.3 Dichromatometry — Potassium Dichromate Titrations

16.3.1 Properties of $K_2Cr_2O_7$

Potassium dichromate ($K_2Cr_2O_7$) is a strong but less powerful oxidizing agent than $KMnO_4$ ($E° = +1.33$ V vs +1.51 V). It has several advantages that make it the preferred titrant in many situations:

- Primary standard: $K_2Cr_2O_7$ can be obtained in high purity, is stable, and can be weighed directly to prepare standard solutions of precisely known concentration — it is a true primary standard.
- Stable solutions: $K_2Cr_2O_7$ solutions are indefinitely stable in light and dark — no decomposition.
- Can be used with HCl: Unlike $KMnO_4$, $K_2Cr_2O_7$ does not readily oxidize Cl^-, so HCl can be used as the acidic medium.
- Selective: Less powerful oxidizing potential makes it more selective — fewer interferences.

Disadvantage: $K_2Cr_2O_7$ is not self-indicating. An external indicator (diphenylamine, diphenylamine sulfonate) or potentiometric detection is required. Also, Cr^{3+} produced is green/grey — the endpoint colour change is from blue/violet to grey-green.

16.3.2 Reaction of $K_2Cr_2O_7$

Dichromate Reduction (Acidic Medium)
$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O$ ($E° = +1.33$ V) Colour: Orange ($Cr_2O_7^{2-}$) → Dark green (Cr^{3+}) With Fe^{2+} (classic titration): $Cr_2O_7^{2-} + 6Fe^{2+} + 14H^+ \rightarrow 2Cr^{3+} + 6Fe^{3+} + 7H_2O$ With Diphenylamine Sulfonate Indicator: • Reduced form: Colourless | Oxidised form: Red-violet • Endpoint: Green → Red-violet (the Cr^{3+} green and the indicator violet overlap to give a distinctive blue-violet)

16.3.3 Comparison: $KMnO_4$ vs $K_2Cr_2O_7$

Property	$KMnO_4$	$K_2Cr_2O_7$
E° (V)	+1.51 (acidic medium)	+1.33 (acidic medium)
Oxidising power	Very high (stronger)	High (slightly weaker)
Primary standard?	No — must be standardized	Yes — primary standard
Stability of solution	Unstable (decomposes slowly)	Very stable (indefinitely)
Indicator	Self-indicating (no external needed)	External indicator required (diphenylamine)
Use with HCl	No (oxidises Cl^-)	Yes (selective; HCl can be used)
Endpoint colour	Colourless → Faint pink	Green → Blue-violet
pH of medium	Strongly acidic (H_2SO_4)	Strongly acidic (H_2SO_4 or HCl)

Toxicity	Moderate	High (Cr^{6+} is a carcinogen — handle with care)

16.3.4 Pharmaceutical Applications of $K_2Cr_2O_7$

- Standardization of sodium thiosulfate solutions (via iodometric method — liberates I_2 from KI).
- Assay of Ferrous Salts (ferrous gluconate, ferrous fumarate) — Fe^{2+} oxidised to Fe^{3+} by $K_2Cr_2O_7$.
- Determination of COD (Chemical Oxygen Demand) in pharmaceutical effluents and water quality monitoring.
- Preparation of certified reference standard solutions for calibration of electrochemical instruments.

16.4 Cerimetry — Ceric Sulfate Titrations

16.4.1 Introduction

Cerimetry uses ceric sulfate ($Ce(SO_4)_2$) or ceric ammonium sulfate as the oxidizing titrant. Ceric ion (Ce^{4+}) is reduced to cerous ion (Ce^{3+}) in the reaction:

Ceric Half-Reaction
$Ce^{4+} + e^- \rightarrow Ce^{3+}$ ($E° = +1.44$ V in H_2SO_4) Colour: Ce^{4+} (yellow) → Ce^{3+} (colourless)

Cerimetry has several advantages over permanganometry:

- Ceric sulfate solutions are extremely stable — they do not decompose over months or years.
- Reactions are rapid and quantitative.
- Can be used in the presence of HCl (like dichromate).
- Ferroin (1,10-phenanthroline-Fe^{2+} complex) is the standard indicator — very sharp endpoint (red to pale blue).
- Pharmaceutical applications: Assay of Ferrous Gluconate, Ferrous Sulfate, Ascorbic Acid by direct cerimetric titration.

16.5 Bromimetry and Bromatometry

Bromine-based titrations are used in the assay of several drug substances, particularly aromatic compounds that undergo electrophilic bromination.

16.5.1 Bromatometry

Potassium bromate ($KBrO_3$) is a strong oxidizing agent ($E° = +1.44$ V) and a primary standard. In acidic KBr solution, it liberates Br_2 in situ:

Reaction
$BrO_3^- + 5Br^- + 6H^+ \rightarrow 3Br_2 + 3H_2O$

The liberated Br_2 reacts with the analyte. Excess Br_2 is detected by the methyl orange indicator (Br_2 bleaches the orange colour — endpoint: orange to colourless), or by iodometric back-titration.

Pharmaceutical Applications of Bromatometry

- Assay of Phenol (by bromination): Phenol + $3Br_2$ → 2,4,6-Tribromophenol↓ + 3HBr
- Assay of Aniline and aniline-containing drugs.
- Assay of 8-Hydroxyquinoline (Oxyquinoline, antiseptic): bromination with $KBrO_3$/KBr.
- Assay of Resorcinol.

16.6 Summary

Chapter 16 Summary

$KMnO_4$ ($E° = +1.51$ V) is a powerful self-indicating oxidant used in acidic medium (H_2SO_4); standardized against oxalic acid/$Na_2C_2O_4$; used for Fe^{2+}, H_2O_2, Ca^{2+} (via oxalate). $K_2Cr_2O_7$ ($E° = +1.33$ V) is a primary standard, stable, can use HCl, but requires external indicator (diphenylamine); used for Fe^{2+} and standardizing thiosulfate. Cerimetry (Ce^{4+}/Ce^{3+}) with ferroin indicator is highly precise. Bromatometry ($KBrO_3$/KBr → Br_2) assays phenol, aniline, and other aromatic compounds.

Review Questions

Short Answer Questions

1. Why is $KMnO_4$ called a self-indicating titrant?
2. Why should HCl not be used as the acid medium in permanganometric titrations?
3. List two advantages of $K_2Cr_2O_7$ over $KMnO_4$ as a titrant.
4. What is the indicator used in cerimetric titrations? State its colour change at endpoint.
5. Write the balanced equation for the reaction of $KMnO_4$ with oxalic acid in acidic medium.

Long Answer Questions

1. Describe the assay of ferrous sulfate by permanganometry as per IP. Include the principle, reagents, procedure, endpoint detection, and calculation.
2. Compare potassium permanganate and potassium dichromate as oxidimetric titrants under the headings: oxidising power, primary standard status, indicator, medium, stability, and pharmaceutical applications.

Multiple Choice Questions

1. Potassium permanganate is standardized against:

(a) NaCl

(b) $Na_2C_2O_4$ (sodium oxalate) — primary standard

(c) $Na_2S_2O_3$

(d) $FeSO_4$

✓Answer: (b) $Na_2C_2O_4$ (sodium oxalate) — primary standard

2. The colour of $KMnO_4$ changes to which colour at the endpoint in acidic medium?

(a) Dark green

(b) Blue-black

(c) Faint pink (first permanent excess)

(d) Brick-red

✓Answer: (c) Faint pink (first permanent excess)

3. Which acid should NOT be used in permanganometric titrations?

(a) H_2SO_4

(b) $HClO_4$

(c) HNO_3

(d) HCl (oxidises Cl^- to Cl_2)

✓Answer: (d) HCl (oxidises Cl^- to Cl_2)

4. $K_2Cr_2O_7$ is a primary standard because:

(a) It is highly coloured

(b) It is available in high purity and is stable — can be directly weighed

(c) It reacts with all reducing agents

(d) It requires no indicator

✓Answer: (b) It is available in high purity and is stable — can be directly weighed

5. In cerimetric titrations, the indicator ferroin changes colour at the endpoint from:

(a) Yellow to colourless

(b) Blue-black to colourless

(c) Red to pale blue

(d) Violet to green

✓Answer: (c) Red to pale blue

UNIT V
GRAVIMETRIC ANALYSIS

Gravimetric analysis is the oldest and most precise branch of quantitative analytical chemistry. It relies on the conversion of the analyte into a pure, stable, weighable form — a precipitate, a residue after ignition, or a gaseous product — and measurement of the mass of that form. Because mass can be measured with extraordinary precision using analytical balances (to ±0.0001 g), gravimetric methods are fundamentally more accurate than titrimetric or instrumental methods when performed correctly. This unit covers the complete theory, methodology, and pharmaceutical applications of gravimetric analysis.

Chapter 17
Principles of Gravimetric Analysis

17.1 Learning Objectives

- Define gravimetric analysis and state its fundamental principle.
- Classify the types of gravimetric methods and give examples of each.
- Explain the theoretical requirements for a gravimetric precipitate.
- Explain the Von Weimarn ratio and its significance in controlling particle size.
- Describe co-precipitation and post-precipitation as sources of error.

17.2 Introduction to Gravimetric Analysis

The word 'gravimetry' is derived from the Latin gravitas (weight) and the Greek metron (measurement). Gravimetric analysis quantitatively determines the amount of a chemical substance by converting it into a form that can be accurately weighed. It is perhaps the most fundamental of all quantitative analytical techniques — requiring only a balance, standard glassware, and careful chemical technique.

Despite the emergence of sophisticated instrumental methods, gravimetric analysis remains widely used in pharmaceutical quality control and pharmacopoeial testing. The Loss on Drying (LOD) test, Residue on Ignition (ROI) test, ash tests, and sulfate ash determination are all gravimetric procedures mandated in the IP, BP, and USP for characterizing pharmaceutical raw materials and dosage forms.

Gravimetric analysis is particularly valuable when: (a) the highest accuracy is required; (b) no suitable titrimetric method exists; (c) the substance being assayed can be converted into a defined, pure, stable compound of known composition; and (d) the analytical concentration of the analyte is relatively high (> 1% w/w).

17.3 Classification of Gravimetric Methods

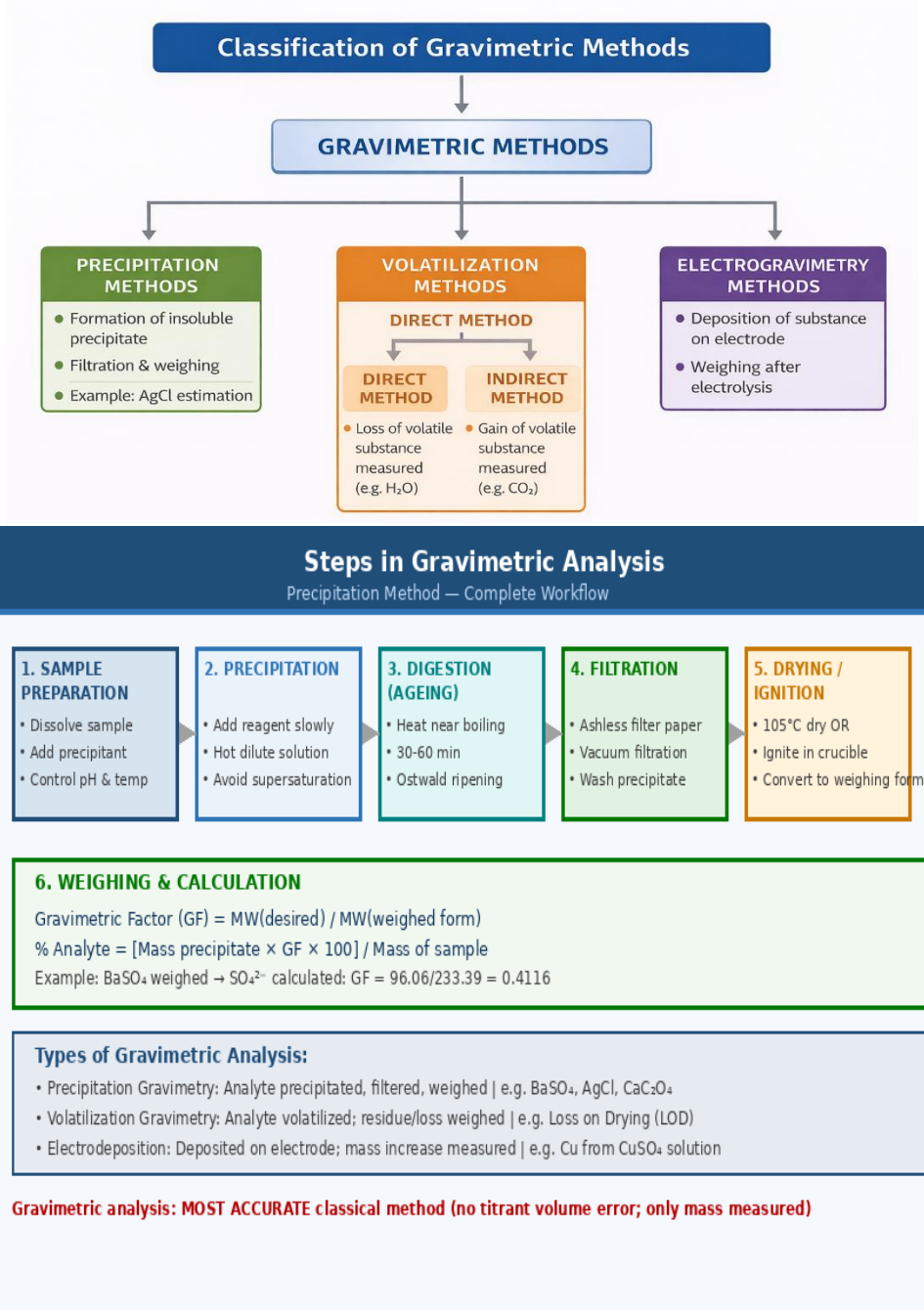


Figure: Steps in Gravimetric Analysis — Complete Workflow with Calculations

Gravimetric methods are classified based on the nature of the conversion step:

17.3.1 Precipitation Gravimetry

The analyte is precipitated from solution as a sparingly soluble compound by adding an appropriate reagent (the precipitating agent). The precipitate is filtered, washed, dried or ignited to a compound of known composition, and weighed. This is the most widely used gravimetric method in pharmaceutical analysis.

Examples of Precipitation Gravimetry

• Barium as $BaSO_4$: $Ba^{2+} + SO_4^{2-} \rightarrow BaSO_4\downarrow$ (weighed as $BaSO_4$) • Chloride as AgCl: $Ag^+ + Cl^- \rightarrow AgCl\downarrow$ (weighed as AgCl) • Calcium as $CaC_2O_4 \rightarrow$ ignited to CaO (weighed as CaO) • Iron as Fe_2O_3 (after precipitation as $Fe(OH)_3$, ignition) • Phosphate as $MgNH_4PO_4 \rightarrow$ ignited to $Mg_2P_2O_7$

17.3.2 Volatilization Gravimetry

The analyte or the matrix is volatilized (evaporated or decomposed to give a gas), and either: (a) the evolved gas is collected and weighed (direct volatilization) or (b) the loss in mass of the residue is measured (indirect volatilization).

- Direct volatilization: CO_2 from carbonates is absorbed in a weighed absorber (Ascarite); increase in absorber mass = mass CO_2.
- Indirect volatilization: Loss on Drying (LOD) — sample is heated at 105°C; loss in mass = moisture content.
- Residue on Ignition (ROI) / Sulfated Ash: Sample is ignited; residue weighed. Used in IP for inorganic impurities.

17.3.3 Electrodeposition (Electrogravimetry)

The analyte is deposited onto a pre-weighed electrode by controlled-potential electrolysis. The mass of the deposited metal equals the increase in electrode weight. Example: Cu^{2+} from $CuSO_4$ solution deposited onto a platinum electrode. Used in assay of copper preparations and in refinery analysis.

17.4 Requirements for a Gravimetric Precipitate

Not every precipitate is suitable for gravimetric analysis. An ideal gravimetric precipitate must fulfil all of the following criteria:

Requirement	Explanation	Consequence if Not Met
Very low solubility (Ksp ≤ 10^{-8})	Ensures that precipitation is essentially complete; < 0.1% remains in solution	Significant loss; positive error in calculation
Known, stable, definite composition	Must be a pure compound of defined formula for calculation	Inaccurate GF; unreliable results
Easily filterable	Particle size should be large enough (coarse crystalline) to pass filtration without clogging	Loss of precipitate; slow filtration
Purity	Low co-precipitation; no adsorption of impurities	Positive error in weight
Chemical stability	Must not change in composition during drying/ignition	Unpredictable stoichiometry
Low formula weight of analyte relative to precipitate	Higher GF → greater sensitivity; small error in weighing has less relative impact	Poor sensitivity for analyte
Insoluble in wash liquid	Precipitate must not dissolve during washing	Analyte loss; negative error

17.5 Solubility Product and Completeness of Precipitation

Precipitation is considered complete when the amount of analyte remaining in solution is less than 0.1 mg (below the sensitivity of an analytical balance). The solubility product (Ksp) determines the theoretical minimum concentration of the analyte remaining in solution at equilibrium:

Completeness of Precipitation

For the precipitate AB: Ksp = $[A^+][B^-]$ If excess precipitating agent B is added: $[A^+]$ = Ksp / $[B^-]$ Example: AgCl, Ksp = 1.8×10^{-10} With 0.01 M excess Ag^+: $[Cl^-] = 1.8 \times 10^{-10} / 0.01 = 1.8 \times 10^{-8}$ M Mass Cl^- remaining in 250 mL = $1.8 \times 10^{-8} \times 250 \times 10^{-3} \times 35.45 \times 1000 = 0.00016$ mg → negligible Conclusion: A small excess of precipitating agent drives precipitation to practical completion.

The common ion effect is the primary tool used to drive precipitation to completion: adding a moderate excess of the precipitating reagent dramatically lowers the solubility of the precipitate. However, excessive excess can sometimes cause problems (peptization, complex formation, or co-precipitation of the excess reagent).

17.6 Particle Size and Von Weimarn Ratio

The particle size of the precipitate is critical because it determines filtration ease, surface area (and hence contamination), and purity. Two types of precipitates are encountered:

- Crystalline precipitates (desired): Large, well-defined crystals with low surface area. Easy to filter; low adsorption of impurities. Examples: $BaSO_4$, CaC_2O_4.
- Colloidal/amorphous precipitates (undesired in gravimetry): Tiny particles that pass through filter paper or clog it. High surface area → more contamination. Examples: $Fe(OH)_3$, $Al(OH)_3$.

The relative supersaturation at the moment of mixing determines which type forms:

Von Weimarn Ratio (RSS)

RSS = (Q − S) / S Where Q = concentration of analyte immediately after mixing (before precipitation equilibrium) S = equilibrium solubility of the precipitate Low RSS → few nuclei → large crystals (preferred for gravimetry) High RSS → many nuclei → colloidal particles (avoid) Strategies to achieve LOW RSS: 1. Precipitate from hot, dilute solutions (increases S) 2. Add precipitant slowly, with stirring (keeps Q low) 3. Adjust pH to control solubility (e.g., avoid too-alkaline conditions) 4. Use homogeneous precipitation (reagent generated in situ)

17.7 Homogeneous Precipitation

Homogeneous precipitation is a technique in which the precipitating agent is generated uniformly throughout the solution by a slow chemical reaction, rather than added externally. This avoids local high-concentration zones that cause high RSS. The result is a more uniform, purer, coarser, and more easily filterable precipitate.

Analyte	Precipitating Agent Generated In Situ	Chemical Reaction
SO_4^{2-}	H_2SO_4 (slowly)	Dimethyl sulfate hydrolysis: $(CH_3)_2SO_4 + H_2O \rightarrow 2CH_3OH + H_2SO_4$
Ca^{2+}	$C_2O_4^{2-}$ (from urea-oxalate)	Urea hydrolysis: $(NH_2)_2CO + H_2O \rightarrow CO_2 + 2NH_3$ → raises pH → CaC_2O_4 ppt
Al^{3+}, Fe^{3+}	OH^- (from urea)	$(NH_2)_2CO + H_2O \rightarrow 2NH_3 + CO_2$; NH_3 slowly raises pH → hydroxide precipitates
Mg^{2+}	HPO_4^{2-} (from triethyl phosphate)	Hydrolysis releases phosphate slowly

17.8 Digestion (Ageing) and Ostwald Ripening

After precipitation, the mixture is heated (near but below boiling) for 30–60 minutes in a process called digestion or ageing. This dramatically improves the quality of the precipitate:

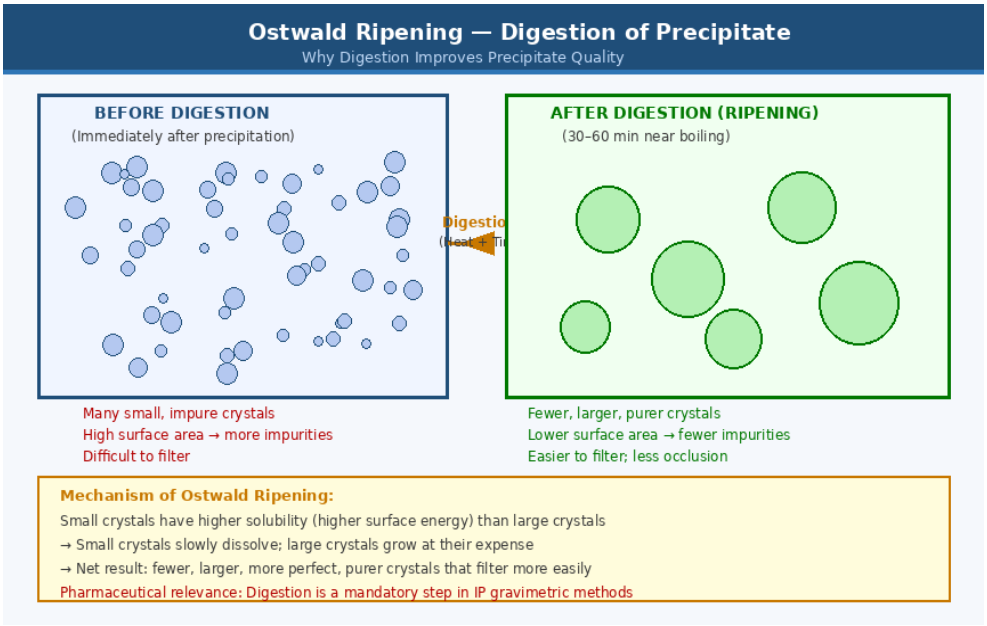


Figure: Ostwald Ripening — How Digestion Improves Precipitate Size and Purity

- Ostwald Ripening: Small crystals have higher solubility (due to higher surface energy per the Kelvin equation) than large crystals. During digestion, small crystals slowly dissolve and their material re-deposits on larger crystals. Net result: fewer but larger, more perfect crystals.
- Reduction of adsorption: As the surface area decreases (fewer, larger crystals), fewer impurities are adsorbed per unit mass of precipitate.
- Expulsion of occluded impurities: Mother liquor trapped inside growing crystals is expelled during the ripening process.
- Increased filterability: Large crystals filter much faster and more completely than small ones.

Pharmaceutical relevance: Digestion is mandated in the IP for methods such as the barium sulfate gravimetric method and the calcium oxalate gravimetric method.

17.9 Contamination of Precipitates

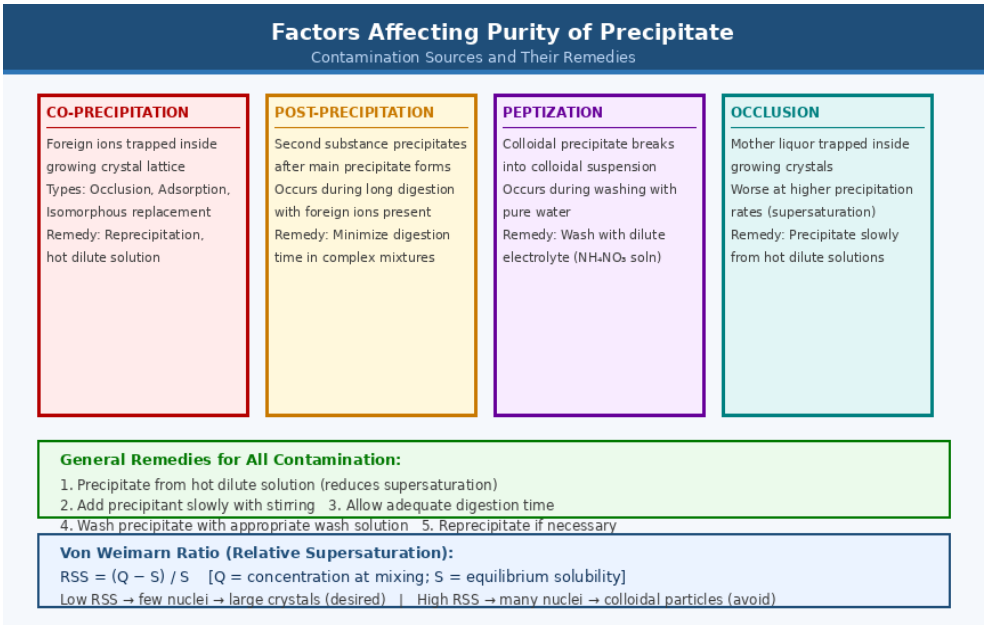


Figure: Factors Affecting Purity of Gravimetric Precipitates — Contamination and Remedies

17.9.1 Co-precipitation

Co-precipitation occurs when normally soluble foreign substances are carried down with the desired precipitate. It is the most important source of error in gravimetric analysis.

- Surface Adsorption: Ions are adsorbed onto the crystal surface due to electrostatic attraction. The type of ion adsorbed follows the Paneth-Fajans-Hahn rule: ions that form insoluble compounds with the lattice ions are preferentially adsorbed.
- Occlusion (Mechanical Entrapment): Mother liquor or foreign ions are physically trapped inside the growing crystal lattice as it grows rapidly. Occurs especially at high RSS (fast precipitation).
- Isomorphous Replacement (Mixed Crystal Formation): Foreign ions of similar size and charge replace lattice ions within the crystal structure. e.g., Pb^{2+} can replace Ba^{2+} in $BaSO_4$ because they are similar in size.
- Inclusion: A foreign ion is trapped by the growing crystal as it grows over an adsorbed layer.

17.9.2 Post-precipitation

Post-precipitation is a different phenomenon: a second substance precipitates on top of the original precipitate during prolonged digestion in the presence of its own ions. Unlike co-precipitation, post-precipitation does not occur during the initial precipitation but develops with time.

Example: CaC_2O_4 precipitates first; if MgC_2O_4 is present and digestion is prolonged, Mg^{2+} post-precipitates. Remedy: Minimize digestion time when interfering ions are present.

17.9.3 Peptization

Peptization is the reversal of coagulation: a coagulated colloidal precipitate breaks up into colloidal particles when washed with pure water. The coagulating electrolyte (normally present in the precipitation medium) is washed away, and the coagulated precipitate reverts to colloidal form.

Remedy: Wash precipitate with dilute electrolyte solution (e.g., 0.1% NH_4NO_3 or dilute HNO_3) rather than pure water. The electrolyte maintains the ionic strength and prevents peptization. The electrolyte chosen must be volatile so it can be removed during drying.

17.10 Summary

Chapter 17 Summary

Gravimetric analysis quantifies analytes by weighing. Types: precipitation, volatilization, electrogravimetry. Ideal precipitate: low Ksp, stable composition, coarse crystalline, pure, chemically stable. Von Weimarn ratio (RSS) determines crystal size — low RSS gives large crystals (use hot dilute solution, slow addition). Homogeneous precipitation gives superior precipitates. Digestion causes Ostwald ripening — smaller crystals dissolve, large ones grow. Contamination sources: co-precipitation (adsorption, occlusion, isomorphous replacement), post-precipitation, peptization; remedies include reprecipitation, appropriate washing solution.

Review Questions

Short Answer Questions

1. What is the Von Weimarn ratio? How does it influence precipitate particle size?
2. Explain Ostwald ripening. Why is digestion important in gravimetric analysis?
3. Distinguish between co-precipitation and post-precipitation.
4. Why should the wash liquid contain a volatile electrolyte when washing amorphous precipitates?
5. What is meant by homogeneous precipitation? Give one example.

Long Answer Questions

1. Classify the types of gravimetric methods. Explain the requirements of an ideal gravimetric precipitate with reasons. Give two pharmaceutical examples.
2. Describe the sources of contamination (co-precipitation, post-precipitation, peptization) in gravimetric analysis. Discuss remedies for each.

Multiple Choice Questions

1. Gravimetric analysis determines analyte content by measuring:

(a) Volume of titrant

(b) Mass of a pure compound derived from the analyte

(c) Absorbance of light

(d) Electrode potential

✓***Answer: (b) Mass of a pure compound derived from the analyte***

2. Von Weimarn ratio (RSS) = (Q − S)/S. A low RSS gives:

(a) Colloidal particles

(b) Few, large coarse crystals (preferred)

(c) High co-precipitation

(d) Rapid precipitation

✓***Answer: (b) Few, large coarse crystals (preferred)***

3. Peptization in gravimetric analysis refers to:

(a) Excessive precipitation

(b) Dissolution of precipitate in hot water

(c) Breakdown of coagulated colloid into colloidal suspension on washing with pure water

(d) Post-precipitation of a second compound

✓***Answer: (c) Breakdown of coagulated colloid into colloidal suspension on washing with pure water***

4. The process of Ostwald ripening results in:

(a) Smaller, more numerous crystals

(b) Fewer, larger, more perfect crystals

(c) Dissolution of the precipitate

(d) Increased surface adsorption

✓***Answer: (b) Fewer, larger, more perfect crystals***

5. The Loss on Drying (LOD) test is an example of:

(a) Precipitation gravimetry

(b) Electrogravimetry

(c) Indirect volatilization gravimetry

(d) Complexometric gravimetry

✓***Answer: (c) Indirect volatilization gravimetry***

Chapter 18
Precipitation Methods

18.1 Learning Objectives

- Describe the complete, step-by-step procedure for performing a precipitation gravimetric analysis.
- Explain the role of each step: dissolution, precipitation, digestion, filtration, washing, drying, ignition, and weighing.
- Select appropriate filter media for different types of precipitates.
- Describe the preparation and use of sintered glass crucibles and filter paper.
- Explain the difference between drying and ignition and when each is used.

18.2 Complete Step-by-Step Procedure in Gravimetric Analysis

A gravimetric analysis comprises eight sequential, carefully controlled steps. Each step must be performed with precision to avoid errors that propagate to the final result.

Step 1: Sample Preparation and Dissolution

The sample is accurately weighed on an analytical balance (to ±0.1 mg) and dissolved completely in an appropriate solvent. The choice of solvent depends on the nature of the sample:

- Aqueous soluble salts: Dissolve in distilled water.
- Insoluble in water: Dissolve in dilute HCl, HNO_3, or H_2SO_4.
- Refractory materials: Fusion with Na_2CO_3 or $K_2S_2O_7$ followed by dissolution.

The solution must be homogeneous and free from particulate matter before precipitation. The volume should be appropriate — typically 100–300 mL to minimize solubility losses.

Step 2: Precipitation

The precipitating agent is added to the sample solution under optimized conditions to achieve:

- Completeness: Use a slight excess (10–20%) of precipitating agent to exploit the common ion effect.
- Large particle size (low RSS): Use dilute solutions, add precipitant slowly with stirring, precipitate from hot solution.
- Selectivity: Choose a precipitating agent that is selective for the analyte under the chosen conditions.

The mixture is then covered and allowed to stand for the precipitate to coagulate before digestion.

Step 3: Digestion

The mixture is heated at 80–100°C (just below boiling) for 30–60 minutes with stirring. This promotes Ostwald ripening (crystal growth), reduces surface area, and expels occluded impurities. For amorphous precipitates like $Fe(OH)_3$, digestion in the hot supernatant (not at full boil to avoid peptization) helps compact the precipitate.

Step 4: Filtration

The precipitate is separated from the mother liquor by filtration. The choice of filtration medium depends on the nature of the precipitate and the subsequent treatment (drying vs. ignition):

Filter Medium	Pore Size	Used For	Treatment After
Ashless filter paper (Whatman 41, 42)	Coarse–medium	Coarse crystalline precipitates ($BaSO_4$, AgCl)	Ignition in crucible (paper burns away)
Ashless filter paper (Whatman 42)	Medium	Crystalline precipitates with small particles	Ignition (ash negligible: < 0.1 mg)
Sintered glass crucible (G3)	Medium	Precipitates sensitive to ignition (AgCl, organic ppts)	Drying at 105–120°C
Sintered glass crucible (G4)	Fine	Very fine precipitates	Drying at 105–120°C
Gooch crucible (asbestos)	Variable	Historical use; now replaced by sintered glass	Drying or gentle ignition

The precipitate is transferred quantitatively to the filter using a wash bottle. A rubber policeman is used to scrape residual precipitate from the walls of the beaker.

Step 5: Washing

The precipitate is washed 6–8 times with small portions (5–10 mL each) of the appropriate wash liquid to remove mother liquor and adsorbed impurities without dissolving the precipitate:

- Crystalline precipitates (AgCl, $BaSO_4$): Wash with cold water or dilute acid to minimize solubility loss and prevent peptization.
- Amorphous precipitates ($Fe(OH)_3$, $Al(OH)_3$): Wash with dilute electrolyte (e.g., 0.1% NH_4NO_3) to prevent peptization.

Test for completeness of washing: Apply the appropriate ion test to the last wash water — e.g., add $AgNO_3$ to washing from a chloride precipitation; if no cloudiness, washing is complete.

Step 6: Drying or Ignition

The washed precipitate, still on the filter medium, is converted to a form of known, stable composition:

- Drying (105–130°C): Removes adsorbed water only. Used for precipitates that decompose on ignition (AgCl, $BaSO_4$ at moderate temperatures, organic precipitates). Use sintered glass crucible.
- Ignition (600–1200°C): Decomposes unstable precipitates and burns off filter paper. Converts the precipitate to a thermally stable form. Use porcelain or platinum crucible. The crucible is pre-ignited (to constant weight) before use.

Precipitate Formed	Ignition Product	Temperature	Example
$Fe(OH)_3$	Fe_2O_3	850°C	Iron assay
$CaC_2O_4 \cdot H_2O$	CaO (then → $Ca^3(PO_4)_2$ if converted)	900°C	Calcium assay
$MgNH_4PO_4$	$Mg_2P_2O_7$	1000°C	Magnesium assay
$BaSO_4$	$BaSO_4$ (stable — no change)	600°C	Sulfate assay; barium assay
AgCl	AgCl (heat at 130°C only — do not ignite; converts to Ag)	130°C (dry only)	Chloride assay

Step 7: Cooling and Weighing

The crucible (with dried/ignited precipitate) is cooled in a desiccator (containing anhydrous $CaCl_2$ or silica gel as desiccant) to room temperature before weighing. Cooling in air is not permitted because:

- Hygroscopic precipitates absorb moisture from air.
- Hot crucibles create air convection currents above the balance pan, causing a false reading.

The crucible is weighed on an analytical balance to ±0.1 mg. It is re-ignited/re-dried and re-weighed until constant weight is achieved (two consecutive weighings agree within 0.3 mg — 'constant weight' criterion).

Step 8: Calculation Using the Gravimetric Factor

The amount of analyte is calculated from the mass of the precipitate using the gravimetric factor:

Gravimetric Factor (GF) and Calculation

GF = (a × MW_analyte) / (b × MW_precipitate) Where a and b are the stoichiometric coefficients relating precipitate to analyte. % Analyte = (mass of precipitate × GF × 100) / mass of sample Example 1: Chloride as AgCl GF = MW(Cl) / MW(AgCl) = 35.45 / 143.32 = 0.2474 If 0.2500 g sample → 0.2895 g AgCl: % Cl^- = (0.2895 × 0.2474 × 100) / 0.2500 = 28.65% Example 2: Sulfate as $BaSO_4$ GF = MW(SO_4^{2-}) / MW($BaSO_4$) = 96.06 / 233.39 = 0.4116 If 0.5000 g sample → 0.4320 g $BaSO_4$: % SO_4^{2-} = (0.4320 × 0.4116 × 100) / 0.5000 = 35.56%

Gravimetric Factors — Pharmaceutical Applications

GF = MW(Analyte) / MW(Weighed Form) | % Analyte = (mass ppt × GF × 100) / sample wt

% Analyte = (mass ppt × GF × 100) / sample wt

Analyte Desired	Weighed Form	GF	Application
SO_4^{2-}	$BaSO_4$	0.4116	Sulfate in barium preparations
Cl^-	AgCl	0.2474	Chloride; saline; drug HCl salts
Fe (as Fe^{3+})	Fe_2O_3	0.6994	Iron content in ferric compounds
Ca^{2+}	$CaCO_3$	0.4004	Calcium gluconate, lactate
Ca^{2+}	CaO	0.7147	Calcium after ignition to CaO
Mg^{2+}	$Mg_2P_2O_7$	0.2185	Magnesium in Mg preparations
Ba^{2+}	$BaSO_4$	0.5884	Barium content
Al^{3+}	Al_2O_3	0.5293	Aluminium in antacid preparations
PO_4^{3-}	$Mg_2P_2O_7$	0.8535	Phosphate content
Bi^{3+}	Bi_2S_3	0.8098	Bismuth in Bi preparations

Calculation Example:

Sample: 0.5000 g $BaCl_2$ dissolved; precipitated as $BaSO_4$; mass of $BaSO_4$ = 0.4320 g

% Ba^{2+} = (0.4320 × 0.5884 × 100) / 0.5000 = 50.87% [GF for Ba from $BaSO_4$ = 137.33/233.39 = 0.5884]

Figure: Gravimetric Factors Table — Pharmaceutical Applications with Calculations

18.3 Filtration Apparatus

18.3.1 Sintered Glass Crucible

The sintered glass crucible (also called a fritted glass crucible) is the modern preferred filtration device in gravimetric pharmaceutical analysis. It consists of a glass crucible with a sintered glass disc fused at the bottom. Advantages over filter paper:

- No ash: Sintered glass contributes no mass to the residue (unlike filter paper which leaves a small ash on ignition).

- Reusable: Can be cleaned and reused hundreds of times.
- No ignition needed for many precipitates: Precipitates can be dried in an oven at 105–130°C without requiring a muffle furnace.
- Pore size control: Available in 5 grades (G1 to G5) for different precipitate sizes.
- Limitation: Cannot be used for precipitates that require ignition above 400°C (sintered glass softens and deforms at high temperatures).

18.3.2 Gooch Crucible (Historical)

The Gooch crucible is a porcelain crucible with a perforated bottom, lined with a mat of asbestos fibres. It has been largely replaced by sintered glass crucibles due to the carcinogenicity of asbestos. It is still mentioned in older pharmacopoeial methods.

18.4 Pharmacopoeial Gravimetric Tests

18.4.1 Loss on Drying (LOD) — IP Method

Loss on Drying is one of the most universally applied gravimetric tests in pharmaceutical quality control. It measures the percentage of volatile matter (primarily water, but also other volatile substances) in a pharmaceutical raw material.

LOD Procedure

1. Accurately weigh ~1.00 g of sample in a tared glass dish (pre-dried to constant weight) 2. Dry at the temperature specified in the IP monograph (typically 100–105°C) 3. Cool in a desiccator for 30 minutes 4. Weigh 5. Repeat until constant weight (within 0.5 mg) LOD (%) = [(Initial wt − Final wt) / Initial wt] × 100 IP limits (examples): Aspirin: NMT 0.5% | Paracetamol: NMT 0.5% | Lactose: NMT 0.5% | $MgSO_4$: NMT 1.0%

18.4.2 Residue on Ignition (ROI) / Sulfated Ash — IP Method

Residue on Ignition measures the non-volatile inorganic matter remaining after complete combustion of an organic pharmaceutical substance. The residue is treated with H_2SO_4 to convert metal oxides to stable sulfates (hence 'sulfated ash').

Sulfated Ash Procedure

1. Accurately weigh ~1.00 g of sample in a pre-ignited, tared platinum/silica crucible 2. Ignite gently to carbonise; cool 3. Moisten with 1 mL of H_2SO_4; heat gently to remove H_2SO_4 4. Ignite at 600°C in muffle furnace until white ash obtained 5. Cool; weigh 6. If residue is not white, add another drop of H_2SO_4 and repeat ignition Sulfated Ash (%) = (mass of residue / mass of sample) × 100 IP limits: Aspirin: NMT 0.1% | Paracetamol: NMT 0.1% | Most organics: NMT 0.1%

18.4.3 Total Ash, Acid-Insoluble Ash — Pharmacognosy Methods

These tests are used in the quality control of crude drugs (herbal materials):

- Total Ash: Sample is ignited to complete ash; residue weighed. Includes both physiological ash (from plant tissue) and non-physiological ash (soil, sand contamination).
- Acid-Insoluble Ash: Total ash is boiled with 25 mL of dilute HCl; the insoluble residue (mainly SiO_2 from sand/soil) is filtered, washed, ignited, and weighed. A specific indicator of siliceous earth contamination.
- Water-Soluble Ash: Total ash minus the residue insoluble in water. Used to detect exhausted drugs.

18.5 Summary

Chapter 18 Summary

Gravimetric analysis involves 8 steps: dissolution, precipitation (low RSS, dilute, hot, slow addition), digestion (Ostwald ripening), filtration (ashless paper/sintered glass), washing (volatile electrolyte wash), drying (105°C) or ignition (600–1000°C), cooling in desiccator, and weighing to constant weight. Gravimetric Factor (GF) = MW(analyte)/MW(precipitate); % analyte = (mass ppt × GF × 100)/sample wt. Pharmacopoeial tests: LOD (moisture), ROI/Sulfated Ash (inorganic residue), Total Ash, Acid-Insoluble Ash.

Review Questions

Short Answer Questions

1. What is 'constant weight'? Why is it necessary in gravimetric analysis?
2. Why is a desiccator used to cool the crucible before weighing?
3. Distinguish between Loss on Drying (LOD) and Residue on Ignition (ROI).
4. What is the difference between ashless filter paper and sintered glass crucible? When is each preferred?

Long Answer Questions

1. Describe the complete step-by-step procedure for the gravimetric determination of sulfate as barium sulfate. Include dissolution, precipitation conditions, digestion, filtration, drying, and calculation.
2. Explain the Sulfated Ash (Residue on Ignition) test as per IP. Why is H_2SO_4 added during the procedure? What does a high residue indicate?

Multiple Choice Questions

1. Ashless filter paper is used in gravimetric analysis because:

(a) It has no pores

(b) It burns completely with negligible ash on ignition (< 0.1 mg)

(c) It dissolves in the wash liquid

(d) It adsorbs all impurities

✓***Answer: (b) It burns completely with negligible ash on ignition (< 0.1 mg)***

2. The gravimetric factor for determining Cl^- from AgCl precipitate is:

(a) 143.32 / 35.45 = 4.04

(b) 35.45 / 143.32 = 0.2474

(c) 35.45 / 107.87 = 0.329

(d) 1.0

✓***Answer: (b) 35.45 / 143.32 = 0.2474***

3. In the Loss on Drying test, the sample is dried at:

(a) 60°C

(b) 200°C

(c) 105°C (or as specified in the monograph)

(d) 400°C

✓***Answer: (c) 105°C (or as specified in the monograph)***

4. Sintered glass crucibles cannot be used above ~400°C because:

(a) The precipitate dissolves

(b) The glass softens and distorts

(c) It reacts with the precipitate

(d) It adsorbs moisture

✓***Answer: (b) The glass softens and distorts***

5. Constant weight in gravimetric analysis means:

(a) Exactly the same as the tare weight

(b) Two consecutive weighings agree within 0.3 mg

(c) Weight does not change in 24 hours

(d) Weight equals the theoretical value

✓***Answer: (b) Two consecutive weighings agree within 0.3 mg***

Chapter 19
Gravimetric Calculations and Pharmaceutical Applications

19.1 Learning Objectives

- Perform gravimetric factor calculations for various analyte-precipitate systems.
- Calculate the percentage purity, analyte content, and formula weight from gravimetric data.
- Describe specific pharmacopoeial gravimetric assay procedures for pharmaceutical substances.
- Apply gravimetric principles to pharmaceutical quality control testing.

19.2 Gravimetric Factor — Theory and Calculations

The gravimetric factor (GF) is the mathematical conversion factor that relates the mass of the weighed form (precipitate) to the mass of the analyte. It accounts for the stoichiometric relationship between the two species:

Gravimetric Factor Formula

GF = (a × FW_analyte) / (b × FW_weighed form) Where: a = moles of analyte per mole of weighed form b = moles of weighed form per mole of analyte (usually 1) FW = formula weight General Calculation: Mass of analyte = mass of precipitate × GF % Analyte = (mass of precipitate × GF × 100) / mass of sample taken

Important: The GF is unitless (g/g). Its value is characteristic of the analyte-precipitate pair and independent of the experimental conditions. However, the same analyte may have different GFs depending on the weighed form chosen (e.g., Ca^{2+} determined as CaO has a different GF from Ca^{2+} determined as $Ca_3(PO_4)_2$).

Worked Problem 19.1 — Sulfate Determination as $BaSO_4$

Problem and Solution

A 0.5000 g sample of a pharmaceutical grade sodium sulfate (Na_2SO_4) was dissolved, and the sulfate precipitated as $BaSO_4$. The precipitate was filtered, washed, ignited at 600°C and weighed: 0.5460 g. GF(SO_4 from $BaSO_4$) = MW(SO_4^{2-}) / MW($BaSO_4$) = 96.06 / 233.39 = 0.4116 GF(Na_2SO_4 from $BaSO_4$) = MW(Na_2SO_4) / MW($BaSO_4$) = 142.04 / 233.39 = 0.6087 % SO_4^{2-} = (0.5460 × 0.4116 × 100) / 0.5000 = 44.95% % Na_2SO_4 = (0.5460 × 0.6087 × 100) / 0.5000 = 66.55% Theoretical % Na_2SO_4 in pure Na_2SO_4: 100% Actual: 66.55% → Sample contains other sodium salts or moisture.

Worked Problem 19.2 — Iron Determination as Fe_2O_3

Problem and Solution

0.4000 g of ferrous ammonium sulfate was dissolved in dilute H_2SO_4, oxidised to Fe^{3+} with HNO_3, precipitated as $Fe(OH)_3$ with NH_3, filtered, washed and ignited at 850°C to Fe_2O_3. Mass of Fe_2O_3 = 0.0894 g. Note: 2 Fe^{3+} → 1 Fe_2O_3; GF(Fe from Fe_2O_3) = (2 × 55.85) / 159.69 = 0.6994 % Fe = (0.0894 × 0.6994 × 100) / 0.4000 = 15.62% MW of $Fe(NH_4)(SO_4)_2 \cdot 6H_2O$ = 392.14; % Fe (theoretical) = 55.85/392.14 × 100 = 14.24% Discrepancy → possible contamination or incomplete dissolution.

Worked Problem 19.3 — Chloride Determination as AgCl

Problem and Solution

An ephedrine hydrochloride tablet was dissolved in water; Cl^- precipitated with $AgNO_3$. AgCl precipitate dried at 130°C (not ignited) in a sintered glass crucible. Mass of AgCl = 0.1438 g; sample weight = 0.1500 g. GF(Cl from AgCl) = 35.45 / 143.32 = 0.2474 % Cl^- = (0.1438 × 0.2474 × 100) / 0.1500 = 23.72% Ephedrine HCl (MW = 201.70): Theoretical % Cl = 35.45 / 201.70 × 100 = 17.58% If sample was pure ephedrine HCl, expected Cl ≠ 23.72% → Indicates non-Cl impurities in calculation, or need to use correct GF for ephedrine HCl itself: GF(Ephedrine HCl from AgCl) = 201.70 / 143.32 = 1.4073 % Ephedrine HCl = (0.1438 × 1.4073 × 100) / 0.1500 = 134.9% — indicates excess halide present.

19.3 Pharmaceutical Applications of Gravimetric Analysis

Test/Assay	Substance	Gravimetric Method	IP Limit
Loss on Drying	Aspirin	Dry at 105°C; loss in mass	NMT 0.5%
Loss on Drying	Paracetamol	Dry at 105°C	NMT 0.5%
Loss on Drying	Sodium Bicarbonate	Dry at 105°C	NMT 0.3%
Loss on Drying	Lactose monohydrate	Dry at 80°C (to preserve monohydrate form)	NMT 0.5%
Residue on Ignition	Aspirin	Sulfated ash at 600°C	NMT 0.1%
Residue on Ignition	Paracetamol	Sulfated ash at 600°C	NMT 0.1%
Total Ash	Dried Ginger	Ignition at 500°C	NMT 6.0% (IP)

Acid-Insoluble Ash	Senna leaf	HCl-insoluble residue	NMT 2.0% (IP)
Sulphate Ash	Ranitidine	Sulfated ash	NMT 0.1%
Chloride as AgCl	Sodium Chloride Inj.	Gravimetric (argentometric)	95–105% w/v
Ca^{2+} as CaO	Calcium Gluconate	Via CaC_2O_4, ignite to CaO	99.0–101.0%

19.4 Advantages and Limitations of Gravimetric Analysis

19.4.1 Advantages

- Highest accuracy and precision of all classical methods — mass can be measured to ±0.1 mg.
- Absolute method — no calibration curves, no reference standards needed for the measurement step.
- Results are independent of the instrument (only a calibrated balance required).
- Versatile — applicable to many anions, cations, and organic functional groups.
- Permanent record — the weighed precipitate can be archived and re-examined.

19.4.2 Limitations

- Time-consuming — typically requires several hours for a complete determination.
- Requires skill and experience to perform correctly (especially filtration, washing, ignition).
- Not suitable for trace-level analysis (< 1 mg of analyte is difficult to weigh accurately).
- Selectivity: Many substances form precipitates with the same reagent; separation steps may be needed.
- Not amenable to automation or high-throughput analysis.

19.5 Summary

Chapter 19 Summary

Gravimetric factor (GF) = (a × MW_analyte)/(b × MW_precipitate). % Analyte = (mass ppt × GF × 100)/sample wt. Pharmaceutical applications include LOD (moisture), ROI/Sulfated Ash (inorganic residue), Total Ash, Acid-Insoluble Ash, and specific precipitation gravimetric assays. Gravimetric analysis is the most accurate classical method but is time-consuming and not suitable for trace analysis. Pharmacopoeial gravimetric tests (LOD, Sulfated Ash, Ash tests) are mandatory quality control tests for most drug substances under IP/BP/USP.

Review Questions

Short Answer Questions

1. Calculate the GF for determining Ca^{2+} from CaO (MW Ca = 40.08; MW CaO = 56.08).
2. A 0.2500 g sample gave 0.3125 g of $BaSO_4$. Calculate % SO_4^{2-} (GF = 0.4116).
3. What is the difference between Total Ash and Acid-Insoluble Ash?
4. Why is gravimetric analysis NOT suitable for trace-level analysis?

Long Answer Questions

1. A 0.5000 g sample of impure $BaCl_2$ was dissolved and the chloride precipitated as AgCl; mass of AgCl = 0.6500 g. (a) Calculate % Cl^-; (b) Calculate % $BaCl_2$ in the sample. [MW $BaCl_2$ = 208.23; MW AgCl = 143.32; MW Cl = 35.45]
2. Discuss the advantages and disadvantages of gravimetric analysis compared to titrimetric and instrumental methods.

Multiple Choice Questions

1. The gravimetric factor (GF) for sulfate (SO_4^{2-}) weighed as $BaSO_4$ is:

(a) 233.39/96.06 = 2.43

(b) 96.06/233.39 = 0.4116

(c) 137.33/233.39 = 0.5884

(d) 1.0

✓***Answer: (b) 96.06/233.39 = 0.4116***

2. In the Sulfated Ash test, H_2SO_4 is added to the residue to:

(a) Dissolve the residue completely

(b) Convert metal oxides to stable, non-hygroscopic sulfates for weighing

(c) Neutralize the alkaline ash

(d) React with carbon residues

✓***Answer: (b) Convert metal oxides to stable, non-hygroscopic sulfates for weighing***

3. IP limit for Loss on Drying of Aspirin is:

(a) NMT 1.0%

(b) NMT 0.5%

(c) NMT 2.0%

(d) NMT 0.1%

✓***Answer: (b) NMT 0.5%***

4. Acid-Insoluble Ash measures:
(a) Total inorganic content
(b) Siliceous earth (SiO_2) contamination — non-physiological mineral impurities
(c) Water-soluble salts
(d) Total heavy metal content
✓Answer: (b) Siliceous earth (SiO_2) contamination — non-physiological mineral impurities

5. Gravimetric analysis is most accurate when analyte concentration is:
(a) < 0.001%
(b) 0.001–0.01%
(c) 0.01–1%
(d) Greater than 1% (major component)
✓Answer: (d) Greater than 1% (major component)

UNIT VI
INSTRUMENTAL METHODS — UV-VISIBLE SPECTROPHOTOMETRY

Instrumental analysis represents the frontier of modern pharmaceutical quality science. In this unit, we explore UV-Visible spectrophotometry — the most widely used instrumental analytical technique in pharmaceutical laboratories worldwide. From the simple quantitative assay of a tablet dissolution sample to the complex multi-component analysis of a pharmaceutical formulation, UV-Vis spectrophotometry is indispensable. This unit covers the electromagnetic spectrum, electronic transitions, the Beer-Lambert law, instrumentation, and the full range of pharmaceutical applications.

Chapter 20
Principles of UV-Visible Spectrophotometry

20.1 Learning Objectives

- Describe the electromagnetic spectrum and identify the UV-Visible region.
- Explain the concept of interaction of electromagnetic radiation with matter.
- Describe the types of electronic transitions relevant to UV-Vis spectroscopy.
- Define and distinguish between chromophores, auxochromores, bathochromic shift, hypsochromic shift, hyperchromic and hypochromic effects.
- Explain the relationship between absorption and molecular structure.
- Describe the factors affecting UV absorption.

20.2 Introduction

UV-Visible spectrophotometry is an analytical technique based on the absorption of ultraviolet (UV, 200–400 nm) or visible (400–700 nm) electromagnetic radiation by molecules in solution. It is arguably the most important routine analytical tool in pharmaceutical quality control — virtually every pharmaceutical QC laboratory in the world has a UV-Vis spectrophotometer.

The technique is used for: quantitative assay of drug substances and formulations, identification of substances (by λmax comparison), dissolution testing, content uniformity, impurity profiling, and stability studies. The IP, BP, and USP all specify UV spectrophotometric methods for the assay of hundreds of drug substances.

UV-Vis spectrophotometry combines simplicity, speed, high sensitivity, reasonable cost, and the availability of validated pharmacopoeial methods — making it ideal for routine pharmaceutical analysis.

20.3 The Electromagnetic Spectrum

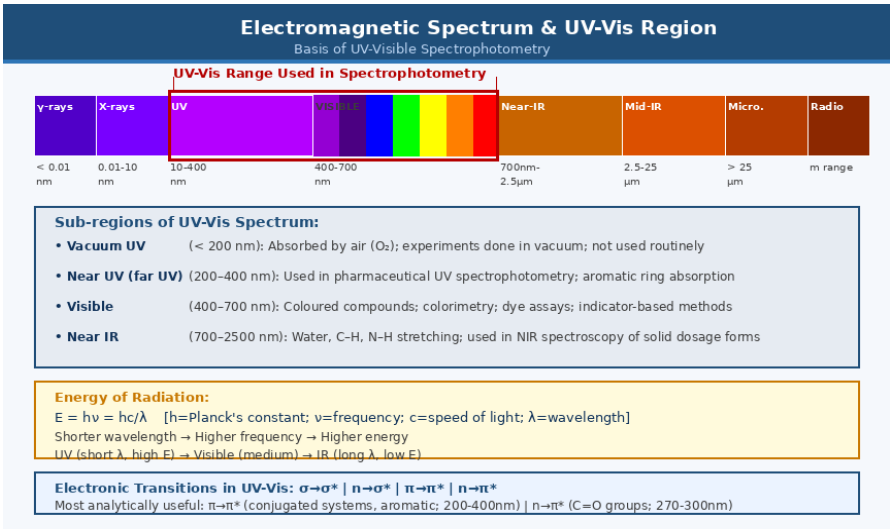


Figure: Electromagnetic Spectrum — UV-Vis Region and Electronic Transitions

Electromagnetic radiation is a form of energy that travels through space as oscillating electric and magnetic fields, perpendicular to each other and to the direction of propagation. It is characterized by:

- Wavelength (λ): The distance between successive wave peaks; measured in nanometres (nm) for UV-Vis.
- Frequency (ν): The number of cycles per second; $\nu = c/\lambda$ where $c = 3 \times 10^8$ m/s (speed of light).
- Energy (E): $E = h\nu = hc/\lambda$ where h = Planck's constant (6.626×10^{-34} J·s). Shorter wavelength = higher energy.

The UV-Visible region spans from approximately 200 nm to 700 nm:

Region	Wavelength Range	Energy Level	Analytical Use
Vacuum UV	< 200 nm	Very high	Not used routinely (absorbed by air and glass)
Near UV	200–400 nm	High	Main pharmaceutical UV spectrophotometry range
Visible	400–700 nm	Moderate	Coloured compounds; colorimetry; dye assays
Near IR	700–2500 nm	Low	NIR spectroscopy of solid pharmaceutical dosage forms

20.4 Nature of Absorption — Electronic Transitions

When UV-Visible radiation interacts with a molecule, photons are absorbed only when the photon energy exactly matches the energy difference between molecular orbital energy levels. This causes electrons to be promoted from a lower-energy (ground state) orbital to a higher-energy (excited state) orbital — an electronic transition.

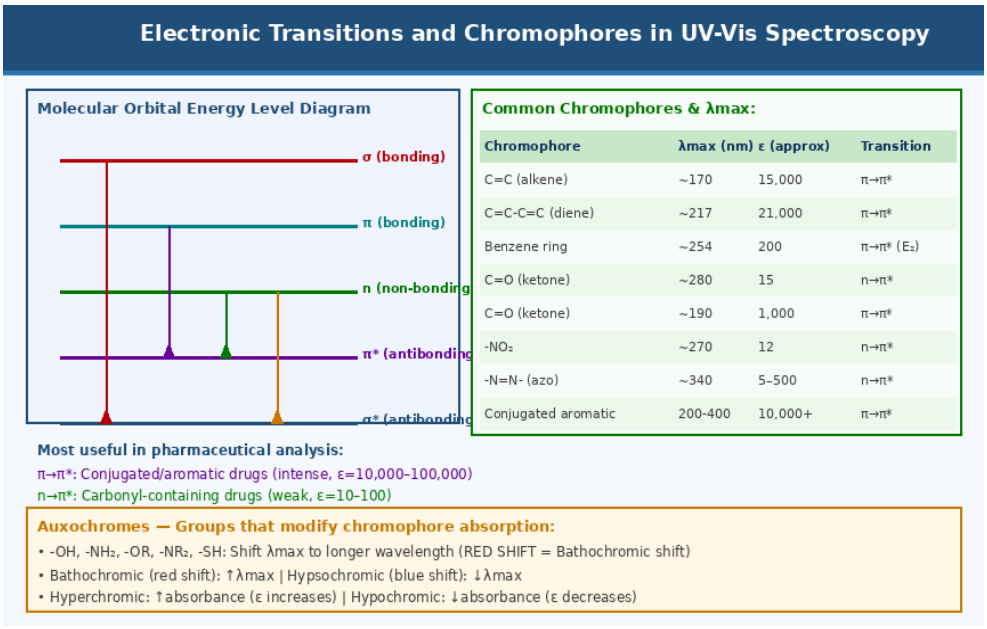


Figure: Electronic Transitions, Chromophores and Auxochromores in UV-Vis Spectroscopy

The types of electronic transitions relevant to UV-Vis spectroscopy are:

Transition	Type	λ Range	ε (approx)	Pharmaceutical Relevance
σ → σ*	C–C, C–H sigma bonds	< 150 nm	Very high	Vacuum UV; not useful (glass absorbs)
n → σ*	Non-bonding → σ*	150–250 nm	~100–3000	Saturated compounds with N, O, S, halogens
π → π*	π bonding → π*	200–300 nm	1,000–100,000	Conjugated systems, aromatic rings — MOST USEFUL
n → π*	Non-bonding → π*	270–400 nm	10–100	Carbonyl compounds (C=O), azo groups — weaker

20.4.1 Chromophores

A chromophore is a structural group in a molecule responsible for absorption of UV-Visible radiation. The word derives from the Greek 'chroma' (colour) and 'phoros' (bearer). In UV spectrophotometry, chromophores need not produce visible colour — they absorb in the UV region.

Chromophore	Structure	λmax (nm)	ε	Transition	Example Drugs
Isolated C=C	–CH=CH–	~170	15,000	π→π*	Simple alkenes (not in UV range)
Conjugated diene	–CH=CH–CH=CH–	217	21,000	π→π*	Retinol (Vitamin A)
Aromatic ring (benzene)	C_6H_6	254	200	π→π*	All benzene-containing drugs
Carbonyl (ketone) C=O	–C(=O)–	~280	~15	n→π*	Steroid hormones, paracetamol

Carboxylic acid –COOH	–COOH	~210	50	n→π*	Aspirin, ibuprofen
Nitro group –NO_2	–NO_2	~270	12	n→π*	Chloramphenicol, nitrofurantoin
Azo group –N=N–	–N=N–	~340	Variable	n→π*	Azo dyes; some sulfonamides

20.4.2 Auxochromic Groups

Auxochromes are substituent groups that, by themselves, do not absorb in the UV-Vis region, but when attached to a chromophore they modify its absorption — typically causing a bathochromic shift (red shift to longer wavelength) and often an increase in intensity (hyperchromic effect). Common auxochromes include: –OH, –OR, –NH_2, –NHR, –NR_2, –SH, –Cl, –Br.

Mechanism: Auxochromes donate electron pairs by resonance to the chromophore, extending the conjugation and lowering the energy gap between bonding and antibonding orbitals → λmax shifts to longer wavelength (bathochromic/red shift).

20.4.3 Spectral Shifts — Definitions

Term	Effect on λmax	Effect on ε (intensity)	Cause
Bathochromic (Red) Shift	Increases (shifts to longer λ)	May increase	Addition of auxochrome; increased conjugation; pH change in ionisable chromophore
Hypsochromic (Blue) Shift	Decreases (shifts to shorter λ)	May decrease	Loss of conjugation; protonation of auxochrome
Hyperchromic Effect	No change in λmax	Increases (ε increases)	Increased number of absorbing groups; structural change increasing delocalization
Hypochromic Effect	No change in λmax	Decreases (ε decreases)	Steric hindrance to coplanarity; structural distortion

20.5 Solvent Effects on UV Absorption

The choice of solvent can significantly affect both the position (λmax) and intensity (ε) of UV absorption:

- π→π* transitions: The λmax undergoes a HYPSOCHROMIC (blue) shift in polar solvents because polar solvents stabilize the ground state more than the excited state (larger energy gap). Example: acetone in hexane: λmax 279 nm; in ethanol: λmax 272 nm.
- n→π* transitions: The λmax undergoes a BATHOCHROMIC (red) shift in polar solvents — the opposite. Polar solvents hydrogen-bond to the non-bonding lone pair in the ground state, raising its energy, and reducing the transition energy.
- Pharmaceutical consequence: UV assay methods specify the solvent precisely. Switching from one solvent to another can shift λmax by 5–20 nm and change ε significantly, rendering the method non-comparable.

Pharmaceutical Solvent	Use in UV Spectrophotometry	Notes
Distilled/purified water	Most widely used; transparent from 200 nm	Preferred when drug is sufficiently soluble
Methanol	Transparent from 205 nm	Common for water-insoluble drugs
Ethanol (95%/absolute)	Transparent from 210 nm	IP/BP frequently specify 'ethanol 96%'
0.1 M HCl	For basic drugs; ionized form	Used for many alkaloids, antihistamines
0.1 M NaOH	For acidic drugs; ionized form	Paracetamol, barbiturates
Acetonitrile	Transparent from 200 nm	HPLC mobile phase UV work; expensive

20.6 pH Effects on UV Absorption

Many drugs are weak acids or bases whose UV absorption changes with pH because protonation/deprotonation alters the extent of conjugation or the electronic distribution:

Example: Phenolphthalein
Acidic solution (pH < 8): Lactone form (colourless, λmax ~260 nm, low ε) Alkaline solution (pH 9–11): Quinonoid dianion (pink, λmax ~552 nm, high ε) Pharmaceutical significance: • Some drugs must be assayed at a specific pH to ensure the same ionization state • pH changes may shift λmax significantly (e.g., sulfonamides, phenols, barbituric acid) • Isosbestic points (where absorbance is independent of pH) are useful for identifying two interconverting species

20.7 Summary

Chapter 20 Summary
UV-Visible spectrophotometry exploits the absorption of UV-Vis radiation (200–700 nm) by electronic transitions. Most analytically useful: π→π* (conjugated, aromatic; intense) and n→π* (C=O, weaker). Chromophores absorb radiation; auxochromic groups modify absorption (bathochromic shifts). Spectral terms: bathochromic (red shift, ↑λ), hypsochromic (blue shift, ↓λ), hyperchromic (↑ε), hypochromic (↓ε). Polar solvents cause hypsochromic shift for π→π* and bathochromic shift for n→π*. pH affects ionisable chromophores. Pharmaceutical analysis uses 200–400 nm range for most drug assays.

Review Questions

Short Answer Questions

1. Define chromophore and auxochrome. Give two examples of each.
2. What is a bathochromic shift? Describe one structural change that causes it.
3. Explain why pH affects the UV absorption of some drug substances.
4. Why is the vacuum UV region (< 200 nm) not useful in routine pharmaceutical analysis?

Long Answer Questions

1. Describe the types of electronic transitions relevant to UV-Vis spectrophotometry. Discuss the nature of chromophores and auxochromic groups with examples from pharmaceutical chemistry.
2. Explain the effect of solvents on UV absorption spectra. Why is the specification of solvent critical in pharmacopoeial UV assay methods?

Multiple Choice Questions

1. The most analytically useful and intense UV transition in aromatic drug molecules is:

(a) σ → σ*
(b) n → σ*
(c) π → π*
(d) n → π*

✓***Answer: (c) π → π****

2. A bathochromic (red) shift refers to:

(a) Shift of λmax to shorter wavelength
(b) Decrease in ε
(c) Shift of λmax to longer wavelength
(d) Increase in ε without change in λmax

✓***Answer: (c) Shift of λmax to longer wavelength***

3. Auxochromes cause bathochromic shifts because:

(a) They absorb UV radiation directly
(b) They donate electron pairs to the chromophore, extending conjugation and lowering transition energy
(c) They increase the molecular weight
(d) They change the pH of the solution

✓ ***Answer: (b) They donate electron pairs to the chromophore, extending conjugation and lowering transition energy***

4. For n→π* transitions in polar solvents, the λmax undergoes a:

(a) Hypsochromic shift
(b) Bathochromic shift
(c) Hyperchromic effect
(d) No change

✓***Answer: (b) Bathochromic shift***

5. The UV-Vis region most used in pharmaceutical spectrophotometry covers:

(a) 50–200 nm
(b) 200–400 nm (near UV) and 400–700 nm (visible)
(c) 700–2500 nm
(d) > 2500 nm

✓***Answer: (b) 200–400 nm (near UV) and 400–700 nm (visible)***

Chapter 21
Beer-Lambert Law

21.1 Learning Objectives

- State and derive the Beer-Lambert law from its two component laws.
- Define absorbance, transmittance, molar absorptivity, and specific absorptivity.
- Construct and use a calibration curve for quantitative UV-Vis analysis.
- Identify and explain the causes of deviations from Beer's law.
- Apply the Beer-Lambert law to pharmacopoeial calculations.

21.2 Lambert's Law and Beer's Law

21.2.1 Lambert's Law (1760)

Lambert's Law states that when monochromatic radiation passes through a homogeneous absorbing medium, the fraction of radiation absorbed is independent of the intensity of the incident radiation, and the absorbance is directly proportional to the path length (thickness) of the absorbing medium.

Lambert's Law

$-dI/dl = k_1 \times I \rightarrow \ln(I_0/I) = k_1 \times l \rightarrow \log(I_0/I) = k_1' \times l$ Absorbance (A) $\propto$ l (path length)

21.2.2 Beer's Law (1852)

Beer's Law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species, when measured with monochromatic radiation at constant path length.

Beer's Law

$\log(I_0/I) = k_2 \times c$ Absorbance (A) $\propto$ c (concentration)

21.2.3 Beer-Lambert Law (Combined)

Beer-Lambert Law — Full Derivation

Combining Lambert's and Beer's laws: $A = \log(I_0/I) = \varepsilon \times c \times l$ Where: A = Absorbance (dimensionless; also called Optical Density, OD) I_0 = Intensity of incident radiation I = Intensity of transmitted radiation ε = Molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$) — characteristic of the molecule at a given λ c = Molar concentration ($mol \cdot L^{-1}$) l = Path length (cm) — usually 1 cm in pharmaceutical spectrophotometry Additional relationships: T (Transmittance) = I/I_0 (0 to 1, or 0–100%) $A = -\log T = \log(1/T)$ $A = 2 - \log(\%T)$ Specific absorptivity (a): $A = a \times c \times l$ (c in g/L; a in $L \cdot g^{-1} \cdot cm^{-1}$) $A^{1}\%_{1}cm$: Absorbance of 1% (w/v) solution in 1-cm cell — used in IP/BP when MW is uncertain

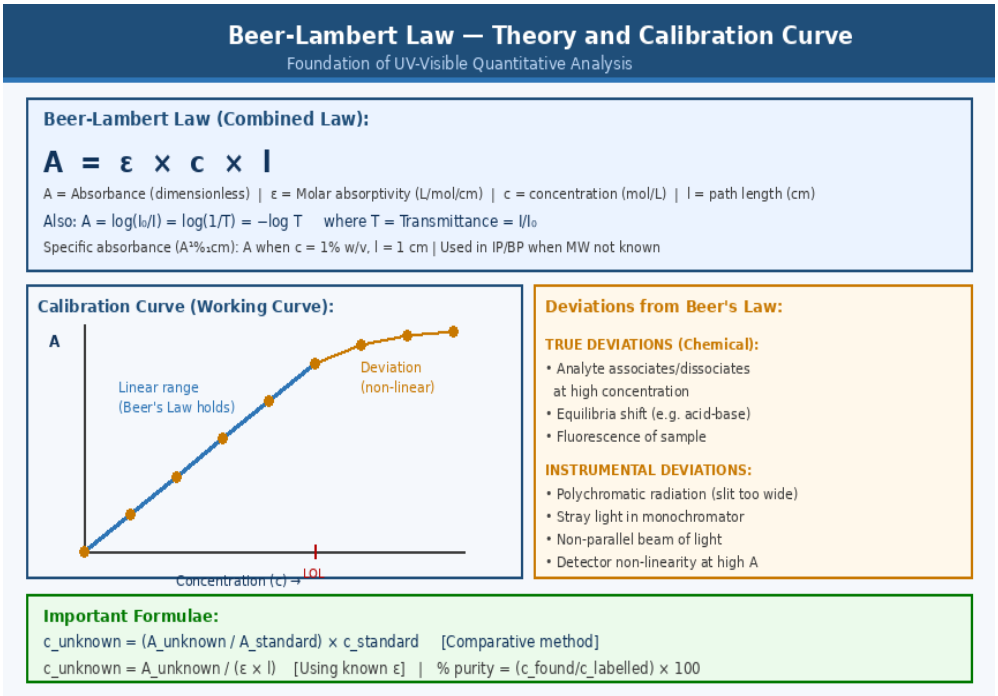


Figure: Beer-Lambert Law — Calibration Curve, Linear Range, and Deviations

21.3 Key Parameters — Definitions and Relationships

Parameter	Symbol	Formula / Units	Significance
Absorbance	A	$A = \log(I_0/I)$; dimensionless	Primary analytical readout; additive for mixtures
Transmittance	%T	$\%T = (I/I_0) \times 100$; %	Useful for colorimetric work; non-linear with conc.
Molar Absorptivity	ε	$L \cdot mol^{-1} \cdot cm^{-1}$	Measure of how strongly compound absorbs; characteristic
Specific Absorptivity	a	$L \cdot g^{-1} \cdot cm^{-1} = \varepsilon/MW$	Used when MW not known
$A^{1}\%_{1}cm$	$A^{1}\%_{1}cm$	Absorbance of 1% w/v soln, 1 cm cell	Used in IP/BP for drug assay; species-specific
Path length	l	cm (usually 1 cm)	Fixed by cuvette design

Concentration	c	mol/L or g/L	The quantity being determined

Worked Example 21.1

A paracetamol tablet was dissolved and diluted to 100 mL; 2 mL of this was further diluted to 100 mL. The final solution was measured in a 1-cm cell at 243 nm and showed A = 0.682. $A^{1}\%_{1}$cm for paracetamol at 243 nm = 715 (IP value) Using A = $A^{1}\%_{1}$cm × c × l: c = A / ($A^{1}\%_{1}$cm × l) = 0.682 / (715 × 1) = 9.538×10^{-4} %w/v Actual concentration in original solution: = 9.538×10^{-4} × (100/2) × (100) = 47.69 mg per tablet If labelled content = 500 mg: % of label = (47.69/500) × 100 = 9.54% Note: Dilution factor must be incorporated correctly. If tablet dissolved in 100 mL → 2 mL → 100 mL: Mass = 9.538×10^{-4} g/100 mL × (100 mL / 2 mL) × 100 mL = 0.04769 g = 47.69 mg IP limit for paracetamol tablets: 95.0–105.0% of labelled content.

Worked Example 21.2 — Comparative Method

Standard solution: 10 µg/mL of aspirin in 0.1 M NaOH → A_std = 0.420 at 280 nm Sample solution: Unknown aspirin conc. → A_samp = 0.378 c_unknown = (A_sample / A_standard) × c_standard c_unknown = (0.378 / 0.420) × 10 µg/mL = 9.0 µg/mL Alternative: If original tablet dissolved in 200 mL then 5 mL → 100 mL: Amount per tablet = 9.0 × (100/5) × 200 / 1000 = 360 mg If labelled 500 mg: % Label = (360/500) × 100 = 72% → out of specification → investigate.

21.4 Calibration Curve (Working Curve)

The calibration curve is a plot of absorbance (y-axis) against concentration (x-axis), constructed using a series of standard solutions of known concentration. It serves as the reference for converting absorbance readings of unknown samples into concentration values.

Construction of a calibration curve:

1. Prepare a series (minimum 5) of standard solutions of the analyte spanning the expected concentration range of samples.
2. Measure the absorbance of each standard at the chosen λmax using the blank as reference.
3. Plot A vs. concentration; draw the best straight line (linear regression).
4. Calculate the slope (m) and intercept (b) of the regression line: A = m × c + b
5. Read off the concentration of unknown from the graph, or calculate: c_unknown = (A_unknown − b) / m
6. Verify linearity: $R^2 \geq 0.999$ indicates excellent linearity.

Typical Calibration Curve Parameters
Linear range: 5–50 µg/mL (example for paracetamol at 243 nm) Correlation coefficient R^2: ≥ 0.999 for acceptable linearity Limit of Detection (LOD) ≈ 3.3 × (SD of blank) / slope Limit of Quantification (LOQ) ≈ 10 × (SD of blank) / slope The calibration curve should be validated for linearity, range, accuracy, precision, LOD, and LOQ as per ICH Q2(R1) guidelines.

21.5 Deviations from Beer-Lambert Law

Beer's law predicts a linear relationship between A and c. In practice, deviations occur at high concentrations or under certain conditions. Understanding deviations is essential for method validation and troubleshooting.

21.5.1 True (Fundamental) Chemical Deviations

- Chemical equilibria: If the analyte associates, dissociates, or reacts at high concentration (e.g., dimerization of dye molecules), the effective absorbing species changes — causing non-linearity. Solution: Work at concentrations where species is stable.
- Ionisation: pH-dependent drugs that partially ionise at the assay concentration — different species (HA vs A^-) have different ε values. Solution: Use buffered solutions at a defined pH.
- Interaction with solvent: Some analytes complex with the solvent at high concentrations, shifting ε.

21.5.2 Apparent Chemical Deviations — Concentration

- At high concentrations (A > 1.5): Beer's law assumes the inter-molecular distance is large compared to the wavelength. At high concentrations, molecules are close enough to alter each other's charge distribution, effectively changing ε. Solution: Work in the A range 0.1–1.0 (recommended).

21.5.3 Instrumental Deviations

- Polychromatic radiation (stray light): If the radiation is not truly monochromatic (wide bandpass), different wavelengths are absorbed to different extents. The overall response is non-linear. This causes the calibration curve to flatten at high concentrations (negative deviation).
- Stray light: Light reaching the detector without passing through the sample causes apparent transmittance to be higher than actual, causing apparent absorbance to be lower — particularly significant at high A values or at wavelengths where the lamp output is low (< 220 nm).
- Detector non-linearity: At very high or very low light intensities, detectors may not respond linearly.

- Fluorescence: If the sample fluoresces, the emitted radiation adds to the transmitted beam, making I appear higher than actual — A is underestimated.

Practical Recommendations
Work within absorbance range A = 0.2 – 1.5 for maximum accuracy and linearity At A < 0.1: Error in %T reading causes large error in A At A > 1.5: Stray light and concentration effects cause negative deviations Optimal A = 0.434 (corresponding to exactly 36.8% T — theoretically minimum photometric error) IP/BP methods are designed to give A in 0.2–0.8 range for best accuracy

21.6 Spectrophotometric Methods for Multi-Component Analysis

When a sample contains two or more absorbing species that both absorb at the assay wavelength, the total absorbance is the sum of the individual absorbances (additivity of Beer's law):

Multi-Component Analysis
For a two-component system (X and Y) at two wavelengths (λ_1, λ_2): $A(\lambda_1) = \varepsilon X(\lambda_1) \times cX \times l + \varepsilon Y(\lambda_1) \times cY \times l$ $A(\lambda_2) = \varepsilon X(\lambda_2) \times cX \times l + \varepsilon Y(\lambda_2) \times cY \times l$ Two equations; two unknowns (cX and cY) → solve simultaneously Requirement: The two wavelengths should be chosen where the difference in ε for X and Y is maximum (best selectivity). Pharmaceutical application: Simultaneous assay of Paracetamol + Ibuprofen in a combination tablet without HPLC separation.

21.7 Summary

Chapter 21 Summary
Beer-Lambert Law: A = ε × c × l (molar absorptivity × concentration × path length). A = log(I_0/I) = −log T. Specific absorptivity a = ε/MW; $A^{1}\%_{1}cm$ = IP/BP standard parameter. Calibration curve: A vs c; linear range $R^2 \geq 0.999$; work in A range 0.2–1.5. Deviations: chemical (equilibria, ionisation), concentration (high c), instrumental (stray light, polychromatic radiation, detector non-linearity). Multi-component analysis exploits additivity of absorbances at two wavelengths. LOD = 3.3σ/S; LOQ = 10σ/S.

Review Questions

Short Answer Questions

1. State the Beer-Lambert law and define each symbol.
2. What is $A^{1}\%_{1}cm$? How is it used in IP assay calculations?
3. Why is the recommended working absorbance range A = 0.2 to 1.5?
4. Explain stray light and its effect on the calibration curve.

Long Answer Questions

1. Derive the Beer-Lambert law from Lambert's law and Beer's law. Discuss the causes of deviations from Beer's law (chemical and instrumental) with remedies.
2. A 500 mg paracetamol tablet was dissolved, diluted to 250 mL. 1 mL was taken and diluted to 100 mL. The solution showed A = 0.540 at 243 nm ($A^{1}\%_{1}cm$ = 715). Calculate the amount of paracetamol in the tablet and determine if it meets the IP specification of 95.0–105.0% of label claim.

Multiple Choice Questions

1. Beer-Lambert law states A equals:

(a) ε / (c × l)
(b) ε × c × l
(c) c / (ε × l)
(d) l / (ε × c)

✓Answer: (b) ε × c × l

2. If %T = 50%, the absorbance is:

(a) 0.500
(b) 0.301
(c) 0.699
(d) 1.000

✓Answer: (b) 0.301

3. Stray light in a spectrophotometer causes:

(a) Bathochromic shift
(b) Negative deviation from Beer's law at high absorbances
(c) Positive deviation from Beer's law
(d) Increased sensitivity

✓Answer: (b) Negative deviation from Beer's law at high absorbances

4. The $A^{1}\%_{1}cm$ value for paracetamol at 243 nm (IP) is:

(a) 250
(b) 480
(c) 715
(d) 1000

✓Answer: (c) 715

5. For minimum photometric error in UV spectrophotometry, the optimal absorbance is:

(a) 0.0

(b) 0.434 (corresponding to 36.8% T)

(c) 1.000

(d) 2.000

✓***Answer: (b) 0.434 (corresponding to 36.8% T)***

Chapter 22
Instrumentation and Applications of UV-Vis Spectrophotometry

22.1 Learning Objectives

- Describe the components of a UV-Vis spectrophotometer and the function of each.
- Distinguish between single-beam and double-beam spectrophotometers.
- Explain the principle and use of different types of radiation sources, monochromators, and detectors.
- Describe the pharmacopoeial applications of UV spectrophotometry in pharmaceutical analysis.
- Explain the procedure for calibration and validation of a UV spectrophotometer.

22.2 Components of a UV-Vis Spectrophotometer

Every UV-Vis spectrophotometer — whether simple or sophisticated — consists of five essential components arranged in a specific sequence:

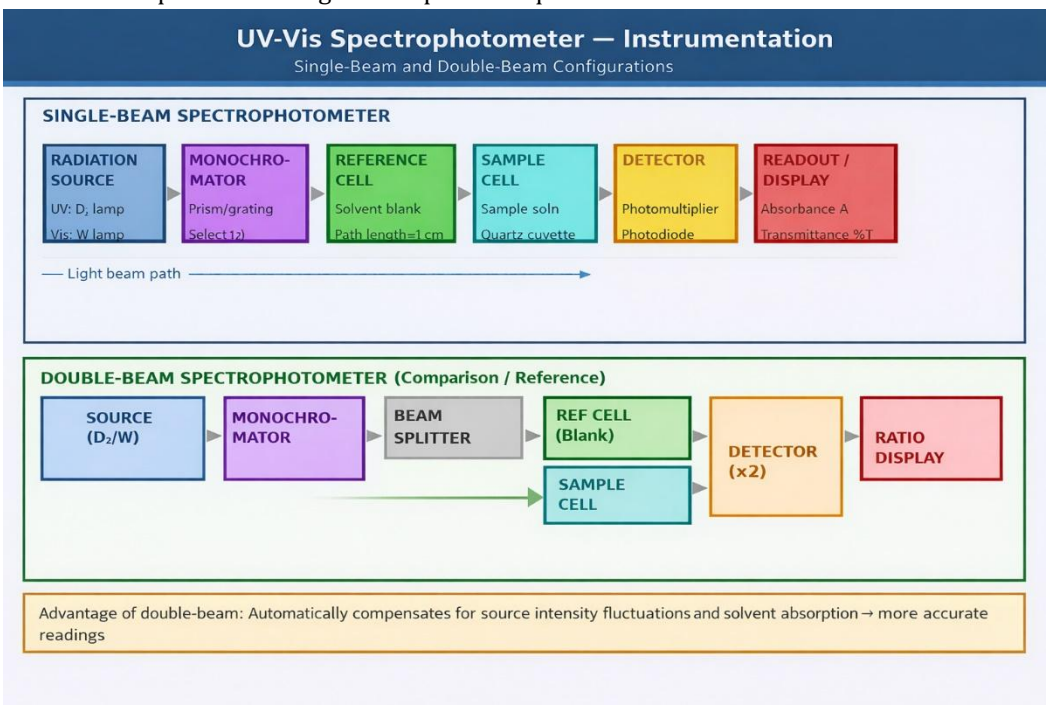


Figure: UV-Vis Spectrophotometer Components — Single-Beam and Double-Beam Designs

22.2.1 Radiation Source

The radiation source must provide continuous radiation of sufficient intensity across the UV-Vis range. Two lamps are used because no single lamp covers the entire 200–800 nm range:

Lamp Type	Wavelength Range	Use	Principle
Deuterium (D_2) Lamp	200–380 nm	UV region	Deuterium discharge; continuous spectrum; high intensity UV
Tungsten Halogen (W) Lamp	380–2500 nm	Visible and near-IR	Incandescent filament; halogen retards blackening
Xenon Arc Lamp	200–1000 nm (pulsed)	UV and visible	High intensity; used in some modern instruments
Light Emitting Diodes (LEDs)	Specific wavelengths	Point measurements	Low power; used in portable field instruments

The switch from D_2 to W lamp is automatic at approximately 340–380 nm in modern instruments.

22.2.2 Monochromator

The monochromator isolates a narrow band of wavelengths from the polychromatic source radiation. It consists of:

- Entrance slit: Limits the width of the radiation beam entering the monochromator.
- Collimating lens or mirror: Renders the beam parallel.
- Dispersing element: Separates wavelengths — either a prism (glass for visible; quartz for UV) or a diffraction grating (ruled or holographic). Modern instruments use holographic gratings almost exclusively.
- Focusing element: Focuses the dispersed beam onto the exit slit.
- Exit slit: Isolates the desired wavelength band (bandwidth). The narrower the slit, the more monochromatic the radiation but the lower the intensity. Spectral bandwidth (SBW): typically 0.5–2 nm for analytical instruments.

22.2.3 Sample Compartment and Cuvettes

The sample compartment holds the cuvette (cell) containing the sample and blank solutions. Cuvettes must be:

- Transparent to the radiation wavelength: Glass/plastic cuvettes are acceptable for visible (400–700 nm); quartz cuvettes are mandatory for UV (200–400 nm) because glass absorbs UV radiation.
- Matched pairs: Reference (blank) and sample cuvettes must be optically matched (same path length, same transmittance of walls) to minimize baseline error. Path length is typically 1 cm.
- Clean: Any contamination (fingerprints, solution residues) on the cuvette walls causes significant errors. Clean with lens tissue; never scratch with abrasive materials.

22.2.4 Detector

The detector converts transmitted light intensity into an electrical signal:

Detector Type	Wavelength Range	Principle	Use
Photomultiplier Tube (PMT)	UV-Vis (200–900 nm)	Photoelectric effect + electron cascade amplification	High sensitivity; most widely used in UV-Vis spectrophotometry
Photodiode Array (PDA)	UV-Vis	Array of Si photodiodes; detects all λ simultaneously	Fast scanning; good for diode-array instruments
Charge-Coupled Device (CCD)	Visible-NIR	Semiconductor; high quantum efficiency	Used in modern multichannel spectrometers

22.2.5 Readout Device

Modern instruments digitize the detector signal and display absorbance, % transmittance, or concentration directly on a screen. Computer software allows: wavelength scanning, kinetic measurements, multi-wavelength analysis, spectral library matching, data storage, and report generation.

22.3 Single-Beam vs Double-Beam Spectrophotometers

Feature	Single-Beam	Double-Beam
Configuration	One beam path; measure blank and sample separately	Beam split; reference and sample measured simultaneously
Source fluctuation compensation	None (manual re-zeroing required)	Automatic (ratio of sample to reference beam)
Baseline correction	Manual; operator dependent	Automatic; more reliable
Accuracy	Good for fixed wavelength; less for scanning	Better; especially for spectral scanning
Complexity and cost	Simpler; lower cost	More complex; higher cost
Application	Routine fixed-λ assays in QC labs	Research, spectral scanning, kinetics, multi-λ work
Lamp drift compensation	Poor	Excellent

22.4 Calibration and Qualification of Spectrophotometers

A UV-Vis spectrophotometer used in pharmaceutical quality control must be periodically calibrated and qualified. The IP and BP specify the following qualification tests:

22.4.1 Wavelength Accuracy

Verified using standard materials with known absorption maxima:

- Holmium oxide glass filter: Certified λmax values at multiple points across UV-Vis range.
- Benzene vapour: Sharp absorption bands at 254.0 nm (reference standard).
- Didymium glass filter: Multiple certified peaks.
- Mercury arc lamp emission lines: 253.65 nm, 296.73 nm, 365.01 nm (wavelength standard for precision calibration).

Acceptance criterion (IP): Wavelength accuracy ≤ ±1 nm in UV; ≤ ±3 nm in visible.

22.4.2 Photometric Accuracy (Absorbance Accuracy)

Verified using certified reference solutions of known absorbance:

- Potassium dichromate ($K_2Cr_2O_7$) in 0.005 M H_2SO_4: IP specifies exact A values at 235, 257, 313, and 430 nm. These are used to verify that the instrument reads absorbance correctly.
- NIST-certified neutral density filters: Glass filters with certified absorbance values.

22.4.3 Stray Light Test

- 5% potassium iodide (KI) solution: Should have A > 2.0 at 220 nm (cuts off below 220 nm; stray light appears as transmission).
- 1.2% potassium chloride (KCl) solution: Should have A > 2.0 at 198 nm.

22.4.4 Resolution Test

The instrument should resolve the two peaks of toluene vapour at 268.5 nm and 269.0 nm or similar closely spaced bands. Slit width must be ≤ 1 nm for this test.

22.5 Pharmaceutical Applications of UV-Vis Spectrophotometry

22.5.1 Assay of Drug Substances and Formulations (IP Method)

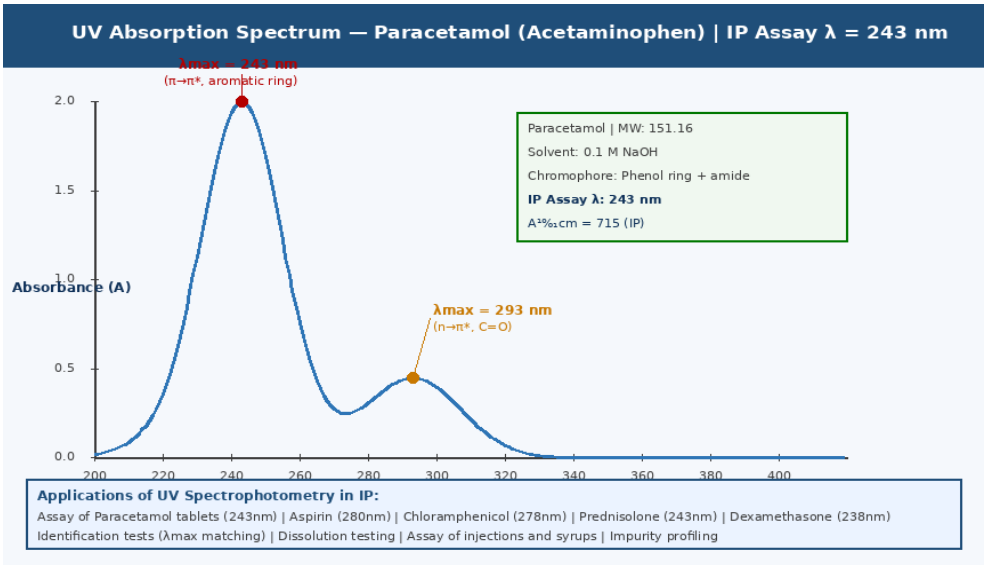


Figure: UV Absorption Spectrum of Paracetamol — λmax = 243 nm (IP Assay Wavelength)

Drug Substance	λmax (nm)	Solvent	$A^{1}\%_{1}cm$ (IP)	Limit (IP)
Paracetamol (Acetaminophen)	243	0.1 M NaOH	715	95.0–105.0%
Aspirin (Acetylsalicylic Acid)	280	0.1 M NaOH	~95	Not less than 99.5% (anhydrous basis)
Chloramphenicol	278	Water	298	97.0–103.0%
Prednisolone	243	Ethanol	415	97.0–103.0%
Dexamethasone	238	Ethanol	391	97.0–103.0%
Rifampicin	475	Methanol	187	97.0–102.0%
Metronidazole	277	0.1 M HCl	~377	99.0–101.0%
Chloroquine Phosphate	343	0.1 M H_2SO_4	~460	98.0–102.0%
Tetracycline HCl	269	0.01 M HCl	~390	95.0–102.0%
Erythromycin Ethylsuccinate	~290	Methanol	~variable	97.0–102.0%

22.5.2 Identification Tests

UV spectrophotometry is used as an identification test by verifying that:

- The absorption spectrum of the sample (λmax and shape) matches that of a reference standard within defined tolerances (typically ±2 nm for λmax).
- The ratio of absorbances at two wavelengths ($A(\lambda_1)/A(\lambda_2)$) is within a specified range — this is less susceptible to concentration error than a single λmax comparison.

IP Identification by UV

IP Example — Paracetamol: 'Dissolve 5 mg in 0.1 M NaOH and dilute to 100 mL. Dilute 10 mL to 100 mL with 0.1 M NaOH. The solution shows a maximum at 257 nm and a minimum at 226 nm. At 243 nm the absorbance is about 0.66.' The matching of λmax AND the characteristic ratio of absorbances at specified wavelengths provides a two-parameter identity test.

22.5.3 Dissolution Testing

UV-Vis spectrophotometry is the dominant analytical technique for dissolution testing of tablets and capsules. In a dissolution apparatus (USP Type I or II), samples withdrawn at defined time intervals are assayed by UV to determine the amount of drug released. Regulatory requirements mandate that dissolution testing is performed for all immediate-release solid oral dosage forms.

Advantages for dissolution: Rapid analysis (< 30 seconds per sample), non-destructive, can be done on-line or in-line, no complex sample preparation, and direct absorbance measurement correlates to drug concentration.

22.5.4 Content Uniformity / Uniformity of Dosage Units

UV spectrophotometry is used to assay individual dosage units (tablets, capsules) for content uniformity testing (IP Chapter 2.4.31 / BP Appendix XII C). Typically 10 dosage units are individually assayed and the results must comply with the RSD limit (IP: NMT 6% for most dosage forms).

22.5.5 Impurity Profiling and Purity Assessment

The UV spectrum of an impure drug substance may show unexpected peaks or absorptionss at wavelengths where the pure compound does not absorb. By comparing spectra of test and standard solutions, UV spectrophotometry can detect and quantify UV-absorbing impurities.

- Related substance testing: IP/BP limit tests for specific UV-absorbing impurities use UV spectrophotometry with threshold concentration comparisons.
- Ratio of absorbances: Comparing A at two wavelengths serves as a purity index. Any deviation from the expected ratio indicates the presence of an impurity absorbing at one wavelength.

22.5.6 Colorimetric Assays

Many drug substances do not absorb significantly in the UV region but can be converted to coloured products by reaction with specific chromogenic reagents, and then assayed by visible spectrophotometry. This approach extends UV-Vis spectrophotometry to non-UV-absorbing analytes:

Assay Method	Chromogenic Reagent	Substance Determined	λmax
Biuret reaction	$CuSO_4$ + NaOH + Na-K tartrate	Proteins / amino acids	540 nm (violet)
Folin-Ciocalteu	Folin reagent (phosphomolybdic acid)	Total phenolic content; proteins	750 nm (blue)
Lowry method	Folin + biuret reagent	Protein quantification	660 nm
Diazonium coupling	Sodium nitrite + coupling agent	Primary aromatic amines	Various
Nessler's reagent	K_2HgI_4 in KOH	Ammonium (NH_4^+)	420 nm (yellow)
Vanillin-H_2SO_4	Vanillin + conc H_2SO_4	Glycyrrhizin; terpenes	Various (visible)
Iron(III) thiocyanate	Fe^{3+} + SCN^-	Aspirin (as salicylate)	530 nm (red)

22.5.7 Kinetic Spectrophotometric Methods

Kinetic methods measure the rate of a chemical reaction rather than the equilibrium absorbance. The rate is proportional to the concentration of the analyte. UV-Vis is used to monitor the reaction in real time:

- Initial rate method: The rate of change of absorbance at early time points (linear region) is measured. $c \propto dA/dt$.
- Fixed-time method: Absorbance is measured at a fixed time after initiation; the difference ΔA correlates with concentration.
- Variable-time method: Time required to reach a fixed absorbance is measured; $c \propto 1/t$.

Application: Determination of glucose (enzyme-based kinetic assay using glucose oxidase); paracetamol overdose monitoring (kinetic method using plasma samples).

22.6 Difference Spectrophotometry

Difference spectrophotometry measures the difference in absorbance between two solutions of the same substance under different conditions (e.g., different pH) rather than the absolute absorbance. This technique eliminates the background absorbance of interferences that do not change between the two conditions.

Principle
A_difference = A(condition 1) − A(condition 2) Example: Phenolphthalein is colourless at pH 5 and pink at pH 11. A difference spectrum shows the net absorption due to the pH-induced structural change. Pharmaceutical application: Simultaneous determination of drugs in formulations where one component is pH-sensitive and the other is not — the pH-insensitive component cancels out; the pH-sensitive component contributes to the difference spectrum.

22.7 Derivative Spectrophotometry

Derivative spectrophotometry plots the first, second, or higher-order derivatives of the absorbance spectrum ($dA/d\lambda$, $d^2A/d\lambda^2$, etc.) against wavelength. Derivatives enhance resolution between overlapping peaks and can reveal shoulders invisible in the zero-order spectrum.

- First derivative ($dA/d\lambda$): Zero crossing coincides with λmax of zero-order spectrum. Peaks of first derivative are at inflection points of zero-order.
- Second derivative ($d^2A/d\lambda^2$): Shows a negative peak at λmax of zero-order. Doubles the number of peaks relative to zero-order.
- Fourth derivative: Further resolution enhancement; used for analysis of complex mixtures.

Pharmaceutical applications: Resolution of overlapping UV spectra in multi-component preparations; detection of impurities with slightly different λmax; sensitivity enhancement.

22.8 Summary

Chapter 22 Summary
UV-Vis spectrophotometer components: Radiation source (D_2 for UV; W lamp for visible), monochromator (diffraction grating), sample compartment (quartz cuvettes for UV), detector (PMT, photodiode array), readout. Single-beam: simpler, routine assay; double-beam: better accuracy, automatic correction. Calibration: wavelength accuracy (holmium glass, Hg lines), photometric accuracy ($K_2Cr_2O_7$ reference solutions), stray light (KI, KCl solutions). Applications: assay (paracetamol 243 nm, chloramphenicol 278 nm), identification, dissolution, content uniformity, colorimetric assays, kinetic methods, difference and derivative spectrophotometry.

Review Questions

Short Answer Questions

1. Why are quartz cuvettes used for UV measurements, while glass cuvettes are acceptable for visible spectrophotometry?

2. What is the purpose of the deuterium lamp in a UV spectrophotometer? What lamp is used for the visible region?
3. Describe the stray light test used to qualify a UV spectrophotometer as per IP.
4. What is derivative spectrophotometry? What advantage does the second derivative offer?
5. State two pharmacopoeial quality control tests in which UV spectrophotometry is used.

Long Answer Questions

1. Describe the components of a UV-Vis spectrophotometer with a labelled diagram. Distinguish between single-beam and double-beam instruments in terms of design, accuracy, and application.
2. Discuss the pharmaceutical applications of UV-Vis spectrophotometry in detail, including assay, identification, dissolution testing, content uniformity, and colorimetric assays. Give specific examples from the Indian Pharmacopoeia.
3. Explain the procedure for the UV spectrophotometric assay of Paracetamol tablets as per IP. Include sample preparation, assay conditions, calculation, and acceptance criterion.

Multiple Choice Questions

1. The radiation source used in the UV region (200–380 nm) of a spectrophotometer is:

(a) Tungsten halogen lamp
(b) Xenon lamp only
(c) Deuterium (D_2) lamp
(d) Mercury lamp

✓***Answer: (c) Deuterium (D_2) lamp***

2. For UV spectrophotometric measurements below 320 nm, the cuvette material must be:

(a) Polystyrene
(b) Borosilicate glass
(c) Fused silica (quartz)
(d) Perspex (acrylic)

✓***Answer: (c) Fused silica (quartz)***

3. The wavelength accuracy of a UV-Vis spectrophotometer per IP is verified using:

(a) Potassium dichromate solutions
(b) Holmium oxide glass filter or benzene vapour
(c) KI solution
(d) Starch-iodine complex

✓***Answer: (b) Holmium oxide glass filter or benzene vapour***

4. The λmax for paracetamol assay as per IP is:
(a) 243 nm in 0.1 M NaOH
(b) 254 nm in water
(c) 280 nm in HCl
(d) 340 nm in ethanol
✓***Answer: (a) 243 nm in 0.1 M NaOH***

5. In double-beam spectrophotometry, the reference cell beam compensates for:
(a) Sample fluorescence
(b) Concentration changes of analyte
(c) Fluctuations in source intensity and absorption by solvent/cuvette
(d) Stray light only
✓***Answer: (c) Fluctuations in source intensity and absorption by solvent/cuvette***

Chapter 23
Limit Tests and Pharmaceutical Quality Control

23.1 Learning Objectives

- Define limit test and distinguish it from a quantitative assay.
- Explain the principle of limit tests for chloride, sulfate, iron, arsenic, heavy metals, and ammonium.
- Describe the complete IP procedures for each limit test with reactions and calculations.
- Explain the concept of pharmaceutical quality control (QC) and its regulatory framework.
- Describe the role of analytical methods in each phase of pharmaceutical manufacturing.
- Discuss the analytical requirements for Good Manufacturing Practice (GMP) compliance.

23.2 Introduction to Limit Tests

A limit test is a semi-quantitative analytical procedure that determines whether the amount of a specific impurity in a pharmaceutical substance exceeds a specified, permissible maximum level. Unlike an assay — which gives an exact numerical value for the amount of a substance — a limit test gives a binary result: PASS (impurity is within the allowed limit) or FAIL (impurity exceeds the allowed limit).

Limit tests are among the most important pharmacopoeial tests applied to pharmaceutical raw materials and drug substances. They protect patient safety by ensuring that potentially toxic inorganic impurities — such as lead, arsenic, mercury, iron, chlorides, and sulfates — are present only at safe, negligible levels that cannot cause harm at therapeutic doses.

The principle of all chemical limit tests is the same: the test solution (prepared from the pharmaceutical sample) is compared with a standard solution prepared to contain exactly the permissible limit of the impurity. If the appearance (cloudiness, colour, or precipitate) of the test solution does not exceed that of the standard, the sample PASSES.

Fundamental Principle of Limit Tests

Test Solution: Prepared from a specified amount of pharmaceutical substance Standard Solution: Prepared to contain exactly the limit concentration of impurity Comparison: Test vs Standard (turbidity / colour / precipitate) If Test ≤ Standard → PASS (impurity within permissible limit) If Test > Standard → FAIL (impurity exceeds limit; reject batch) The method is semi-quantitative because it only determines whether the impurity is above or below the limit, not its exact amount.

23.3 Limit Test for Chloride (IP Method)

23.3.1 Principle

Chloride ions react with silver nitrate ($AgNO_3$) in acidic (dilute nitric acid) medium to form a white, opalescent precipitate of silver chloride (AgCl). The opalescence produced in the test solution is compared with that of a standard chloride solution (typically NaCl dissolved in water) treated identically.

Reaction

$Ag^+ + Cl^- \rightarrow AgCl$ (white opalescence/precipitate) Medium: Dilute HNO_3 (to prevent interference from phosphates, sulfates, carbonates which would also precipitate with $AgNO_3$ in neutral/basic conditions)

23.3.2 IP Procedure

1. Dissolve the specified amount of substance in the required solvent as directed in the individual monograph.
2. Add 10 mL of dilute nitric acid (to acidify; prevents interference from other anions).
3. Add 1 mL of silver nitrate solution ($AgNO_3$, 17.5% w/v or as specified).
4. Allow to stand for 5 minutes protected from light.
5. Compare opalescence in diffuse daylight: test solution vs standard solution containing the limit of Cl^-.

Standard Preparation and Calculation

Standard: A volume of standard chloride solution (NaCl in HNO_3) equivalent to the permissible limit of Cl^- Example: If the IP limit is 50 ppm Cl^- and 2.0 g of sample is taken: Permissible mass of Cl^- = 50 ppm × 2.0 g = 0.10 mg = 100 µg Cl^- Standard: 1.0 mL of 0.01% NaCl contains 60 µg Cl; adjust volume to contain 100 µg Cl^- Common IP Cl^- limits: Aspirin: NMT 100 ppm | Paracetamol: NMT 100 ppm | Glucose: NMT 100 ppm

23.3.3 Interferences and Precautions

- Phosphates, carbonates, and oxalates also precipitate with $AgNO_3$ — eliminated by acidifying with HNO_3.
- Do not use HCl to acidify (introduces Cl^- contaminant) or H_2SO_4 (introduces SO_4^{2-}).
- Comparison must be in identical cylinders (Nessler tubes) against a white background, in diffuse daylight.
- Turbid samples must be filtered before the test.

23.4 Limit Test for Sulfate (IP Method)

23.4.1 Principle

Sulfate ions react with barium chloride ($BaCl_2$) in acidic medium (dilute hydrochloric acid) to form a white, turbid precipitate of barium sulfate ($BaSO_4$). The turbidity is compared visually with a standard sulfate solution.

Reaction

$Ba^{2+} + SO_4^{2-} \rightarrow BaSO_4\downarrow$ (white turbidity) Medium: Dilute HCl (prevents interference from phosphates, carbonates) Key: $BaSO_4$ is sparingly soluble ($Ksp = 1.1 \times 10^{-10}$) → even small amounts of SO_4^{2-} produce detectable turbidity

2

3.4.2 IP Procedure

1. Dissolve the specified amount of substance in water.
2. Add 2 mL of dilute hydrochloric acid.
3. Add 2 mL of 25% $BaCl_2$ solution.
4. Stir and allow to stand for 10 minutes.
5. Compare turbidity with standard sulfate solution (K_2SO_4 standard) in Nessler tubes.

Common IP Sulfate Limits

Aspirin: NMT 0.06% (600 ppm) | Paracetamol: NMT 0.02% (200 ppm) Sodium Bicarbonate: NMT 0.015% | Glucose: NMT 200 ppm Calculation same principle as chloride: prepare standard to contain exactly the limit amount of SO_4^{2-}

23.5 Limit Test for Iron (IP Method)

23.5.1 Principle

Iron (Fe^{3+}) forms an intense blood-red complex with thiocyanate ions (SCN^-) in acidic medium. The colour intensity of the test solution is compared with a standard iron solution.

Reaction

$Fe^{3+} + SCN^- \rightarrow [Fe(SCN)]^{2+}$ (blood-red complex) Note: Fe^{2+} must be oxidised to Fe^{3+} first (with H_2O_2 or HNO_3) before the test. The complex $Fe(SCN)^{63-}$ gives maximum colour intensity.

23.5.2 IP Procedure

1. Dissolve the sample in dilute HCl. Add 2 mL of H_2O_2 (to oxidise Fe^{2+} to Fe^{3+}).
2. Add 3 mL of thioglycollic acid (alternative method — iron reacts to give red colour).
3. Alternatively: Add 3 mL of KSCN solution (10% w/v) and compare red colour.
4. Compare colour in matched Nessler tubes with iron standard.

Common IP Iron Limits
Aspirin: NMT 20 ppm | Glucose: NMT 5 ppm | Calcium Carbonate: NMT 200 ppm

23.6 Limit Test for Arsenic — Gutzeit Method (IP)

23.6.1 Principle

Arsenic (as As^{3+} or As^{5+}) is reduced to arsine gas (AsH_3) by zinc and hydrochloric acid. The AsH_3 reacts with mercuric chloride ($HgCl_2$) paper to produce a yellow-to-brown stain. The stain intensity is compared with that produced by a standard arsenic solution under identical conditions.

Reactions
Step 1 — Reduction: $As^{3+} + 3Zn + 6HCl \rightarrow AsH_3\uparrow + 3ZnCl_2 + H_2O$ As^{5+} is first reduced to As^{3+} by KI + Sn^{2+} before this step Step 2 — Detection: $AsH_3 + 3HgCl_2 \rightarrow As(HgCl)_3$ (yellow) + 3HCl Excess AsH_3: $As(HgCl)_3 + AsH_3 \rightarrow [AsHg_2Cl]_2$ (orange-brown) Endpoint: Compare stain on $HgCl_2$ paper with standard arsenic paper stain

23.6.2 Procedure

1. Place sample in Gutzeit apparatus flask with KI (10%), $SnCl_2$ in HCl, and granulated zinc.
2. AsH_3 gas generated passes through lead acetate cotton wool (removes H_2S interference) into $HgCl_2$ paper.
3. The stain produced is compared with the standard arsenic solution stain under identical conditions.

Common IP Arsenic Limits
Most pharmaceutical APIs: NMT 2 ppm (IP: NMT 2 ppm = NMT 2 µg/g) Alternative method: Silver diethyldithiocarbamate (SDDC) method — AsH_3 reacts with SDDC in pyridine to give red colour measured at 520 nm

23.7 Limit Test for Heavy Metals (IP Method)

23.7.1 Principle

Heavy metals (principally lead, but also Pb, Bi, Cu, Hg, Sb, Sn) react with hydrogen sulfide (H_2S) or sodium sulfide (Na_2S) in slightly acidic conditions (acetate buffer pH 3.5) to form dark brown or black sulfide precipitates. The colour produced is compared with a standard lead solution.

Reaction
$Pb^{2+} + H_2S \rightarrow PbS\downarrow$ (black-brown sulfide) + $2H^+$ All heavy metal sulfides (HgS, CuS, Bi_2S_3, SnS) are also dark and are detected collectively. Buffer: pH 3.5 acetate buffer (ensures H_2S is partially ionised; prevents FeS from forming)

23.7.2 IP Method A (for Most Substances)

1. Dissolve the sample in water; adjust pH to 3.5 with acetate buffer.
2. Add freshly prepared hydrogen sulfide water (or thioacetamide reagent).
3. Allow to stand for 2 minutes; compare brown colour with standard lead solution.

ICH Q3D Elemental Impurities — Updated Approach
ICH Q3D (2014) replaced the traditional heavy metals test with a risk-based, element-specific approach: • Oral daily exposure limits (PDEs) defined for 24 elements: Pb, Cd, Hg, As, Co, Ni, V, Cu, Mo, etc. • Analysis by ICP-MS or ICP-OES (inductively coupled plasma) — more sensitive and specific • Traditional heavy metals test (H_2S colour) is being phased out in USP/Ph.Eur. • IP still uses both traditional (limit test) and ICP methods depending on substance

Common Heavy Metal Limits (IP)
Most organic APIs: NMT 20 ppm (as Pb) | Calcium preparations: NMT 10 ppm Parenteral preparations: NMT 1 ppm | Ophthalmic preparations: NMT 5 ppm

23.8 Limit Test for Ammonium Ions

23.8.1 Principle

Ammonium ions (NH_4^+) react with Nessler's reagent (dipotassium tetraiodomercurate(II), K_2HgI_4 in KOH) to produce a yellow-to-brown coloured complex. The colour is compared with a standard ammonium solution.

Reaction
$NH_4^+ + 2K_2HgI_4 + 4KOH \rightarrow Hg_2NI{\cdot}H_2O\downarrow$ (brown ppt at high conc.) or yellow colour Nessler's Reagent: $K_2HgI_4 + 2KOH$ Precaution: The test must be performed in alkaline conditions (NaOH added first to liberate NH_3) Interference: Proteins, amines, and other nitrogen compounds may interfere — preliminary distillation may be needed

23.8.2 Procedure

1. Dissolve sample in water. Add 1 mL of potassium hydroxide (10% w/v).
2. Add 1 mL of Nessler's reagent.
3. Allow to stand for 5 minutes. Compare yellow/brown colour with standard (NH_4Cl solution).

Common IP Ammonium Limits
Sodium Chloride: NMT 2 ppm | Magnesium Sulfate: NMT 20 ppm | Potassium Chloride: NMT 10 ppm

23.9 Pharmaceutical Quality Control — Overview

Quality control (QC) in the pharmaceutical industry is the totality of measures taken to ensure that pharmaceutical products consistently meet defined specifications of safety, efficacy, purity, identity, and quality. It encompasses all analytical, microbiological, physical, and chemical tests performed throughout the product life cycle.

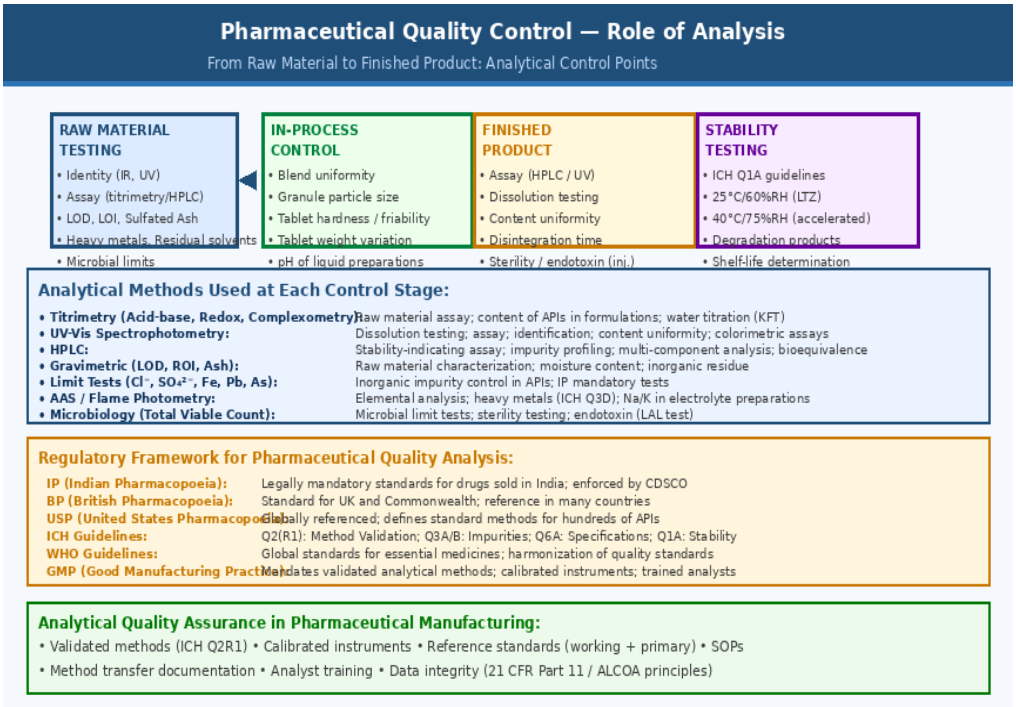


Figure: Pharmaceutical QC — Analytical Control Points from Raw Material to Finished Product

23.9.1 Phases of Pharmaceutical Quality Control

Phase	Analytical Tests	Responsible Body	Regulatory Basis
Raw Material Release	Identity (IR, UV), Assay, LOD, Ash, Heavy metals, Residual solvents	QC Laboratory	IP/BP/USP monographs + GMP
In-Process Control	Blend uniformity, weight variation, hardness, friability, pH	Production QC	IP/GMP in-process specifications
Finished Product Release	Assay, dissolution, content uniformity, disintegration, sterility	QA + QC Lab	IP/BP/USP finished product specs
Stability Testing	Degradation products, potency, dissolution, physical appearance	QC Stability Lab	ICH Q1A–Q1F guidelines
Market Surveillance	Periodic random testing of market samples	CDSCO / State Drug Authorities	Drugs and Cosmetics Act

23.9.2 GMP Requirements for Analytical Laboratories

Good Manufacturing Practice (GMP) mandates a rigorous analytical framework for pharmaceutical manufacturing. Key GMP requirements for QC laboratories include:

- Validated analytical methods: All test methods must be validated per ICH Q2(R1) before use for product release.
- Calibrated, qualified instruments: Spectrophotometers, HPLC systems, balances, pH meters must be calibrated and qualified (IQ, OQ, PQ).
- Reference standards: Working standards must be traceable to primary pharmacopoeial standards (IP Reference Substances, USP RS, BP CRS).
- Standard Operating Procedures (SOPs): All analytical procedures must be documented in validated SOPs.
- Data Integrity: Raw analytical data must be attributable, legible, contemporaneous, original, and accurate (ALCOA+ principles). Electronic records per 21 CFR Part 11.
- Out-of-Specification (OOS) investigations: Any result outside the specification must be investigated per SOP; results cannot be discarded without documented investigation.
- Analyst training and qualification: All analysts must be trained, competency-tested, and regularly updated.

23.10 Summary

Chapter 23 Summary

Limit tests are semi-quantitative tests comparing test solution vs standard for specific inorganic impurities. Key tests: Chloride ($AgNO_3/HNO_3$, white opalescence), Sulfate ($BaCl_2$/HCl, white turbidity), Iron ($KSCN/H_2O_2$, red complex), Arsenic (Gutzeit method, $AsH_3/HgCl_2$ paper stain), Heavy Metals (H_2S/acetate buffer, brown sulfides), Ammonium (Nessler's reagent, yellow colour). All compare with standard at the limit concentration. Pharmaceutical QC encompasses raw material, in-process, and finished product testing within a GMP framework governed by IP, ICH guidelines, and the Drugs & Cosmetics Act.

Review Questions

Short Answer Questions

1. What is the purpose of using dilute HNO_3 in the limit test for chloride?
2. Describe the principle of the Gutzeit test for arsenic.
3. What is the principle of the heavy metals limit test? Why is acetate buffer (pH 3.5) used?
4. Explain the Nessler's reagent test for ammonium ions.
5. What is ALCOA in the context of GMP data integrity?

Long Answer Questions

1. Describe the IP limit test for sulfate in detail: principle, reagents, procedure, precautions, and calculation for an aspirin sample (IP limit: 200 ppm SO_4^{2-}).
2. Write a comprehensive note on pharmaceutical quality control. Include the phases of QC (raw material, in-process, finished product, stability), the analytical methods used at each stage, and the GMP requirements for analytical laboratories.

Multiple Choice Questions

1. In the limit test for chloride, dilute HNO_3 is used to:

(a) Dissolve the sample

(b) Prevent interference from phosphates, sulfates, and carbonates which precipitate with $AgNO_3$

(c) Oxidise chloride to chlorine gas

(d) Stabilise the AgCl precipitate

✓ ***Answer: (b) Prevent interference from phosphates, sulfates, and carbonates which precipitate with $AgNO_3$***

2. In the Gutzeit method for arsenic, arsine (AsH_3) is detected by:
(a) Silver nitrate solution
(b) Starch indicator
(c) Mercuric chloride ($HgCl_2$) paper (yellow-brown stain)
(d) Nessler's reagent
✓Answer: (c) Mercuric chloride ($HgCl_2$) paper (yellow-brown stain)

3. The heavy metals limit test uses which buffer system to control pH at 3.5?
(a) Phosphate buffer
(b) Acetate buffer (acetic acid + sodium acetate)
(c) Citrate buffer
(d) Borate buffer
✓Answer: (b) Acetate buffer (acetic acid + sodium acetate)

4. The reagent used in the limit test for ammonium ions is:
(a) Fehling's solution
(b) Nessler's reagent (K_2HgI_4/KOH)
(c) KSCN solution
(d) $AgNO_3$ solution
✓Answer: (b) Nessler's reagent (K_2HgI_4/KOH)

5. ICH Q3D guidelines deal with:
(a) Impurities in new drug substances
(b) Elemental impurities in pharmaceutical products (PDE limits)
(c) Method validation
(d) Stability testing protocols
✓Answer: (b) Elemental impurities in pharmaceutical products (PDE limits)

Chapter 24
Non-Aqueous Titrations and Analytical Method Validation

24.1 Learning Objectives

- Explain the theoretical basis for non-aqueous titrations and the role of solvent in enhancing ionisation.
- Describe the preparation, standardization, and use of perchloric acid titrant in glacial acetic acid.
- Detail the pharmacopoeial procedure for non-aqueous titration of weak bases.
- Define analytical method validation and state the regulatory requirements (ICH Q2(R1)).
- Explain each validation parameter: specificity, linearity, accuracy, precision, LOD, LOQ, range, and robustness.
- Design a method validation protocol for a UV spectrophotometric assay.
- Describe the concept of system suitability testing (SST) in chromatographic methods.

24.2 Non-Aqueous Titrations — Deep Theory

24.2.1 Theoretical Background

Most drug substances that contain basic functional groups (amines, alkaloids, antihistamines, antipsychotics) are classified as weak bases. In aqueous solution, these substances are too weakly basic to be titrated with a strong acid (HCl) to give a sharp, detectable endpoint. The equilibrium constant for their protonation in water is simply too small to achieve complete reaction at the equivalence point.

Non-aqueous titrations overcome this limitation by exploiting the properties of organic solvents. The solvent glacial acetic acid (AcOH) is a protogenic (proton-donating) and levelling solvent for bases — it behaves as a stronger acid than water, so it protonates weak bases much more effectively. The result is a greatly enhanced basicity of the drug substance relative to its behaviour in water, giving a dramatically steeper pH jump at the equivalence point and a clear, sharp endpoint.

Why Glacial Acetic Acid Enhances Basicity

In water: $B + H_2O \rightleftharpoons BH^+ + OH^-$ (K very small for weak base — poor endpoint) In AcOH: $B + AcOH \rightarrow BH^+ + AcO^-$ (K much larger — good endpoint) Reason: AcOH is a stronger proton donor than H_2O → protonates weak bases completely AcOH also suppresses the self-ionisation of $HClO_4$ → perchloric acid acts as a stronger acid in AcOH than in water Net effect: The acid-base difference between $HClO_4$ and the drug base is magnified in AcOH → sharp, quantitative reaction

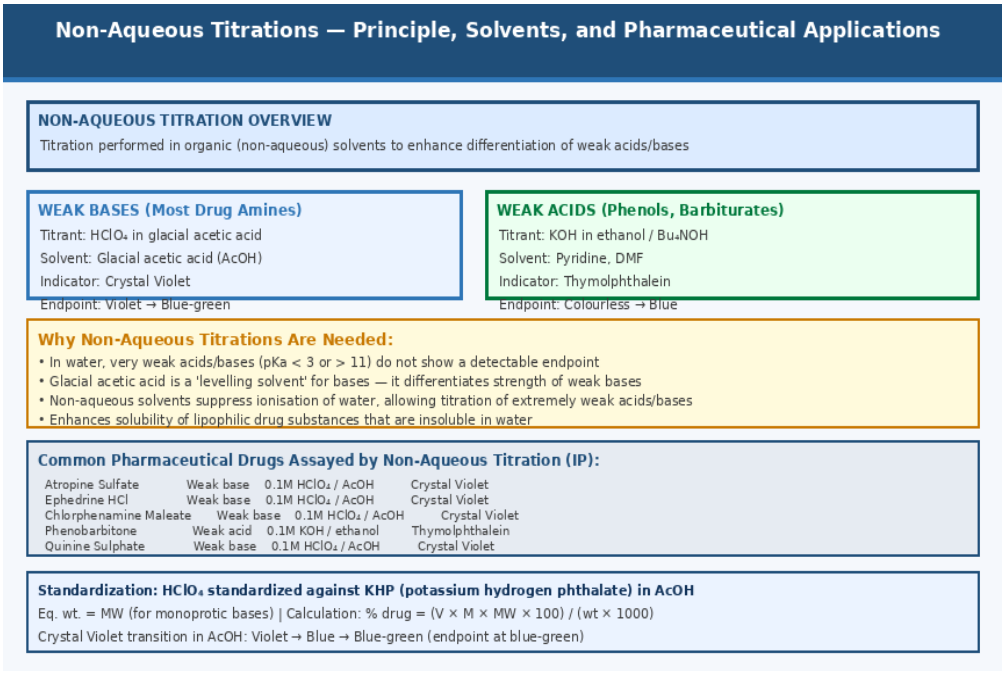


Figure: Non-Aqueous Titrations — Principle, Solvents, and Drug Applications

24.2.2 Choice of Titrant: Perchloric Acid ($HClO_4$)

Perchloric acid ($HClO_4$) in glacial acetic acid is the standard titrant for non-aqueous titration of bases. It is chosen because:

- Perchloric acid is the strongest common acid — it is a non-oxidising, non-reducing acid that reacts completely and quantitatively with weak bases even in non-aqueous media.
- The perchlorates of drug bases ($BH^+ClO_4^-$) are soluble in glacial acetic acid — preventing precipitation during the titration.
- $HClO_4$ solutions in AcOH are stable and can be standardized accurately.
- The 0.1 M $HClO_4$ titrant is prepared by diluting 70% $HClO_4$ with glacial acetic acid, with the addition of a calculated amount of acetic anhydride to convert any water present to acetic acid (water reduces the titration sensitivity).

Preparation of 0.1 M $HClO_4$ in Glacial Acetic Acid

Add 8.5 mL of 70% $HClO_4$ slowly to 500 mL of glacial acetic acid. Add the calculated volume of acetic anhydride to neutralise water: Volume Ac_2O = (% water in $HClO_4$ × density × volume) / (MW × 2) Mix; dilute to 1 litre with glacial acetic acid. Allow to stand 24 hours; standardize against KHP. Caution: $HClO_4$ + organic solvents at elevated temperature → EXPLOSION RISK Never heat concentrated $HClO_4$ with organic material; keep equipment clean.

24.2.3 Standardization of $HClO_4$ — Against Potassium Hydrogen Phthalate (KHP)

KHP ($C_8H_5KO_4$, MW 204.22) is used as the primary standard for standardizing $HClO_4$ in glacial acetic acid. KHP is treated as a monobasic acid (acting as a base in AcOH medium — the phthalate anion accepts a proton from $HClO_4$):

Standardization Reaction

KHP + $HClO_4$ → K^+ + ClO_4^- + H-Phthalic acid (in AcOH) Procedure: 1. Dry KHP at 120°C for 2 hours; cool in desiccator 2. Accurately weigh ~200 mg into a conical flask 3. Dissolve in 20 mL glacial acetic acid with gentle warming 4. Cool; add 2 drops of 0.1% crystal violet indicator 5. Titrate with $HClO_4$ until colour changes from violet to blue-green 6. Calculate: M_HClO_4 = (Wt KHP) / (V_HClO_4 × 204.22 / 1000)

24.2.4 Endpoint Detection in Non-Aqueous Titrations

Indicator/Method	Colour Change in AcOH	Notes
Crystal Violet	Violet → Blue → Blue-green	Most widely used; endpoint at blue-green
Malachite Green	Blue-green → Yellow	For very weak bases
Methyl Violet	Violet → Blue → Green-blue	Similar to crystal violet
Quinaldine Red	Red → Colourless	For strongly basic compounds
Potentiometric	Inflection point in mV-volume plot	Most accurate; no colour interference; IP referee method
Oracet Blue	Blue → Pink	Used for very weak bases in anhydrous conditions

24.2.5 Calculation for Non-Aqueous Titration

General Calculation

For a monobasic drug substance (1 mol drug reacts with 1 mol $HClO_4$): % Drug = (V × M × MW × 100) / (Sample weight in mg × 1000) where V = volume of $HClO_4$ (mL); M = molarity; MW = molecular weight of drug Example: 0.2500 g Atropine Sulfate titrated with 0.1 M $HClO_4$; V = 12.45 mL; MW Atropine Sulfate = 694.83 Note: Atropine Sulfate = $(Atropine)_2 \cdot H_2SO_4$; 2 basic nitrogen atoms → 2 moles $HClO_4$ per mole salt % = (12.45 × 0.1 × 694.83/2 × 100) / (250.0) = 99.26% [Divide MW by 2 because 2 mol $HClO_4$ per mol salt]

24.2.6 Pharmacopoeial Applications of Non-Aqueous Titrations

Drug(IP Monograph)	Molecular Formula	MW	Titrant	Indicator	Factor (mg/mL $HClO_4$)
Atropine Sulfate	$(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$	694.83	0.1M $HClO_4$	Crystal Violet	34.74 (each mL 0.1M)
Ephedrine Hydrochloride	$C_{10}H_{15}NO \cdot HCl$	201.70	0.1M $HClO_4$	Crystal Violet	20.17
Chlorphenamine Maleate	$C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$	390.86	0.1M $HClO_4$	Crystal Violet	39.09
Quinine Sulphate	$(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4$	782.93	0.05M $HClO_4$	Crystal Violet	19.57
Codeine Phosphate	$C_{18}H_{21}NO_3 \cdot H_3PO_4$	397.37	0.1M $HClO_4$	Crystal Violet	39.74
Phenobarbitone	$C_{12}H_{12}N_2O_3$	232.23	0.1M KOH/ethanol	Thymolphthalein	23.22
Promethazine HCl	$C_{17}H_{20}N_2S \cdot Hcl$	320.88	0.1M $HClO_4$	Crystal Violet	32.09

24.3 Analytical Method Validation — ICH Q2(R1)

24.3.1 Introduction to Method Validation

Analytical method validation is the process of demonstrating, through systematic experimental studies, that an analytical procedure is suitable for its intended purpose. Regulatory authorities (CDSCO, FDA, EMA) require that all analytical methods used for drug registration, quality control, and pharmaceutical manufacturing be fully validated. The primary international guideline governing analytical method validation is ICH Q2(R1) — 'Validation of Analytical Procedures: Text and Methodology.' This guideline defines the validation characteristics that must be evaluated for different types of analytical procedures.

Types of Analytical Procedures and Validation Requirements per ICH Q2(R1)
1. Identification tests: Specificity only 2. Tests for impurities (limit tests): Specificity + LOD 3. Tests for impurities (quantitative): Specificity + Linearity + Accuracy +

Precision + LOD + LOQ + Range 4. Assay (content/potency): Specificity + Linearity + Accuracy + Precision + Range (+ Robustness)

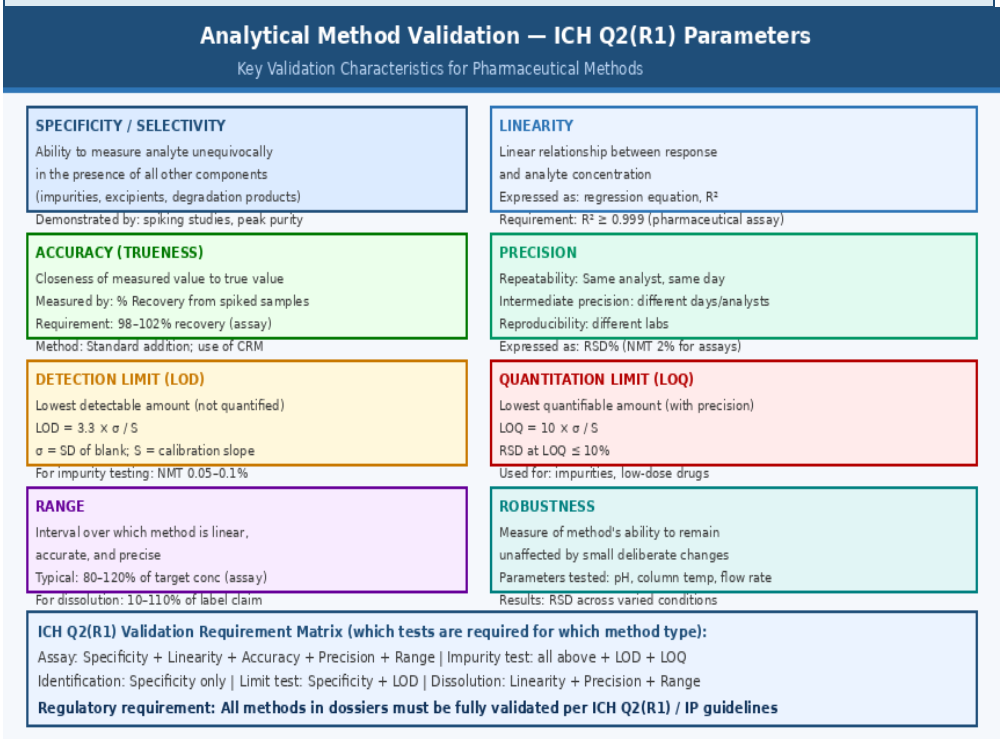


Figure: ICH Q2(R1) Analytical Method Validation Parameters — All 8 Characteristics

24.3.2 Specificity / Selectivity

Specificity is the ability of an analytical method to measure the analyte unambiguously in the presence of all other components that are likely to be present in the sample — excipients, impurities, degradation products, process residuals, and matrix components. Specificity is demonstrated by:

- Spiking studies: Adding known amounts of potential interfering substances to the sample and verifying that the response is not affected.
- Forced degradation (stress testing): Subjecting the drug to acid/base hydrolysis, oxidation, UV light, heat, and humidity; demonstrating that the method detects degradation products separately from the parent drug (stability-indicating capability).
- Peak purity (HPLC with PDA detector): Confirming that the peak at the analyte retention time is spectrally pure (not co-eluting with an impurity).

24.3.3 Linearity

Linearity is the ability of the method to give test results that are directly proportional to the concentration (or amount) of the analyte over a specified range.

Linearity is established by:

- Preparing a minimum of 5 concentrations spanning the intended range.
- Plotting the response (e.g., absorbance, peak area) vs concentration.
- Performing linear regression: $y = mx + b$. The correlation coefficient (R^2) should be ≥ 0.999 for assay methods.
- Evaluating residuals — the differences between observed and predicted values.

Acceptance Criteria for Linearity

Assay (100% of target): Range typically 80–120% $R^2 \geq 0.999$; y-intercept ≈ 0 (or negligible); slope consistent with expected sensitivity Impurity testing: 50–150% of limit; or LOQ to 120% of limit Disso: 10–110% of label claim

24.3.4 Accuracy

Accuracy expresses the closeness of the measured value to the true (accepted reference) value. For pharmaceutical methods, accuracy is typically assessed using:

- Standard Addition Method: Known amounts of standard (three different levels, typically 80%, 100%, 120% of target concentration) are spiked into the matrix (pre-assayed blank formulation); the recovery is measured.
- Comparison with Reference Method: Results compared with those of an established, validated method.
- Use of Certified Reference Material (CRM): Sample assayed against a material of certified composition.

Acceptance Criteria for Accuracy

Assay methods: % Recovery = 98.0–102.0% at each concentration level Impurity methods: % Recovery = 90.0–108.0% (or 80–110% for trace impurities) Calculation: % Recovery = (Amount found / Amount added) × 100

24.3.5 Precision

Precision expresses the degree of agreement among a series of measurements obtained under prescribed conditions. Three levels of precision are distinguished:

Type of Precision	Conditions	Expression	Typical Acceptance
Repeatability (intra-assay)	Same analyst, same equipment, same day, minimum 6 replicates at 100% level	RSD (%)	≤ 2.0% RSD for assay
Intermediate Precision	Different analysts and/or different days within same laboratory	RSD (%)	≤ 3.0% RSD
Reproducibility	Different laboratories (collaborative study)	RSD (%)	Depends on method; typically ≤ 5%

24.3.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is the smallest amount of analyte in a sample that can be detected but not necessarily quantified. LOQ is the smallest amount that can be quantitatively determined with acceptable precision and accuracy.

ICH Q2(R1) Formulas

Based on standard deviation of response (σ) and slope of calibration curve (S): LOD = 3.3σ / S LOQ = 10σ / S Alternative approaches: Signal-to-noise ratio: LOD = S/N ≥ 3:1; LOQ = S/N ≥ 10:1 Based on residual standard deviation of regression line Acceptance criteria: LOQ: RSD ≤ 10% at LOQ concentration; % Recovery 80–120% LOD: Visual detection confirmed at stated concentration

24.3.7 Range

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which the procedure is linear, accurate, and precise. Range is derived from linearity studies and encompasses the intended working range.

Procedure Type	Minimum Range
Assay of drug substance or formulation	80–120% of the test concentration
Content uniformity	70–130% of test concentration
Dissolution testing	± 20% over specified limits (e.g., 10–110% of label)
Impurity testing	Reporting threshold to 120% of specification limit
Potency (biological)	50–200% of test concentration

24.3.8 Robustness

Robustness is a measure of the method's capacity to remain unaffected by small, deliberate variations in method parameters. It provides an indication of the method's reliability during normal usage. Robustness evaluation should be considered during method development.

Parameters typically evaluated for chromatographic methods:

- Mobile phase composition (± 5% variation in organic modifier)
- pH of mobile phase (± 0.1 unit)
- Column temperature (± 5°C)
- Flow rate (± 0.1 mL/min)
- Wavelength (± 2 nm for UV detection)
- Column lot-to-lot and brand variation

24.3.9 System Suitability Testing (SST)

System suitability testing is performed prior to running a series of samples to verify that the analytical system (instrument + column + reagents) is performing within established parameters on the day of analysis. SST is particularly important for HPLC methods.

SST Parameter	Requirement	Calculation
Theoretical plates (N)	N ≥ 2000 (typical; per method specification)	$N = 16(tR/W)^2$ or $N = 5.545(tR/W½)^2$
Resolution (Rs)	Rs ≥ 2.0 between critical peak pairs	$Rs = 2(tR_2 - tR_1)/(W_1+W_2)$
Tailing factor (T)	0.8 ≤ T ≤ 1.5 (USP); T ≤ 2.0 (EP)	T = (a+b) / 2a (a = leading half-width)
RSD of replicate injections	RSD ≤ 1.0% for 5–6 injections	Standard injections of reference standard
Capacity factor (k')	k' > 2 (peak not too close to void)	$k' = (tR - t_0) / t_0$

24.4 Method Transfer and Revalidation

When an analytical method is transferred from a development laboratory to a QC laboratory (or between manufacturing sites), a method transfer study must be performed to demonstrate that the receiving laboratory can successfully run the method and reproduce results consistent with those at the originating laboratory.

- Comparative testing: Both sites analyze the same samples; results are statistically compared using t-test and F-test.
- Revalidation triggers: A validated method requires full or partial revalidation when: the drug substance changes (new supplier, new synthesis route), when the dosage form changes, when equipment changes significantly, or when the method is transferred to a different analytical technology.
- ICH Q2(R1) Section 5 specifies which validation parameters need to be re-evaluated based on the nature of the change.

24.5 Summary

Chapter 24 Summary

Non-aqueous titrations use $HClO_4$ in glacial acetic acid as titrant for weak bases (alkaloids, antihistamines, amines). Glacial acetic acid enhances basicity → sharper endpoint. Crystal Violet indicator; standardize $HClO_4$ against KHP. Analytical method validation (ICH Q2(R1)) encompasses 8 parameters: Specificity, Linearity ($R^2 \geq 0.999$), Accuracy (98–102% recovery), Precision (RSD≤2%), LOD ($3.3\sigma/S$), LOQ ($10\sigma/S$), Range (80–120% for assay), Robustness. System suitability testing (SST) ensures HPLC system performance before each run: N, Rs, tailing factor, RSD.

Review Questions

Short Answer Questions

1. Why is glacial acetic acid used as the solvent in non-aqueous titrations of weak bases?
2. How is $HClO_4$ standardized for non-aqueous titration? Write the reaction with KHP.
3. Define specificity in the context of analytical method validation. How is it demonstrated for a stability-indicating HPLC method?
4. What is system suitability testing? List four SST parameters for HPLC.
5. Distinguish between LOD and LOQ with ICH formulas.

Long Answer Questions

1. Describe the non-aqueous titration of ephedrine hydrochloride as per IP. Include the principle, reagents, indicator, endpoint, and calculation. [MW Ephedrine HCl = 201.70]
2. Design a complete method validation protocol for a UV spectrophotometric assay of paracetamol tablets at 243 nm. State the validation parameters, experimental design, and acceptance criteria for each parameter.

Multiple Choice Questions

1. In non-aqueous titration, glacial acetic acid enhances the basicity of weak drugs because:

(a) It is a stronger proton donor than water, protonating weak bases more completely
(b) It dissolves all drugs
(c) It acts as a reducing agent
(d) It increases the molecular weight of the drug

✓Answer: (a) It is a stronger proton donor than water, protonating weak bases more completely

2. $HClO_4$ (perchloric acid) is used in non-aqueous titrations because:

(a) It is a mild oxidising acid
(b) It is the strongest common acid; non-oxidising; drug perchlorates are soluble in AcOH
(c) It changes colour at endpoint
(d) It is a primary standard

✓Answer: (b) It is the strongest common acid; non-oxidising; drug perchlorates are soluble in AcOH

3. For an assay method, the ICH Q2(R1) acceptable % recovery range is:
(a) 90–110%
(b) 98–102%
(c) 80–120%
(d) 95–105%
✓***Answer: (b) 98–102%***

4. System suitability parameter 'tailing factor' for HPLC should be:
(a) > 2.0
(b) 0.8 – 1.5 (USP)
(c) Equal to zero
(d) > 5.0
✓***Answer: (b) 0.8 – 1.5 (USP)***

5. According to ICH Q2(R1), LOQ is defined as:
(a) 3.3σ/S
(b) 10σ/S
(c) σ/S
(d) S/10σ
✓***Answer: (b) 10σ/S***

Chapter 25
Introduction to Chromatography, Flame Photometry, and Atomic Absorption Spectroscopy

25.1 Learning Objectives

- Explain the fundamental principles of chromatographic separation.
- Classify chromatographic methods and describe the basis of separation in each.
- Describe the principles, instrumentation, and pharmaceutical applications of TLC and HPLC.
- Explain the principle of flame photometry and its pharmaceutical applications.
- Describe the principle of atomic absorption spectroscopy (AAS) and compare it with flame photometry.
- Apply chromatographic and elemental analysis methods to pharmaceutical quality control.

25.2 Introduction to Chromatographic Methods

Chromatography is a powerful set of physical separation techniques based on the differential distribution of analytes between a stationary phase and a mobile phase. The word 'chromatography' literally means 'colour writing' — coined by the Russian botanist Mikhail Tswett who separated plant pigments on a chalk column in 1903. Modern chromatographic methods have evolved dramatically from this simple beginning and now represent the most important group of analytical separation techniques used in pharmaceutical science.

Every chromatographic method exploits the same fundamental principle: components of a mixture interact differently with the stationary phase (some are retained more strongly, some weakly). The mobile phase carries the mixture through the stationary phase. Components that interact more weakly with the stationary phase move faster; those that interact more strongly move slower. With sufficient time (or column length), the components are resolved into separate bands that emerge at different times (retention times) or positions.

25.3 Thin-Layer Chromatography (TLC)

25.3.1 Principle

TLC is a form of planar chromatography in which the stationary phase is a thin layer of adsorbent (typically silica gel, alumina, or cellulose) coated on an inert support (glass, aluminium, or plastic sheet). The mobile phase (developing solvent) migrates up the plate by capillary action, carrying the sample components with it. Separation is based on adsorption (normal phase) or partition.

Rf Value — Fundamental TLC Parameter
Rf = Distance travelled by analyte spot / Distance travelled by solvent front Rf ranges from 0 (no migration) to 1 (migrates with solvent front) Optimal Rf: 0.3 – 0.5 (centred on plate; best resolution) Rf is characteristic of a compound under defined conditions (stationary phase, mobile phase, temperature) Comparison method: Co-spot the sample with reference standard on the same plate; if Rf values are identical, the compound is tentatively identified.

25.3.2 TLC Procedure

1. Activate TLC plate at 110°C for 30 minutes before use (silica gel plates).
2. Apply sample and reference solutions as small spots (2–5 mm diameter) using a capillary spotter, 1.5 cm from the bottom edge.
3. Place the plate in the developing tank pre-saturated with the mobile phase vapour; ensure mobile phase level is below the spots.
4. Allow the solvent to develop until the front reaches 15 cm (or as specified). Remove and mark the solvent front immediately.
5. Dry the plate; visualise spots using: UV lamp (254 nm for UV-absorbing compounds), iodine vapour, ninhydrin spray (amino acids), or specific spray reagents.
6. Calculate Rf values; compare with reference standards.

25.3.3 Pharmaceutical Applications of TLC

Application	Procedure	Example
Identification of drug substances	Compare Rf of sample with reference standard in same system	Identity test for paracetamol IP: silica gel, hexane-acetone system
Purity testing / related substances	Apply higher concentration of sample; detect impurity spots below specification	Related substances of aspirin (IP): silica gel G, toluene-glacial AcOH system
Assay of herbal drugs	TLC fingerprinting; densitometric scanning of spots	Standardization of Senna, Rauwolfia, Belladonna extracts
Confirmation of dosage form homogeneity	Multiple tablets extracted and compared by TLC	Batch-to-batch consistency in traditional formulations

25.4 High-Performance Liquid Chromatography (HPLC)

25.4.1 Principle

HPLC is a powerful column chromatographic technique in which the mobile phase (a liquid) is pumped at high pressure through a column packed with very fine particles (3–5 µm) of stationary phase material. The high pressure allows rapid flow through these fine-particle columns, dramatically improving resolution and speed compared to classical column chromatography.

The most widely used HPLC mode in pharmaceutical analysis is Reversed-Phase HPLC (RP-HPLC):

- Stationary phase: Non-polar (C18 — octadecylsilane, ODS; or C8 — octylsilane) bonded to silica particles.
- Mobile phase: Polar — water + organic modifier (methanol, acetonitrile) + buffer (to control pH).
- Separation basis: Hydrophobic interaction — more non-polar compounds are retained longer on the C18 phase; more polar compounds elute first.
- Gradient elution: Mobile phase composition is changed progressively (increasing organic modifier) during the run to elute a wide range of polarities.

25.4.2 HPLC Instrumentation

Component	Function	Details
Solvent reservoir	Contains mobile phase	Degassed with helium or vacuum; HPLC-grade solvents
High-pressure pump	Delivers mobile phase at controlled flow rate	Reciprocating piston; pressure up to 400 bar (isocratic or gradient)
Injector (autosampler)	Introduces sample into mobile phase stream	6-port valve; loop injection; autosampler for high throughput
Column	Separation of analytes	C18/C8 ODS column, 15–25 cm × 4.6 mm, 5 µm particle size; thermostatted
UV Detector (DAD)	Detects separated analytes	Diode-array detector: scans 190–800 nm simultaneously; peak purity
Data system	Records and processes data	Chromatograms; peak area; retention time; calculations; reports

25.4.3 Key Chromatographic Parameters

Important HPLC Parameters

Retention time (tR): Time from injection to peak maximum Capacity factor (k'): $k' = (tR - t_0)/t_0$ — should be 2–10 Theoretical plates (N): $N = 16(tR/w)^2$ — measure of column efficiency; N > 2000 typical Resolution (Rs): $Rs = 2(tR2-tR1)/(w1+w2)$ — should be ≥ 1.5 for baseline resolution; ≥ 2.0 for critical pairs Tailing factor (T): $T = (a+b)/2a$ — should be 0.8–1.5 (USP) Relative retention (α): $\alpha = k'2/k'1$ — selectivity factor; should be > 1.2 Plate height (H): H = L/N — smaller H = more efficient column

25.4.4 Pharmaceutical Applications of HPLC

Application	Method Type	Examples
Assay of drug substance	RP-HPLC, UV detection	Paracetamol (243nm), Amoxicillin (272nm), Atenolol (224nm)
Related substances / Impurity profiling	Stability-indicating gradient HPLC	ICH Q3A/B compliant impurity methods for all APIs
Content uniformity	RP-HPLC	Individual tablet assay; RSD ≤ 6% (IP)
Dissolution testing	HPLC or UV of dissolution samples	Time-point sampling; in-line UV/HPLC monitoring
Bioequivalence studies	LC-MS/MS (plasma samples)	Cmax, Tmax, AUC determination from plasma drug levels
Residual solvent analysis	Headspace GC	ICH Q3C residual solvents: Class 1, 2, 3 solvents in APIs
Enantiomeric purity	Chiral HPLC (cellulose columns)	Chiral drugs: ibuprofen (R/S ratio), clopidogrel
Natural product standardization	HPLC fingerprinting	Curcumin in turmeric; berberine in goldenseal; withanolides in ashwagandha

25.5 Flame Photometry

25.5.1 Principle

Flame photometry (also called Flame Emission Spectrometry, FES) is an analytical technique based on the emission of radiation by atoms in an excited state. When a

solution containing metal ions is aspirated into a high-temperature flame (air-propane or air-acetylene), the metal atoms are excited to higher energy levels. When these excited atoms return to the ground state, they emit radiation at characteristic wavelengths specific to each element. The intensity of this emitted radiation is measured and is proportional to the concentration of the metal in the sample.

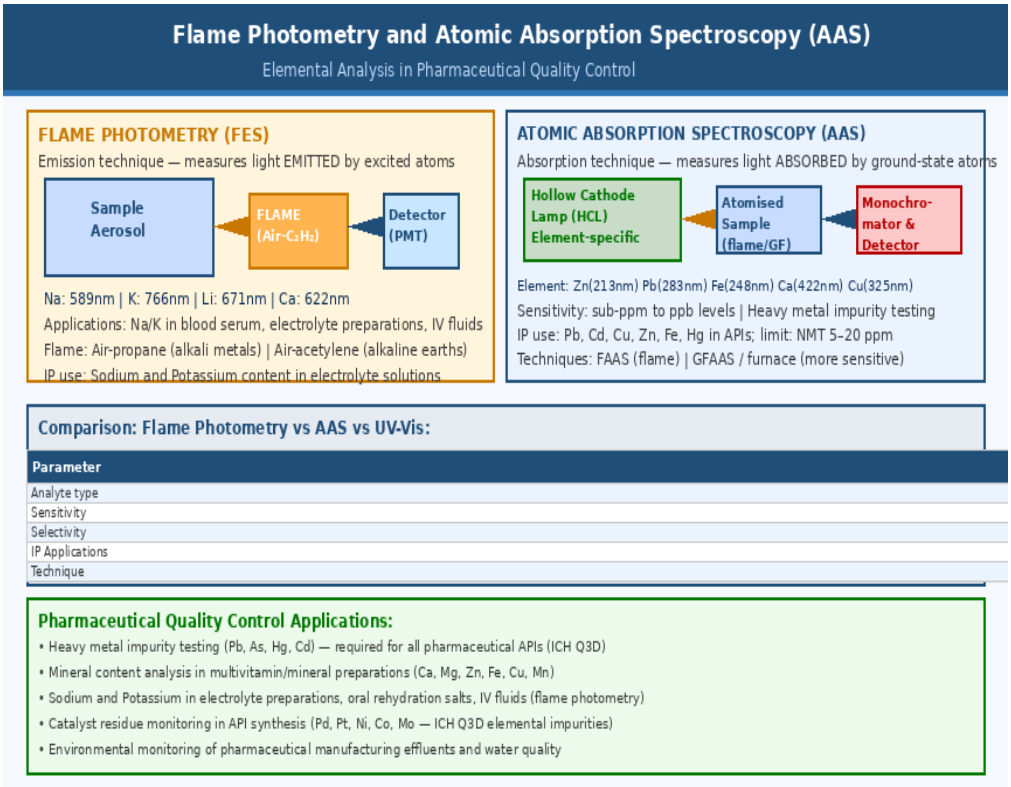


Figure: Flame Photometry and AAS — Principle, Instrumentation, and Pharmaceutical Applications

Principle of Flame Photometry

$M^+(aq) \rightarrow M^0$(excited state) [in flame] $\rightarrow M^0$(ground state) + hν (characteristic emission) Emission wavelengths: Sodium (Na): 589 nm (intense yellow D-line) Potassium (K): 766 nm (violet-red) Lithium (Li): 671 nm (crimson red) Calcium (Ca): 622 nm (orange-red) Barium (Ba): 554 nm (green) Intensity ∝ Concentration (Beer's Law analogy for emission)

25.5.2 Instrumentation

- Nebuliser: Converts liquid sample into a fine aerosol (mist) for introduction into the flame.
- Burner: Air-propane (for alkali metals — lower temperature) or air-acetylene (for alkaline earth metals — higher temperature). The flame atomises and excites the metal atoms.

- Optical filter or monochromator: Isolates the emission line of the element of interest from background radiation.
- Detector (PMT): Measures the intensity of emitted radiation.
- Readout: Concentration displayed directly after calibration with standards.

25.5.3 Interferences in Flame Photometry

Interference Type	Cause	Remedy
Spectral interference	Emission lines of two elements overlap	Use narrower spectral bandpass filter; wavelength selection
Ionisation interference	At high flame temperatures, atoms ionise (lose electron) → reduced emission	Add ionisation suppressor (e.g., large excess of KCl for Na analysis)
Chemical interference	Formation of stable compounds in flame (e.g., phosphates reduce Ca emission)	Add releasing agent (La^{3+} or Sr^{2+} preferentially binds phosphate)
Matrix effects	Complex sample matrix alters nebulisation efficiency	Matrix matching: standards prepared in same matrix as samples

25.5.4 Pharmaceutical Applications of Flame Photometry

- Determination of Sodium and Potassium in oral rehydration salts (ORS), electrolyte preparations, and intravenous fluids — the most important pharmaceutical application.
- Assay of Lithium Carbonate tablets (Li used in bipolar disorder therapy) — Li emission at 671 nm.
- Sodium content in antacid preparations (sodium bicarbonate, sodium alginate).
- Potassium content in potassium chloride tablets, potassium citrate solutions.
- Calcium and Barium determinations in pharmaceutical preparations (less sensitive than AAS).

Flame Photometric Assay of Sodium in ORS — Example
Preparation: Dissolve ORS sachet in 1 L water as directed. Dilute appropriately (1:100 or as needed). Calibration: Prepare Na standard solutions (5, 10, 20, 40, 60 ppm NaCl). Measure emission intensity of standards at 589 nm; plot calibration curve. Measure sample emission; read Na concentration from curve. IP Specification for ORS: Na = 75 mEq/L (= 1725 mg/L = 1.725 g/L) Result: c_Na (mg/L) × dilution factor × volume = total Na in preparation

25.6 Atomic Absorption Spectroscopy (AAS)

25.6.1 Principle

Atomic Absorption Spectroscopy (AAS) is based on the absorption of radiation by ground-state atoms in the gaseous phase. Unlike flame photometry (emission), AAS measures how much light is absorbed by ground-state atoms. A hollow cathode lamp (HCL) emitting the characteristic spectral lines of the element being determined is used as the radiation source. The sample solution is aspirated into a flame (FAAS) or into a heated graphite furnace (GFAAS), atomising the element. Ground-state atoms in the vapour phase absorb radiation from the HCL at the characteristic wavelength.

AAS Principle
Source: Hollow Cathode Lamp (HCL) — element-specific lamp Atomisation: Flame (FAAS) or Graphite Furnace (GFAAS) Measurement: Absorption follows Beer-Lambert Law: $A = \log(I_0/I) = \varepsilon \times c \times l$ Key advantage over flame photometry: Ground-state atoms >> Excited-state atoms at any temperature → AAS is more sensitive and selective than flame photometry → Hollow cathode lamp radiation is element-specific → fewer spectral interferences Sensitivity: FAAS: 0.1–10 ppm range | GFAAS: 0.001–0.1 ppm (ppb range)

25.6.2 Instrumentation Components

Component	Function	Details
Hollow Cathode Lamp (HCL)	Provides element-specific radiation	Different lamp for each element; cathode made of analyte metal
Atomiser (Flame/Furnace)	Converts metal ions to free ground-state atoms	Flame: air-acetylene (2300°C); N_2O-acetylene (2900°C); GF: graphite at 2600°C
Monochromator	Isolates the analytical wavelength	Separates the HCL line from background flame emission
Detector (PMT)	Measures transmitted beam intensity	Background corrector (D_2 or Zeeman) improves accuracy
Readout	Displays A; concentration from calibration	Regression-based concentration calculation

25.6.3 Flame AAS vs Graphite Furnace AAS

Feature	FAAS (Flame)	GFAAS (Graphite Furnace)
Sensitivity	0.1–10 ppm	0.001–0.1 ppm (100–1000× more sensitive)
Sample volume	1–5 mL	5–50 µL (microsampling)
Throughput	High (fast, continuous)	Low (batch; 3–5 min per sample)
Interferences	More	Less (controlled atmosphere in furnace)
Cost	Lower	Higher
Applications	Major elements; routine analysis	Trace metals; toxic elements at ppb levels
Pharmaceutical use	Zn, Cu, Fe, Ca, Mg in supplements	Pb, Cd, As, Hg at ppb in APIs; ICH Q3D

25.6.4 Pharmaceutical Applications of AAS

- Heavy metal impurity testing (ICH Q3D): Determination of Pb, Cd, Hg, As, Co, Ni, V, Cu in APIs and pharmaceutical products. PDEs (Permitted Daily Exposures) defined by ICH Q3D are verified by AAS or ICP-MS.
- Elemental analysis of mineral nutritional supplements: Ca, Mg, Fe, Zn, Mn, Cu, Cr, Se in multivitamin-mineral tablets.
- Catalyst residue monitoring: Pd, Pt, Rh used in pharmaceutical synthesis must be controlled to ICH Q3D limits.
- Preservative testing: Benzalkonium chloride levels in ophthalmic preparations; Ba in barium sulfate preparations.
- Environmental monitoring: Heavy metal monitoring in pharmaceutical effluents and pharmaceutical-grade water.
- Dissolution media validation: Verifying absence of metal ion contamination in dissolution media.

25.7 Comparison of Key Analytical Techniques

Technique	Analyte Type	Sensitivity	Selectivity	Pharmaceutical Application	Cost
Titrimetry	Bulk drugs; functional groups	mg level	Moderate	Assay of APIs; IP/BP primary method	Low
Gravimetry	Salts; inorganic; moisture	mg level	High	LOD; ROI; Ash tests; IP mandatory	Low
UV-Vis	UV-absorbing organics	µg/mL	Moderate	Assay; dissolution; ID; content uniformity	Low
TLC	All separable compounds	µg	Low–moderate	ID; purity; herbal standardization	Very Low
HPLC	Most organic drugs; mixtures	ng/mL	Very high	Assay; impurities; content uniformity; stability	High

GC	Volatile compounds	ng/mL	Very high	Residual solvents; volatile impurities	High
Flame Photometry	Na, K, Li, Ca, Ba	ppm	Moderate (element-specific)	Electrolyte preparations; ORS; Li tablets	Moderate
AAS (FAAS)	All metals	ppm (0.1–10)	Very high	Heavy metals; minerals; catalyst residues	Moderate
AAS (GFAAS)	Trace metals	ppb (0.001–0.1)	Very high	ICH Q3D elemental impurities; Pb, Cd, Hg, As	High
ICP-MS	All elements	ppt (sub-ppb)	Excellent (isotope-specific)	Multi-element ICH Q3D; ultra-trace metals	Very High

25.8 Emerging Analytical Techniques in Pharmaceutical Analysis

25.8.1 Near-Infrared (NIR) Spectroscopy

NIR spectroscopy (700–2500 nm) is increasingly used for non-destructive analysis of solid pharmaceutical dosage forms. It can simultaneously determine moisture content, API concentration, and excipient ratios without sample preparation. NIR is particularly valuable for at-line or in-line process analytical technology (PAT) — monitoring tablet manufacture in real time.

25.8.2 Raman Spectroscopy

Raman spectroscopy exploits inelastic scattering of laser light to provide molecular fingerprint information similar to IR, but with some advantages: aqueous samples can be analyzed directly (water is a poor Raman scatterer), non-destructive analysis, and can penetrate through blister packs or glass containers for counterfe-it drug detection.

25.8.3 Mass Spectrometry (MS) and LC-MS/MS

Mass spectrometry measures the mass-to-charge ratio (m/z) of ions and provides unambiguous molecular identification. Coupled with HPLC (LC-MS/MS), it is the gold standard for: bioequivalence and PK studies (plasma drug levels at ng/mL or pg/mL), impurity identification and structure elucidation, metabolite profiling, and proteomics.

25.8.4 Capillary Electrophoresis (CE)

CE separates charged analytes based on their differential migration in an electric field within a narrow capillary. It is particularly useful for: chiral separations, analysis of large biomolecules (peptides, proteins, nucleotides), and charged drug substances. CE can achieve resolution superior to HPLC for many ionic species with very small sample volumes (nL range).

25.9 Summary

Chapter 25 Summary

Chromatography separates analytes based on differential distribution between stationary and mobile phases. TLC (Rf values; silica gel; UV/iodine visualisation) is used for identification and purity testing. HPLC (RP-C18, UV detection) is the dominant modern analytical technique — assay, impurities, dissolution, stability. Key HPLC parameters: N (efficiency), Rs (resolution ≥1.5), k' (capacity factor 2–10). Flame photometry (emission of excited atoms at 589nm Na, 766nm K) determines Na/K in electrolytes and ORS. AAS (ground-state absorption; element-specific HCL; FAAS for ppm, GFAAS for ppb) is used for heavy metal impurity testing (ICH Q3D) and mineral assay. ICP-MS offers highest sensitivity for elemental impurity profiling.

Review Questions

Short Answer Questions

1. Define Rf in TLC. What is the optimal Rf range for a pharmaceutical TLC assay?
2. What is the stationary phase in RP-HPLC? What type of compounds are more retained?
3. Distinguish between flame photometry and AAS in terms of the measured signal.
4. What is a hollow cathode lamp (HCL)? Why is a separate HCL required for each element in AAS?
5. State two pharmaceutical applications each of (a) HPLC and (b) AAS.

Long Answer Questions

1. Describe the instrumentation of a UV-HPLC system. Explain the principle of reversed-phase HPLC. Discuss three pharmaceutical applications of HPLC with specific drug examples.
2. Compare flame photometry and atomic absorption spectroscopy (AAS) under the headings: principle, instrumentation, element range, sensitivity, selectivity, interferences, and pharmaceutical applications.
3. Write a comprehensive note on the role of chromatographic methods (TLC and HPLC) in pharmaceutical quality control. Include identification, purity testing, impurity profiling, and stability-indicating assays.

Multiple Choice Questions

1. In TLC, Rf = 0.45 means:

(a) The compound has not moved

(b) The compound moved 45% of the distance travelled by the solvent front

(c) The compound moved beyond the solvent front

(d) The compound has 45% purity

✓Answer: (b) The compound moved 45% of the distance travelled by the solvent front

2. In reversed-phase HPLC, the stationary phase is:

(a) Polar (silica gel)

(b) Non-polar (C18 — octadecylsilane bonded to silica)

(c) Ion exchange resin

(d) Cellulose

✓Answer: (b) Non-polar (C18 — octadecylsilane bonded to silica)

3. Flame photometry is based on:

(a) Absorption of radiation by ground-state atoms

(b) Emission of radiation by excited atoms in a flame

(c) Scattering of radiation by atoms

(d) Fluorescence of metal atoms

✓Answer: (b) Emission of radiation by excited atoms in a flame

4. The radiation source in AAS is:

(a) Deuterium lamp

(b) Tungsten filament lamp

(c) Hollow Cathode Lamp (HCL) — element specific

(d) Xenon arc lamp

✓Answer: (c) Hollow Cathode Lamp (HCL) — element specific

5. GFAAS (graphite furnace AAS) is preferred over FAAS when:

(a) Large sample volumes are available

(b) Very high concentration samples are analyzed

(c) Ultra-trace (ppb) level elemental analysis is needed with small sample volumes

(d) Fast throughput analysis is required

✓Answer: (c) Ultra-trace (ppb) level elemental analysis is needed with small sample volumes

Glossary of Important Terms

The terms below are listed alphabetically. Terms are defined as used in pharmaceutical analysis and in the context of this textbook. Where a term appears in multiple chapters, the definition given here is the general working definition; chapter-specific nuances are discussed in the text.

Term	Definition
Absorbance (A)	The logarithm (base 10) of the ratio of incident to transmitted radiant power: $A = \log(I_0/I)$. Directly proportional to concentration under Beer-Lambert conditions. Dimensionless.
Accuracy	The closeness of an analytical result to the true (accepted) value. Expressed as % recovery or % error. Affected by systematic (determinate) errors.
Acidimetry	A form of acid-base titrimetry in which a standard acid solution is used to determine the amount of a base.
Active Pharmaceutical Ingredient (API)	The biologically active component of a pharmaceutical product; the drug substance itself, as distinct from excipients or diluents.
Adsorption indicator	An indicator used in precipitation titrations (Fajans method) that changes colour upon adsorption onto the precipitate surface at the equivalence point. Example: fluorescein.
Alkalimetry	A form of acid-base titrimetry in which a standard base (alkali) is used to determine the amount of an acid.
Argentimetry	A group of precipitation titrations using silver nitrate ($AgNO_3$) as the titrant for determination of halide ions. Includes Mohr, Volhard, and Fajans methods.
Assay	An analytical determination of the amount (potency) of the active substance in a pharmaceutical preparation. Usually expressed as % w/w, % w/v, or mg/unit.
Atomic Absorption Spectroscopy (AAS)	An instrumental technique based on the absorption of characteristic radiation by ground-state atoms in the gaseous phase, used for quantitative determination of metallic elements.

Auxochrome	A functional group (e.g., –OH, $–NH_2$, –OR) that, when attached to a chromophore, shifts the absorption maximum to longer wavelengths (bathochromic shift) and intensifies absorption.
Back-titration	An indirect titrimetric technique in which an excess of standard reagent is added to react completely with the analyte, and the unreacted excess is then titrated with a second standard solution.
Bathochromic shift (Red shift)	A shift of the absorption maximum (λmax) to a longer wavelength (lower energy) caused by structural changes or solvent effects.
Beer-Lambert Law	The combined law stating that absorbance is directly proportional to both the molar concentration of the absorbing species and the path length of radiation through the solution: A = εcl.
Blank	A sample containing all reagents and solvents but no analyte, used to correct for background absorbance or reagent contribution in spectrophotometric and other instrumental measurements.
Buffer solution	A solution that resists significant changes in pH upon addition of small quantities of acid or base. Consists of a weak acid and its conjugate base (or vice versa).
Calibration curve	A graph of instrument response (e.g., absorbance) vs known analyte concentration. Used to determine unknown concentrations by interpolation.
Chelate	A complex formed between a metal ion and a polydentate ligand (chelating agent) that forms two or more coordinate bonds with the metal. EDTA chelates form highly stable 1:1 complexes with most metal ions.
Chromophore	An atom or group of atoms in a molecule responsible for its absorption of UV or visible radiation by undergoing electronic transitions (e.g., C=C, C=O, aromatic rings).
Complexometric titration	A titrimetric method based on the formation of a stable, soluble complex between the analyte (usually a metal ion) and a complexing agent. EDTA titrations are the most common example.

Confidence interval (CI)	A range of values, derived from sample statistics, within which the true population mean is expected to lie with a specified level of probability (e.g., 95%).
Coprecipitation	The contamination of a precipitate by the simultaneous precipitation of normally soluble foreign ions. A major source of error in gravimetric analysis.
Determinate (Systematic) error	An error with a definite, assignable cause that introduces a consistent bias (always in the same direction) into analytical results. Can be identified and eliminated.
Digestion (precipitate)	The process of allowing a freshly formed precipitate to stand in contact with the mother liquor, usually at elevated temperature, to improve crystal size and purity before filtration.
Dissolution testing	A pharmacopoeial in vitro test that measures the rate and extent to which the active ingredient is released from a solid dosage form into a dissolution medium under standardized conditions.
EDTA (Ethylenediaminetetraacetic acid)	A hexadentate chelating agent that forms stable 1:1 complexes with virtually all metal cations. Widely used in complexometric titrations in pharmaceutical analysis.
Eriochrome Black T (EBT)	A metallochromic indicator used in EDTA titrations for hardness determination (Ca^{2+} + Mg^{2+}); forms wine-red complex with metals, turns blue at the endpoint.
Equivalence point	The theoretical point in a titration at which the amount of titrant added is stoichiometrically equivalent to the amount of analyte. Not directly observable; approached by the endpoint.
Fajans method	A precipitation titration using adsorption indicators (e.g., fluorescein, eosin). The indicator adsorbs onto the precipitate surface and changes colour at the equivalence point.
Flame photometry	An emission spectroscopic technique in which a sample solution is aspirated into a flame, and the intensity of characteristic emission lines of excited metal atoms (Na, K, Li) is measured.

Gravimetric analysis	A classical quantitative method in which the mass of a pure substance chemically related to the analyte is measured. Includes precipitation, volatilization, and electrodeposition gravimetry.
Gravimetric factor (GF)	The ratio of the molar mass of the analyte to the molar mass of the weighed form, multiplied by the appropriate stoichiometric coefficient. Used to convert the mass of the weighed precipitate to the mass of the analyte.
HPLC (High Performance Liquid Chromatography)	A chromatographic technique in which a liquid mobile phase is pumped at high pressure through a column packed with a stationary phase to separate, identify, and quantify analytes.
Hypsochromic shift (Blue shift)	A shift of the absorption maximum (λmax) to a shorter wavelength (higher energy).
ICH guidelines	Guidelines issued by the International Council for Harmonisation specifying technical requirements for pharmaceutical products. Key guidelines include Q1 (stability), Q2 (validation), Q3 (impurities).
Indicator (acid-base)	A weak acid or base whose conjugate form differs in colour from the undissociated form, allowing visual detection of the endpoint in acid-base titrations.
Indeterminate (Random) error	An error arising from uncontrollable, unpredictable fluctuations in the analytical system. Described by statistics; reduced by replicate measurements.
Iodimetry	A redox titrimetric method in which iodine (I_2) is used as the oxidising titrant in a direct titration of reducing analytes (e.g., ascorbic acid, sodium thiosulfate).
Iodometry	An indirect redox titrimetric method in which an oxidising analyte liberates iodine from potassium iodide, and the liberated iodine is back-titrated with sodium thiosulfate.
Karl Fischer titration	A specific titrimetric method for the quantitative determination of water content in pharmaceutical substances and preparations, based on the reaction of water with iodine in the presence of sulfur dioxide and a base.
Limit of Detection (LOD)	The lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under the stated

	analytical conditions. Typically defined as signal-to-noise ratio ≥ 3.
Limit of Quantification (LOQ)	The lowest amount of analyte that can be quantitatively determined with acceptable precision and accuracy. Typically defined as signal-to-noise ratio ≥ 10.
Limit test	A semi-quantitative pharmacopoeial test that compares the colour or turbidity of a test solution to that of a standard reference, to verify that the concentration of an impurity does not exceed a specified limit.
Mean (Arithmetic mean, $\bar{x}$)	The sum of all measured values divided by the number of measurements. The most common measure of central tendency in analytical data.
Metalochromic indicator	An indicator used in complexometric titrations that forms a coloured complex with metal ions. Free in solution, it has a different colour from the metal complex (e.g., EBT, murexide).
Mohr method	A direct argentimetric precipitation titration for halides (Cl^-, Br^-) using $AgNO_3$ as titrant and potassium chromate (K_2CrO_4) as indicator. Conducted in neutral solution.
Molar absorptivity (ε)	The proportionality constant in the Beer-Lambert law: A = εcl. Units: L mol^{-1} cm^{-1}. A fundamental property of the absorbing species at a given wavelength.
Molarity (M)	The number of moles of solute per litre of solution. The standard expression of concentration in titrimetric analysis.
Mother liquor	The supernatant solution remaining after the formation and settling of a precipitate.
Murexide	A metalochromic indicator used in EDTA titrations for calcium (alone, at pH 12) and copper/nickel. Changes from red-violet (metal complex) to violet (free indicator) at the endpoint.
Non-aqueous titration	A titrimetric method conducted in non-aqueous solvents (e.g., glacial acetic acid, acetonitrile) for the assay of substances too weakly acidic or basic to be titrated in water.
Normal distribution (Gaussian distribution)	A symmetrical, bell-shaped probability distribution characterising the distribution of random errors in repeated

	analytical measurements. Described by mean (μ) and standard deviation (σ).
Normality (N)	The number of gram-equivalents of solute per litre of solution. Older concentration unit used in titrimetry; largely replaced by molarity in modern practice.
Permanganometry	A redox titrimetric method using potassium permanganate ($KMnO_4$) as the oxidising titrant. Self-indicating in acidic solution (purple → colourless).
pH	The negative logarithm (base 10) of the hydrogen ion (or hydronium ion) activity: pH = –log[H^+]. A measure of acidity/alkalinity of a solution.
Pharmacopoeia	An official, legally recognised compendium of standards for drug substances, excipients, and pharmaceutical preparations, including tests, assays, and reference standards.
Precipitate	The insoluble solid formed in a solution as a result of a chemical reaction (precipitation). In gravimetric analysis, the precipitate is a chemically defined compound related stoichiometrically to the analyte.
Precision	The closeness of agreement among replicate measurements made under the same conditions. Expressed as standard deviation (SD), relative standard deviation (RSD), or coefficient of variation (CV). Affected by random errors.
Primary standard	A highly pure, stable compound of accurately known composition, used to prepare or standardize solutions of accurately known concentration.
Q-test (Dixon Q-test)	A statistical test used to identify and reject outliers in a small data set, based on the ratio of the gap between the suspect value and its nearest neighbour to the range of the data set.
Redox titration	A titrimetric method based on oxidation-reduction reactions between the analyte and the titrant. Includes permanganometry, dichromatometry, iodimetry, and iodometry.
Relative standard deviation (RSD)	Standard deviation expressed as a percentage of the mean: RSD (%) = (SD / $\bar{x}$) × 100. Also called coefficient of variation (CV). A dimensionless measure of precision.

Reversed-phase HPLC (RP-HPLC)	The most common form of HPLC, using a non-polar stationary phase (typically C18) and a polar aqueous-organic mobile phase. Non-polar analytes are retained longer; polar analytes elute first.
Rf value	Retardation factor in TLC: the ratio of the distance travelled by the analyte spot to the distance travelled by the solvent front from the origin. Values range from 0 to 1.
Secondary standard	A solution whose concentration has been determined by standardization against a primary standard. Used routinely as a working titrant.
Significant figures	The number of meaningful digits in a measured or calculated value, indicating the precision of the measurement. Determined by specific rules for zeros and arithmetic operations.
Spectrophotometry	An analytical technique based on the measurement of the intensity of radiation absorbed or transmitted by a sample as a function of wavelength. UV-Visible spectrophotometry is widely used in pharmaceutical assay.
Standard deviation (SD)	A measure of the spread of replicate data around the mean. Calculated as the square root of the variance: $SD = \sqrt{[\Sigma(x_i - \bar{x})^2 / (n-1)]}$ for a sample.
Standardization	The process of accurately determining the concentration of a solution by titration against a primary standard or a solution of accurately known concentration.
Student's t-test	A statistical test used to compare a sample mean to a known value or to compare two sample means, to determine whether any difference is statistically significant. Uses the t-distribution for small sample sizes.
t-statistic	The ratio of the difference between the sample mean and reference value to the standard error of the mean: $t = (\bar{x} - \mu) / (SD/\sqrt{n})$. Compared to tabulated critical values.
Titer	The concentration of a solution expressed as the mass of a specific analyte (e.g., drug substance) that corresponds to a given volume of the solution.
TLC (Thin-Layer Chromatography)	A planar chromatographic technique in which analytes are separated on a thin layer of adsorbent (usually silica gel on

	aluminium or glass) by differential migration driven by capillary action of the mobile phase.
Titrant	The standard solution of known concentration added from a burette during a titration.
Titrimetry (Volumetric analysis)	A classical quantitative analytical method in which the volume of a standard solution (titrant) required to react stoichiometrically with the analyte is measured.
True value	The actual, correct value of the quantity being measured. Approximated by the accepted reference standard value.
UV-Visible spectrophotometry	An analytical technique based on the measurement of absorption of ultraviolet (200–400 nm) and/or visible (400–700 nm) radiation by molecules in solution. Widely used for drug assay.
Variance (s^2)	The square of the standard deviation: $s^2 = \Sigma(x_i - \bar{x})^2 / (n-1)$. A measure of the spread of analytical data.
Volhard method	An indirect argentimetric back-titration for halides, thiocyanate, and phosphate, conducted in acidic solution using iron(III) as indicator and ammonium thiocyanate as back-titrant.
Volumetric glassware	Calibrated glassware (burettes, pipettes, volumetric flasks) used for accurate measurement of volumes of solutions in titrimetric analysis.