Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis III***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.1***

Name of topic/lesson –Atomic absorption spectroscopy

Points – Theory & principle

Atomic absorption spectroscopy (AAS) *is a spectroanalytical procedure for the quantitative determination of chemical elements employing the absorption of optical radiation (light) by free atoms in the gaseous state. In analytical chemistry the technique is used for determining the concentration of a particular element (the analyte) in a sample to be analyzed. AAS can be used to determine over 70 different elements in solution or directly in solid samples employed in pharmacology, biophysics and toxicology research.*

Atomic absorption spectrometry was first used as an analytical technique, and the underlying principles were established in the second half of the 19th century by Robert Wilhelm Bunsen and Gustav Robert Kirchhoff, both professors at the University of Heidelberg, Germany. The modern form of AAS was largely developed during the 1950s by a team of Australian chemists. They were led by Sir Alan Walsh at the CSIRO (Commonwealth Scientific and Industrial Research Organization), Division of Chemical Physics, in Melbourne, Australia.

Principle

The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lambert Law. In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using the Beer-Lambert Law.

References : *1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no.264*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.340

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis III***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.2***

Name of topic/lesson –Atomic absorption spectroscopy

Points – *Instrumentation*

In order to analyze a sample for its atomic constituents, it has to be atomized. The atomizers most commonly used nowadays are flames and electrothermal (graphite tube) atomizers. The atoms should then be irradiated by optical radiation, and the radiation source could be an element-specific line radiation source or a continuum radiation source. The radiation then passes through a monochromator in order to separate the element-specific radiation from any other radiation emitted by the radiation source, which is finally measured by a detector.

The instrument consists of:

1. *A flame*
2. *Lamps to produce the correct wavelength of light*
3. *A detector*
4. *A system to aspirate solutions into the flame*
5. *A computer to control the experiment*

References : *1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no.265*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.340

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis III***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.3***

Name of topic/lesson –Atomic absorption spectroscopy

Points-Instrumentation

Atomizers

The atomizers most commonly used nowadays are (spectroscopic) flames and electrothermal (graphite tube) atomizers. Other atomizers, such as glow-discharge atomization, hydride atomization, or cold-vapor atomization might be used for special purposes.

The oldest and most commonly used atomizers in AAS are flames, principally the air-acetylene flame with a temperature of about 2300 °C and the nitrous oxide (N_2O)-acetylene flame with a temperature of about 2700 °C. The latter flame, in addition, offers a more reducing environment, being ideally suited for analytes with high affinity to oxygen.

A laboratory flame photometer that uses a propane operated flame atomizer.

Liquid or dissolved samples are typically used with flame atomizers. The sample solution is aspirated by a pneumatic analytical nebulizer, transformed into an aerosol, which is introduced into a spray chamber, where it is mixed with the flame gases and conditioned in a way that only the finest aerosol droplets (< 10 µm) enter the flame. This conditioning process is responsible that only about 5% of the aspirated sample solution reaches the flame, but it also guarantees a relatively high freedom from interference.

On top of the spray chamber is a burner head that produces a flame that is laterally long (usually 5–10 cm) and only a few mm deep. The radiation beam passes through this flame at its longest axis, and the flame gas flow-rates may be adjusted to produce the highest concentration of free atoms. The burner height may also be adjusted, so that the radiation beam passes through the zone of highest atom cloud density in the flame, resulting in the highest sensitivity.

References: *1. Principles of Instrumental Analysis by Skoog, 5th edition, p.no.266*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.341

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis III***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.4***

Name of topic/lesson –Atomic absorption spectroscopy

Points-Interferences

1. *Spectral Interferences:Spectral interference can occur due to overlapping lines. e.g. a vanadium line at 3082.11Å interferes in an analysis based upon the aluminum absorption line at 3082.15 Å. This type of interference can be avoid by employing the aluminum line at 3092.7 Å instead.*

   Concentrated solution of elements such as Ti, Zr and W which form refractory oxides can cause spectral interference due to scattering.

2. Chemical Interferences:

Formation of Compounds of Low Volatility: The most common type of interference is by anions that form compounds of low volatility with the analyte and thus reduce the rate at which the analyte is atomized. The decrease in calcium absorbance that is observed with increasing concentrations of sulfate or phosphate. Example of cation interference have also been recognized. Aluminum is found to cause low results in the determination of magnesium, apparently as a result of the formation of a heat-stable aluminum/magnesium compound.

Background absorption and background correction:

Background correction techniques in LS AAS

Deuterium background correction

Zeeman-effect background correction

Refrences: *1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no.267*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.342

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis III***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.5***

Name of topic/lesson –Atomic absorption spectroscopy

Points- Applications of Atomic Absorption Spectroscopy:

It is a sensitive means for the quantitative determination of more than 60 metals or metalloid elements. The resonance lines for the nonmetallic elements are generally located below 200 nm, thus preventing their determination by convenient, nonvacuum spectrophotometers

-water analysis (e.g. Ca, Mg, Fe, Si, Al, Ba content)

- Food analysis

- Analysis of animal feedstuffs (e.g. Mn, Fe, Cu, Cr, Se, Zn)

-analysis of additives in lubricating oils and greases (Ba,Ca, Na, Li, Zn, Mg)

- Analysis of soils

-clinical analysis (blood samples: whole blood, plasma, serum; Ca, Mg, Li, Na, K, Fe)

Refrences: *1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no.268*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.360

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis III***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.6***

Name of topic/lesson – Flame Photometry:

Point-Principle

When an alkali metal salt is drawn into a non luminous flame, it will ionize, absorb energy from the flame and then emit light of a characteristic wavelength as the excited atom decay to the unexcited ground state. The intensity of emission is proportional to the concentration of the element in the solution. A photocell detects the emitted light and converts it to a voltage which can be recorded.

- *Atomic Orbital*
- *Energy States of Atoms*
- *Ions*
- *Atomic Emission*
- *Electrons of atoms reside in concentric spheres known as energy "shells" in which they orbit the nucleus of an atom.*
- *Each shell is assigned a principal quantum number, n.*
- *The value of n is an integer, 1, 2, 3, etc.*
- *This number determines the relative energy of the orbital and relates the distance from t*
- *As the electrons "fall" from the excited state to the ground state, they emit the energy they absorbed in the form of electromagnetic radiation (heat, light, etc.)*

Refrences: *1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no.291*

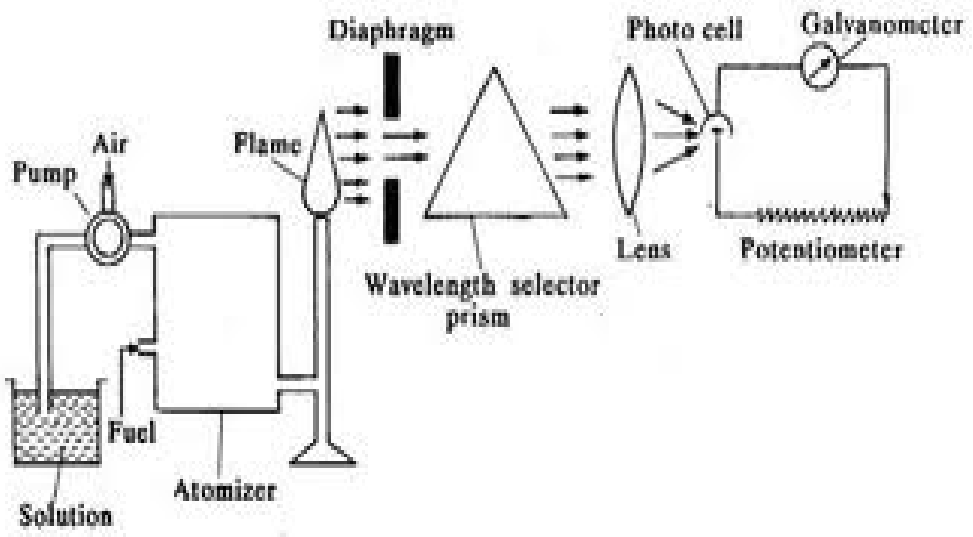
2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.367

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis III***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.7***

Name of topic/lesson – Flame Photometry:

Point-Instrumentation



- *NEBULIZER-means of transporting a homogeneous solution into the flame at a steady rate*
- *BURNER-maintain flame at a constant form and at a constant rate*
- *SIMPLE COLOUR FILTERS(interference type)-means of isolating light of the wavelength to be measured from that of extraneous emissions*
- *PHOTODETECTOR-means of measuring the intensity of radiation emitted by the flame*

Refrences: *1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no.292*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.368

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.8***

Name of topic/lesson – Flame Photometry:

Point-Application

- *To estimate sodium, potassium, calcium, lithium level in sample of serum, urine, CSF and other body fluids.*
- *Sodium is the main cation of plasma and interstitial fluid(extracellular)*
- *Potassium is the main cation in intracellular space*
- *Both ions are important in order to maintain the polarity of membranes of all living cells*
- *Lithium estimation is required in some psychiatric disorder where it is used therapeutically*

Refrences: *1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no.293*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.378

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.9***

Name of topic/lesson – Fluorimetry and phosphorimetry

Point-Introduction

A group of related optical methods, which involve excitation of a molecular analyte to give species whose emission spectra are used for qualitative or quantitative analysis, is known collectively as molecular luminescence spectroscopy.

The excited species of interest is formed either by absorption of photons (fluorescence and phosphorescence, or together called photoluminescence) or by chemical reaction (chemiluminescence). For this course we will limit our attention to the first two methods, and generally concentrate on fluorescence, since it currently affords by far the largest number of applications.

Fluorescence occurs in chemical systems in the gaseous, liquid, or solid state. The 3s electron of vaporized sodium atoms can be excited to the 3p state by light with wavelengths of 5896 and 5890 Å, which is reemitted at the same wavelengths within ten to 1/100 microseconds.This resonance fluorescence also occurs in many molecular species. Molecules, however, more commonly emit light at longer wavelengths than those of the light absorbed. This emission at higher wavelength is known as the Stokes shift.

Electron Spin, Excited Singlets, and Excited Triplets

To understand the difference between fluorescence andphosphorescence, we must recall that the Pauli exclusion principle dictates that no two electrons in an atom or molecule can have the same set of four quantum numbers. This means that no orbital can have more than two electrons, and that any two electrons in the same orbital cannot have the same spin. One physical consequence of this "pairing" of electrons in molecular orbitals is that most molecules have no net magnetic field (i.e., they are diamagnetic, and are not affected by any static external magnetic field).

References: *1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no.443*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.399

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.10***

Name of topic/lesson – Fluorimetry and phosphorimetry

Point-Principle

Absorption and Emission in Photoluminescence

The rate of absorption of photons is tremendous (10-14 to 10-15 seconds), but the rate of fluorescence and phosphorescence emission is considerably less. Excited singlets have relatively short lifetimes (10-5 to10-8 seconds), compared to emission ranges of 10-4 to 10 seconds for excited triplets (phosphorescence). In general, the lifetime of the excited state is inversely proportional to the molar absorptivity of the species for the light corresponding to the excitation. In practice, light is absorbed over a range of wavelengths, since the electron can be excited to one of a

number of higher energy electronic states, as well as to a number of different vibrational states within a given excited electronic state.

Excitation of a ground state electron to an excited triplet state is so rare that it is classified as a forbidden transition, so population of the triplet state comes about by relaxation of an excited singlet electron (higher energy) through a process known as intersystem crossing.

Deactivation Processes

The energy of the excited singlet electron can be dispersed in a number of ways besides though fluorescence or phosphorescence emission.

Vibrational relaxation: as we saw above, an electron can be excited to any of the vibrational states within an excited electron state. Those electrons in the more energetic vibrational states can relax to lower states without radiation of light. These radiationless processes are highly efficient (thus rapid), with the result that when emission of light energy by photoluminescence does occur, the electron is in the ground vibrational energy level of the given excited electron state. (However, it can emit photons of a range of wavelengths as it can relax back to any of a number of vibrational states in the ground electronic state.)

References*: 1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no.444*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.399

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.11***

Name of topic/lesson – Fluorimetry and phosphorimetry

Point-Principle

Internal conversion: the higher excited electronic states (S2 and above) often have vibrational levels that overlap those of a lower electronic state, allowing an electron to pass to a lower state without radiation, (internal conversion: often accompanied by more vibrational relaxation).

External conversion: interaction and energy transfer between the excited molecule and either a solvent or another solute molecule is termed external conversion or collisional quenching. Variations in fluorescent intensity caused by different solvents, as well as increased intensity in conditions that tend to reduce the number of particle collisions (low temperature and increased solution viscosity) are taken as evidence for the process, though the details are not well understood.

Intersystem crossing: as was stated above, excitation of a ground state electron directly to the excited triplet state is a rare occurrence. However, overlap of vibrational levels between the excited singlet and triplet states enhances the possibility of reversal of electron spin.

Electrons excited to higher electronic states often show no emission bands based on relaxation to the ground state from these higher electronic states. Internal conversion between the first excited electronic state and the ground state (S1 - S0), though poorly understood may account for the lack of fluorescence in aliphatic compounds. Internal conversion can result in a phenomenon known as predissociation, in which the electron is transferred to a vibrational level of a lower electronic state that results in rupture of a bond. This is different from dissociation, which results when absorption of a photon excites the electron to a vibrational level energetic.

Quantum Yield or Quantum Efficiency

Whether or nor a molecule will luminesce, as well as the intensity of luminescence when it does occur, is influenced by molecular structure and chemical environment. The ratio of molecules that exhibit either type of photoluminescence to the total number of excited molecules is called
quantum yield or quantum efficiency (φ). This ratio (for fluorescence quantum efficiency) ranges from zero for chemical species that do not fluoresce to any appreciable degree to almost unity for highly fluorescent molecules

References: 1. Principles of Instrumental Analysis by Skoog, 5th edition, p.no.449

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.340

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.12***

Name of topic/lesson – Fluorimetry and phosphorimetry

Point-Principle

The Pauli exclusion principle states that no two electrons in an atom can have the same set of four quantum numbers. This restriction requires that no more than two must have opposed spin states. Because of spin pairing, most molecules exhibit no net magnetic field and are thus said to be diamagnetic. In contrast, free radical, which contain unpaired electrons, have a magnetic moment are said to be paramagnetic.

Transition Types in Fluorescence

To briefly review the types of electron transitions that might result from absorption of a photon by a molecule:

Energy level diagram for transition types*: relative molecular orbital energy levels from lowest to highest:*

σ (bonding) <π (bonding)<n (nonbonding)<π(antibonding)<σ*(antibonding)*

*largest ΔE: σ-σ**

medium ΔE: π-π ≈ n-σ**

*smallest ΔE: n-π**

*One important factor to consider in determination of what wavelength range of light is likely involved in fluorescence is the energy of the absorbed photon. Light in the ultraviolet range is likely to lead to transitions that will be deactivated by the predissociation or dissociation pathways. Consider that light at 200 nm has an energy level of over 140 kcal/mol, which is probably sufficient to rupture a bond in many organic compounds. From this consideration fluorescence emission from σ *-σtransitions are not likely. Generally, emission is confined to the less energetic π *-π or π *-n transition.*

References: 1.Principles of Instrumental Analysis by Skoog, 5th edition, p.no.449

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.340

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.13***

Name of topic/lesson – Fluorimetry and phosphorimetry

Point-Factors affecting fluorescence.

Quantum Efficiency

Whether or nor a molecule will luminesce, as well as the intensity of luminescence when it does occur, is influenced by molecular structure and chemical environment. The ratio of molecules that exhibit either type of photoluminescence to the total number of excited molecules is called

quantum yield or quantum efficiency (φ). This ratio (for fluorescence quantum efficiency) ranges from zero for chemical species that do not fluoresce to any appreciable degree to almost unity for highly fluorescent molecules

$$F = 2.3\Phi\varepsilon bCP_o$$

- Φ = quantum efficiency = # molecules emitting/total # molecules excited
- ε (L/mol-cm) and b (cm) have their usual meanings
- P_o in incident radiant power density (watts/cm^2)

- Linear relationship, F = KC

- *Self-absorption* and *self-quenching* cause negative deviations from linearity (i.e., reduced fluorescence intensity).

- Φ increases with lower temperature, increased structural rigidity, $\pi \longrightarrow \pi^*$ transition, and can be affected by solvent type and pH.

- Electron donating groups (NH_2, OH) tend to enhance fluorescence while electron withdrawing groups (Cl, COOH) tend to inhibit it.

References:: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.449*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.341

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.14***

Name of topic/lesson – Fluorimetry and phosphorimetry

Point- Factors affecting fluorescence.

Structural Considerations

In light of the above, it is not surprising to find that aromatic compounds with low energy $\pi \rightarrow \pi^*$ transitions generally exhibit the most intense and useful fluorescence emissions. To a lesser degree, some aliphatic and alicyclic carbonyl structures and highly conjugated double bond structures exhibit a lesser degree of fluorescence emission. Most unsubstituted aromatic hydrocarbons fluoresce in solution, with quantum yield increasing with the number of rings. Simple heterocyclic compounds such as pyridine, pyrrole, furan, and thiophene do not exhibit

fluorescence, while fused ring structures usually do. [single ring heterocyclic structures p. 405, column 2 top] In nitrogen heterocyclics, the lowest energy transition is thought to involve an $n \rightarrow \pi^*$ system that converts to the triplet state. However, in the fused ring heterocyclics, the benzene ring increases the molar absorptivity of the absorption peak, shortening the lifetime of the excited state and leading to fluorescence emission. [fused ring heterocyclic structures p. 405, column 2 bottom] As we can see in the following table, substitution on the benzene ring can cause shifts in the maximum wavelength of absorbance and fluorescence emission, as well as emission intensity and quantum efficiency. One interesting point is the effect of halogen substitution: presence of a heavy atom such as bromine or iodine is thought to increase the size of spin/orbital interactions in a molecule, thus increasing the likelihood of a change in spin. Substitution of carboxylic acid or carbonyl groups greatly inhibits fluorescence since the energy of the $n \rightarrow \pi^*$ transition is less than that of the $\pi \rightarrow \pi^*$ transition in these compounds.

References: *1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no.450*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.342

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.15***

Name of topic/lesson – Fluorimetry and phosphorimetry

Point- Factors affecting fluorescence.

Structural Rigidity

Another structural feature that has been shown empirically to affect fluorescence intensity is the rigidity of a molecular structure. For example, fluorene and biphenyl[Fluorene & biphenyl structures p. 406 column 1] have similar structures except for the bridge methylene group of fluorene, yet the quantum yield of biphenyl (φ = 0.2) is considerably less than that of fluorene (φ = ~1.0). It is thought that the lack of rigidity leads to an increased rate of deactivation by internal conversion (increased rates of low frequency vibrations with respect to other parts of the molecule account for some energy loss).

Effect of pH

The fluorescence of aromatic compounds with acidic or basic substituents is generally affected by pH. It is thought that this is related to the number of resonance structures associated with the acidic and basic forms of the molecule in question. For example, the strong fluorescence emission of aniline compared to the lack of fluorescence in the anilinium ion is attributed to the stability of the first excited state of the basic form due to resonance as evidenced by its three resonance structures.

Temperature and Solvent Effects

In general, increased temperature is expected to increase the deactivation due to external conversion (increased number of collisions between solute and solvent or other solute molecules). Increased solvent viscosity acts in the opposite way (decreased number of collisions and thus loss of energy through external conversion). Solvents containing heavy atoms will reduce quantum yield by promotion of intersystem crossing in the same way that substitution of bromine or iodine reduces quantum yields of benzene derivatives.

References*:: 1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no.450*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.400

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.16***

Name of topic/lesson – Fluorimetry and phosphorimetry

Point- Factors affecting fluorescence.

Concentration and Fluorescence Intensity

The power of fluorescence emission (F) is proportional to the radiant power of the excitation beam absorbed by the system: (eqn. 15-2 p.407)

where P0 is the power incident on the solution, P is the power of the beam after traversing a length b of the medium, and K' is a constant dependent on the quantum yield. To relate the fluorescence emission to concentration, we employ Beer's law in the form

At concentration high enough that A > 0.05, this linear relationship fails, and F would lie below the extrapolation of a straight-line plot.Two other factors that cause a negative deviation from linearity at high concentration are self-quenching (caused by collisions between excited molecules) and self-absorption (which occurs when the excitation and emission spectra overlap, so that some of the emitted light is reabsorbed by other fluorescent molecules).

Effect of pH

The fluorescence of aromatic compounds with acidic or basic substituents is generally affected by pH. It is thought that this is related to the number of resonance structures associated with the acidic and basic forms of the molecule in question. For example, the strong fluorescenc emission of aniline compared to the lack of fluorescence in the anilinium ion is attributed to the stability of the first excited state of the basic form due to resonance as evidenced by its three resonance structures.

Temperature and Solvent Effects

In general, increased temperature is expected to increase the deactivation due to external conversion (increased number of collisions between solute and solvent or other solute molecules). Increased solvent viscosity acts in the opposite way (decreased number of collisions and thus loss of energy through external conversion). Solvents containing heavy atoms will reduce quantum yield by promotion of intersystem crossing in the same way that substitution of bromine or iodine reduces quantum yields of benzene derivatives.

References: *1.Principles of Instrumental Analysis by Skoog, 5th edition, p.no.453*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.401

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.17***

Name of topic/lesson – Fluorimetry and phosphorimetry

Point- Factors affecting fluorescence.

Concentration and Fluorescence Intensity

The power of fluorescence emission (F) is proportional to the radiant power of the excitation beam absorbed by the system.

Two other factors that cause a negative deviation from linearity at high concentration are self-quenching (caused by collisions between excited molecules) and self-absorption (which occurs when the excitation and emission spectra overlap, so that some of the emitted light is reabsorbed by other fluorescent molecules).

At concentration high enough that A > 0.05, this linear relationship fails, and F would lie below the extrapolation of a straight-line plot.Two other factors that cause a negative deviation from linearity at high concentration are self-quenching (caused by collisions between excited molecules) and self-absorption (which occurs when the excitation and emission spectra overlap, so that some of the emitted light is reabsorbed by other fluorescent molecules).

References: . *1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no.453*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.401

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.17***

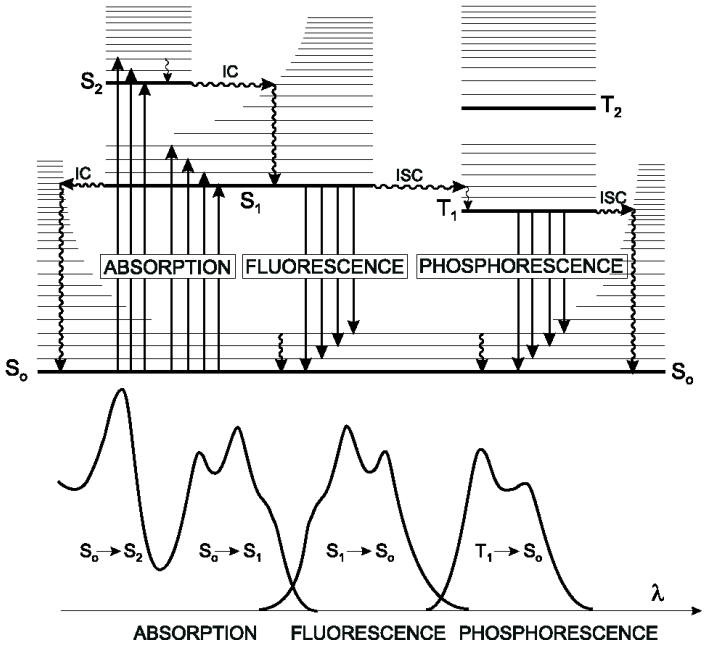
Name of topic/lesson – Fluorimetry and phosphorimetry

Emission and Excitation Spectra:*The figure below shows three types of photoluminescence spectra for phenanthrene. The excitation spectrum (E) is simply an absorbance spectrum for the emitting species (energy must be absorbed to excite the molecule which then emits some or all of the absorbed energy). The*

Fluorescence and phosphorescence spectra (F and P, respectively) involve excitation at a fixed wavelength, while the emission intensity is recorded as a function of wavelength.

Photoluminescence generally takes place at longer wavelengths (lower energy) than excitation. One should also note that phosphorescence emission bands usually occur at longer wavelengths

than fluorescence emission band for the same molecule. This is due to the fact that the excited triplet state is usually lower in energy than the corresponding singlet state. The difference in wavelength between the two bands is, in fact, a good measure of the difference in energy between the two states.



References: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.455*

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No. 17***

Name of topic/lesson – Fluorimetry and phosphorimetry

Instrumentation: Fluorometers and Spectrofluorometers

The components of instruments for measuring photoluminescence are similar to those found in ultraviolet/visible spectrophotometers. Nearly all fluorescence instruments employ double-beam optics to compensate for fluctuations in the power source. The sample beam first passes through an excitation filter or a monochrometer, which transmits radiation that will excite fluorescence but excludes or limits radiation of the wavelength of the fluorescence emission. Fluorescence is propagated from the sample in all directions, but is most conveniently observed at right angles to the excitation beam to minimize errors in measurement of intensity due to scattering of light from the sample itself or the cell walls. Emitted radiation is then passed through a second filter or monochrometer before it reaches a phototransducer. The reference beam is passed through an attenuator to reduce its power to approximately that of the fluorescent radiation (a reduction on the order of 100 times or more). The signals from reference and sample photomultipliers are then fed to a difference amplifier whose output is displayed by a meter or recorder.

References*: 1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.461*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.405

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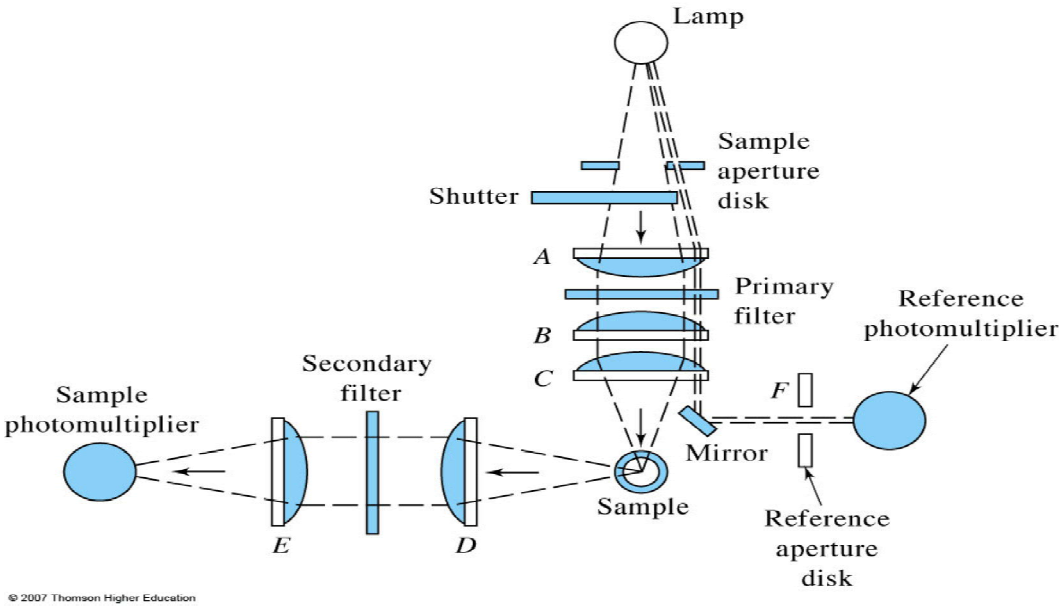
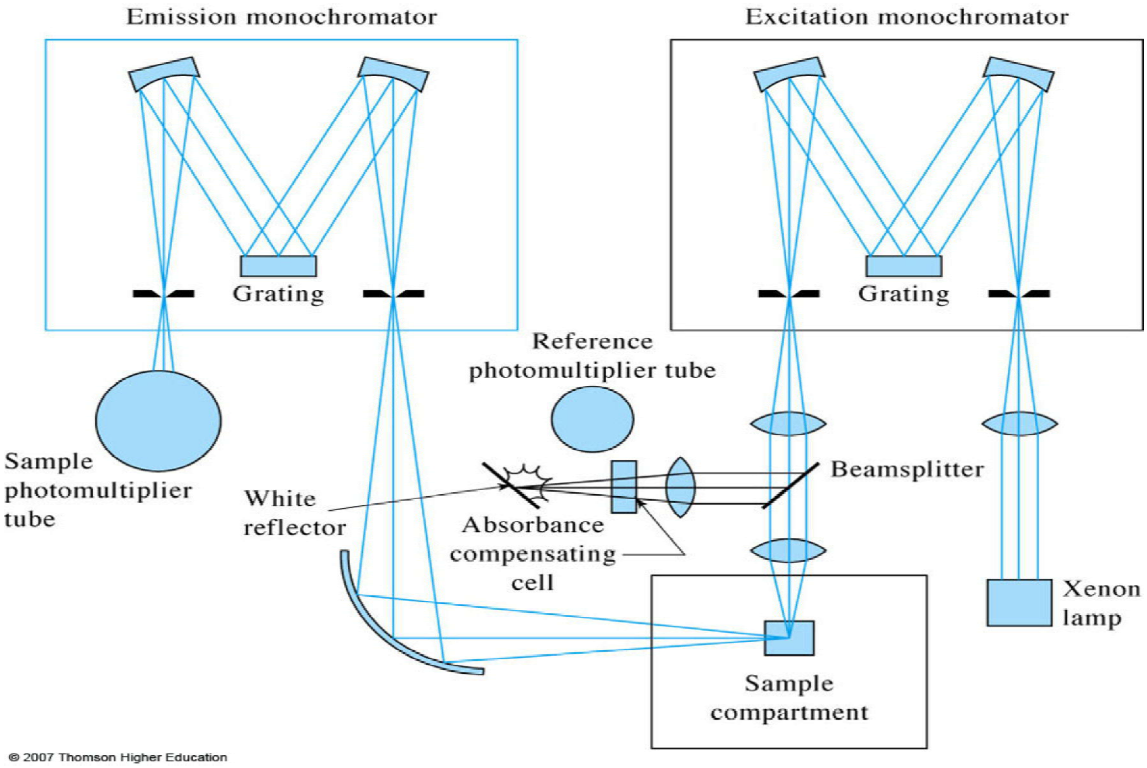
Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No. 18***

Name of topic/lesson – Fluorimetry and phosphorimetry

Instrumentation: Fluorometers and Spectrofluorometers





References: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.461*

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MODERN COLLEGE OF PHARMACY (FOR LADIES), MOSHI, PUNE-412105

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No. 18***

Name of topic/lesson – Fluorimetry and phosphorimetry

Instrumentation: Fluorometers and Spectrofluorometer

Components

Sources: *The most common source for filter fluorometers is a lowpressure mercury vapor lamp equipped with a fused silica window. This source produces useful lines for exciting fluorescence at 254, 302, 313, 546, 578, 691, and 773 nm. Individual lines can be isolated with suitable*

absorption or interference filters. Since fluorescence can be induced in most fluorescing compounds by a variety of wavelengths, at least one of the mercury lines ordinarily proves suitable. In spectrofluorometers, where a source of continuum radiation is required, a 75- to 450-W high-pressure xenon arc lamp is often employed as a source.. The spectrum from this type of lamp is a continuum over the range of about 300 to 1300 nm, approximating that of a blackbody. Some instruments provide higher intensity pulses of light by discharging a capacitor through the lamp at a constant frequency. Another advantage of this design is that signals output by the transducers are then alternating current (ac) signals, which are more readily amplified and processed. Beginning in the 1970's, various types of lasers have also been

used as excitation sources. These sources are more costly, but can be

advantageous when samples are very small (as in microbore chromatography and capillary electrophoresis), when remote sensing is needed, or when a highly monochromatic excitation is needed to minimize the effects of fluorescing interference.

Cells and cell compartments: *both cylindrical and rectangular cells of glass or silica are employed. It is also more critical than in absorbance measurements to avoid fingerprints on cell surfaces since skin oils are often themselves sources of fluorescing agents. Cell compartments must be carefully designed to reduce the amount of scattered radiation, and this is often accomplished by use of baffles.*

Filters and monochrometers: *fluorometers generally employ either absorption or interference filters for wavelength selection, while spectrofluorometers are usually equipped with one or sometimes two grating monochrometers.*

References*: 1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.461*

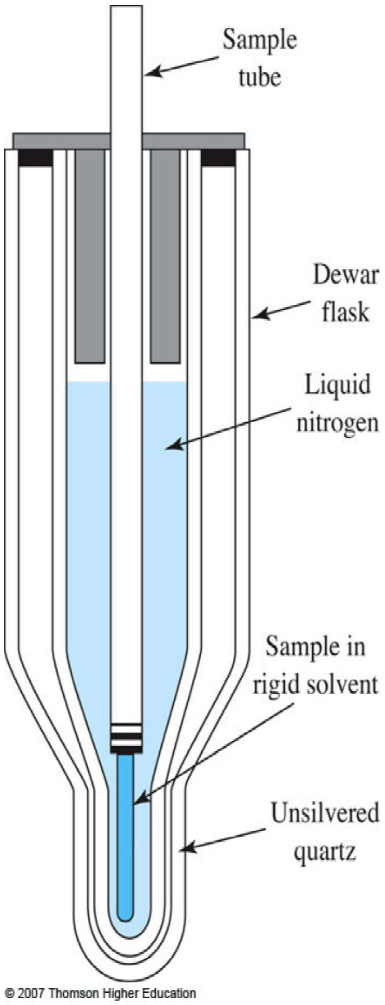
2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.401

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No. 18***

Name of topic/lesson – Fluorimetry and phosphorimetry

Instrumentation: phosphorimetry



Very similar instrumentation to fluorescence except:

1) time delay between excitation and measurement of phosphorescence so no fluorescence is seen

2) sample measured at liquid N_2 temperature (-196 ^{o}C) to minimize collisional deactivation

References*: 1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.461*

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No. 18***

Name of topic/lesson – Fluorimetry and phosphorimetry

Chemiluminescence

Chemilumunescence occurs when a chemical reaction produces an electronically excited product, which then radiates light. This occurs naturally in some biological systems (bioluminescence), such as the firefly and certain jellyfish, bacteria, protozoa, and crustacea. Certain relatively simple organic compounds have also been found to be capable of chemiluminescence

simplest reaction sequence:

$A + B \rightarrow C^* + D$

$C^* \rightarrow C + h\nu$

References*: 1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.469*

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.18***

Name of topic/lesson – Fluorimetry and phosphorimetry

Advantages and Drawbacks of Luminescence Methods

These methods have sensitivities on the order of ten to 1000 times greater that absorption spectroscopy, and detection limits for these methods are typically in the parts per billion range. Photoluminescence methods also have large linear concentrations. This sensitivity can be a drawback if a given sample contains more than one species susceptible to luminescence, but even this "problem" can be turned to an advantage when luminescence measures are combined

with the excellent techniques of separation of modern liquid chromatography and electrophoresis, where fluorescence detectors are often used. Luminescence methods are, however, less widely applicable to quantitative analysis, since the number of molecular species that exhibit photoluminescence upon irradiation with ultraviolet/visible light is much less that the number of species that absorb light in this wavelength range.

References: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.465*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.411

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.18***

Name of topic/lesson – Fluorimetry and phosphorimetry

Applications:

Fluorometric Determination of Inorganic Species

There are two general types of fluorometric methods used in determining inorganic species. Direct methods involve formation of a fluorescing chelate and measurement of its emission. A second, indirect,method involves following the diminution of fluorescence due to the quenching action of the substance under study.

Two factors greatly limit the number of transition-metal ions that form fluorescent chelates:

1) Paramagnetic ions have increased rates of intersystem crossing to the triplet state, which greatly reduces the likelihood of deactivation by fluorescent emission, and 2) transition-metal

complexes tend to have many closely spaced energy levels, which increase the probability of deactivation by internal conversion. Thus it is generally the non-transition metals that have ions with decreased susceptibilities to alternative deactivation pathways that can be determined by these types of fluorometric methods. The best fluorometric reagents for cation analyses have

aromatic structures with two or more donor functional groups that permit chelate formation.

Applications to Organic Species

The number of organic and biochemical species that are susceptible to fluorometric analysis is much greater than inorganic species. More than 200 substances that can be determined by fluorometric methods have been catalogued by Weissler and White, including a wide array of organic compounds, enzymes and coenzymes, medicinal agents, plant products, steroids, and vitamins. The most important applications of fluorometry are in the analyses of food products, pharmaceuticals, clinical samples and natural products.

Luminescence Lifetime Measurements

Measurement of luminescence lifetimes was initially restricted to phosphorescent systems, where decay times are long enough to permit the easy measurement of emission intensity versus time. Modern equipment employing lock-mode lasers to produce pulses of radiation having widths of 70 to 100 ps for excitation, together with fast-rise-time photomultipliers for detection, allow us to study rates of decay on the fluorescent time scale (10-5 to 10-8 s). This type of instrument is useful in studies of energy transfer and quenching, as well as in studies involving mixtures of two or more luminescent species with differing decay rates.

References*: 1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.465*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.411

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.19***

Name of topic/lesson – Fluorimetry and phosphorimetry

Applications:

Analysis for Inorganic Species in the Liquid Phase

Using Chemiluminescence

Many analyses used in the liquid phase use organic chemiluminescing species containing the functional group [functional group structure: p. 425 column 1 middle]

These reagents react with a number of strong oxidizing agents to produce an excited oxidation product.

Fluorometric Determination of H_2O_2 in Water

This method to determine H_2O_2 is based on reaction of scopoletin, a highly fluorescent molecule, with H_2O_2 to produce a non fluorescent product.

scopoletin + H_2O_2 non fluorescing product

I_{ex} = 365 nm I_{em} = 490 nm

Reference: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.465*

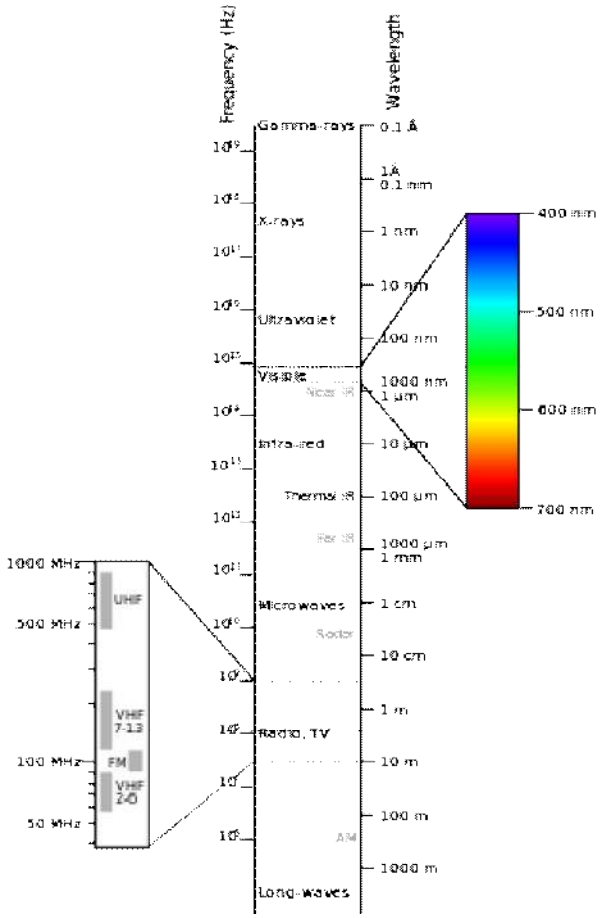
2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.411

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No. 20***

Name of topic/lesson – Electromagnetic spectrum

Types of radiation:



1. *Gamma radiation*
2. *X-ray radiation*
3. *Ultraviolet radiation*
4. *Visible radiation*
5. *Infrared radiation*
6. *Microwave radiation*
7. *Radio waves*

References: *1.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.1.2*

2. Principles of Instrumental Analysis by Skoog, 5th edition,p.no .14

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.21***

Name of topic/lesson – Electromagnetic spectrum

-molecular analysis,

-elemental analysis

-classification of instrumental methods.

UV

NMR

IR

MASS

AAS

Flame

Nephelometry

Fluorimetry & phosphorimetry

Reference: *1.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.1.11*

2. Principles of Instrumental Analysis by Skoog, 5th edition,p.no .14

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.22***

Name of topic/lesson – *Ultraviolet and Visible absorption spectroscopy*

Origin and theory of UV spectra

The UV-Vis Range

UV-Vis Spectroscopy comprises:

- *< 200 nm (Vacuum UV)*
- *200 nm - 450 nm (UV)*
- *400 - 750 nm (visible).*

- *The way electromagnetic radiation affects atoms and molecules depends on the energy of the light. The energy is, in turn, dependent upon the frequency of the light. Ultraviolet and visible light promotes electrons into higher energy orbital. Infrared light excites atomic and molecular vibrations. Microwaves excite atomic and molecular rotation.*

- *Spectrophotometry is the determination of a molecule or compound's identity. It also allows scientists to measure the amount of substance present in a sample. Spectrophotometry in the ultraviolet and visible spectrums has many applications in numerous scientific fields.*

- *Ultraviolet absorption spectra arise from transition of electron or electrons within a molecule or an ion from a lower to a higher electronic energy level and the ultraviolet emission spectra arise from the reverse type of transition. For radiation to cause electronic excitation, it must be in the UV region of the electromagnetic spectrum.*

Reference: 1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no .378

2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.149

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.23***

Name of topic/lesson – *Ultraviolet and Visible absorption spectroscopy*

Principle

Ultraviolet absorption spectra arise from transition of electron or electrons within a molecule or an ion from a lower to a higher electronic energy level and the ultraviolet emission spectra arise from the reverse type of transition. For radiation to cause electronic excitation, it must be in the UV region of the electromagnetic spectrum.

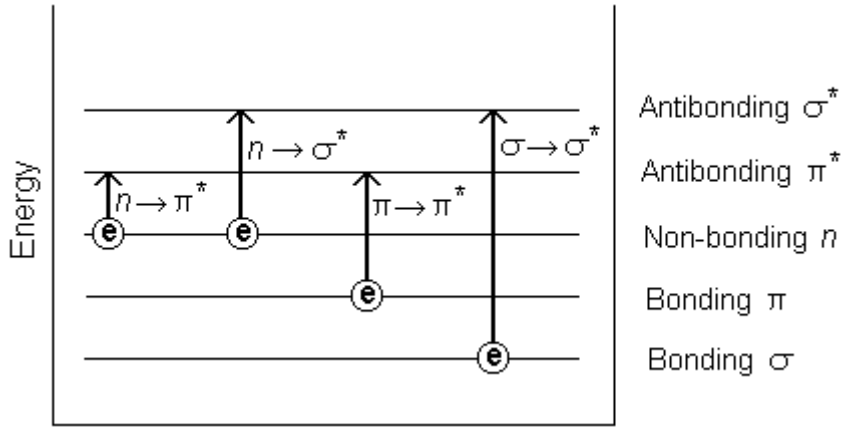
When a molecule absorbs ultraviolet radiation of frequency v sec^{-1}, the electron in that molecule undergoes transition from a lower to a higher energy level or molecule orbital, the energy difference is given by

$$E=hv \text{ erg} \quad - (1)$$

The actual amount of energy required depends on the difference in energy between the ground state E_0 and excited state E_1 of the electrons. Equation (1) becomes as

$$E_1-E_0=hv$$

We know that the total energy of a molecule is equal to the sum of electronic, vibrational and rotational energy. The magnitude of these energies decreases in the following order. E_{elec}, E_{vib}, and E_{rot}.



Reference: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no .378*

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.24***

Name of topic/lesson – *Ultraviolet and Visible absorption spectroscopy*

BEERS LAW.

When a beam of monochromatic light is passing through the transparent medium then decrease in intensity of incident light is directly proportional to concentration of analyte.

LAMBERTS LAW.

When a beam of monochromatic light is passing through the transparent medium then decrease in intensity of incident light is directly proportional to thickness of medium.

Deviations:

Reference: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no .379*

2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.112

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.24***

Name of topic/lesson – *Ultraviolet and Visible absorption spectroscopy*

n→π TRANSITION.*

These types of transitions are shown by unsaturated molecules which contain atoms such as oxygen, nitrogen and sulphur. These transitions exhibit a weak band in their absorption spectrum.

Example: In aldehydes and ketones the band due to the n→π transition generally occurs in the range 270-300 nm. On the other hand, carbonyl compounds having double bonds separated by two or more single bonds exhibit the bands due to the n→π* transitions in the range 300-350 nm.*

π→π TRANSITION.*

A π→π transition corresponds to the promotion of an electron from a bonding π orbital to an antibonding π* orbital. This transition can in principle occur in any molecule having a π electron system. However, selection rule are known which are based on symmetry concepts. These rules determine whether a transition to a particular π* orbital is allowed or forbidden.*

EXAMPLE :For instance, the spectrum of ethylene exhibits an intense band at 174 nm and the weak band at 200 nm. Both of these are due to π→π transitions.*

n→σ TRANSITION: Saturated compounds with lone pair (non-bonding) electrons undergo n→σ* transitions in addition to σ→σ* transitions. The energy required for an n→σ* transition is generally less than the required for a σ→σ* transition and the corresponding absorption bands appear at longer wavelengths in the near ultraviolet (180-200 nm) region. However, some compounds are known which absorb at slightly longer wavelength.*

EXAMPLE:$(CH_3)_3N$, λ_{max}=227 nm for n→σ and σ→σ* for this molecule occurs at 99 nm. When absorption measurements are made in the ultraviolet region, compounds such as a aliphatic alcohols and alkyl halides are commonly used as solvents because they start to absorb at 260 nm.*

σ→σ TRANSITION:These transitions can occur in such compounds in which all the electrons involved in single bonds and there are no lone pairs of electrons. Examples involving such transitions are saturated hydrocarbons. As the energy required for σ→σ* transition is very large, the absorption band occurs in the far ultraviolet region (126-135 nm).*

Reference: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no .378*

2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.150-2.151

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No. 25***

Name of topic/lesson – *Ultraviolet and Visible absorption spectroscopy*

CHROMOPHORE

The term chromophore was previously used to denote a functional group or some other structural feature the presence of which gives a colour to a compound.

EXAMPLE

Nitro group is a chromophore because its presence in a compound gives yellow colour to the compound. But these days the term chromophore is used in a much broader sense which may be defined as any group which exhibits absorption of electromagnetic radiations in the visible or ultraviolet region. It may or may not impart any colour to the compound.

Some of the important chromophores are ethylenic, acetylenic, carbonyls, acids, esters, nitrile group etc. A carbonyl group is important chromophores, although the absorption of light by an isolated group does not give rise to any colour in the ultraviolet spectroscopy.

Reference*: 1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no .411*

2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.156

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No. 25***

Name of topic/lesson – *Ultraviolet and Visible absorption spectroscopy*

CHANGES IN POSITION AND INTENSITY OF ABSORPTION

For isolated chromophore groups such as =C=C= and –C≡C−, absorption takes place in far ultraviolet region which cannot be easily studied. But the position of absorption maximum and the intensity of absorption can be modified in different way by some structural changes or change of solvent as given below.

(1) BATHOCHROMIC SHIFT OR RED SHIFT

It involves the shift of absorption maximum towards longer wavelength because of the presence of certain groups such as OH and NH2 called auxochromes or by change of solvent.

Example: Decreasing polarity of solvent causes a red shift in the n→π absorption of carbonyl compounds bathochromic shift is also produced when two or more chromophores are present in conjugation in a molecule.*

Example: ethylene shows a π→π transition at 170 nm.*

(2) hypsochromic shift or blue shift: this effect involves the shift of absorption maximum towards shorter wavelength and may be caused by removal of conjugation in a system or by change of solvent. The absorption shift towards shorter wavelength is also called blue shift.

(3) hyperchromic effect:This effect involves an increase in the intensity of absorption and is usually brought about by introduction of an auxochrome.

(4) hypochromic effect: it involves a decrease in the intensity of absorption and is brought by groups which are able to distort the geometry of the molecule.

Example: when a methyl group is introduced in position 2 of biphenyl group hypochromic effect occurs because of distortion caused by methyl group.

Auxochrome: It is a group which itself does not acts as a chromophore but when attached to a chromophore it shifts the adsorption maximum towards longer wavelength along with an increase in the intensity of absorption. Some commonly known auxochromic groups are –OH, -NH_2, -OR, -NHR, and NR_2. EX-When the auxochrome –NH_2 group is attached to benzene ring, its absorption changes from λmax255 to λmax 280.

Reference: *1.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.149*

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.26***

Name of topic/lesson – *Ultraviolet and Visible absorption spectroscopy*

Effect of Conjugation

A comparison of the absorption spectrum of 1-pentene, λ_{max} = 178 nm, with that of isoprene (above) clearly demonstrates the importance of chromophore conjugation. Further evidence of this effect is shown below. The spectrum on the left illustrates that conjugation of double and triple bonds also shifts the absorption maximum to longer wavelengths. From the polyene spectra displayed in the center diagram, it is clear that each additional double bond in the conjugated pi-electron system shifts the absorption maximum about 30 nm in the same direction. Also, the molar absorptivity (ε) roughly doubles with each new conjugated double bond. Spectroscopists use the terms defined in the table on the right when describing shifts in absorption. Thus, extending conjugation generally results in bathochromic and hyperchromic shifts in absorption.

The appearance of several absorption peaks or shoulders for a given chromophore is common for highly conjugated systems, and is often solvent dependent. This fine structure reflects not only the different conformations such systems may assume, but also electronic transitions between the different vibrational energy levels possible for each electronic state. Vibrational fine structure of this kind is most pronounced in vapor phase spectra, and is increasingly broadened and obscured in solution as the solvent is changed from hexane to methanol.

Reference: 1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no .385

2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.160

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.27***

Name of topic/lesson – UV

SOLVENT EFFECT

Solvents play an important role in UV spectra. Compound peak could be obscured by the solvent peak. So a most suitable solvent is one that does not itself get absorbed in the region under investigation. A solvent should be transparent in a particular region. A dilute solution of sample is always prepared for analysis.

CHOICE OF SOLVENT

A suitable solvent for ultraviolet spectroscopy should meet the following requirements.

a. It should not itself absorb radiations in the region under investigation.

b. It should be less polar so that it has minimum interaction with the solute molecule.

The most commonly employed solvent is 95% ethanol. It is cheap, has a good dissolving power and does not absorb radiations above 210 nm. It is transparent above 210 nm. Some other solvents which are transparent above 210 nm are n-hexane, cyclohexane, methanol, water and ether. Benzene, chloroform and carbon tetrachloride cannot be used because they absorb in the range of about 240 to 280 nm. Hexane and other hydrocarbons are sometimes preferred to polar solvents because they have minimum interactions with the solute molecules.

Reference: 1.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.160

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.28***

Name of topic/lesson – UV

INSTRUMENTATION

Instruments for measuring the absorption of U.V. or visible radiation are made up of the following components;

1. Sources (UV and visible)

2. Filter or monochromator

3. Sample containers or sample cells

4. Detector

Radiation sources:

1. Tungsten lamp

2. Hydrogen discharge lamp

3. Deuterium lamp

4. Xenon discharge lamp

5. Mercury arc

In ultraviolet spectrometers, the most commonly used radiation sources are hydrogen or deuterium lamps, the xenon discharge lamps and mercury arcs. In all sources, excitation is done by passing electrons through a gas and these collisions between electrons and gas molecules may result in electronic, vibrational and rotational excitation in the gas molecules. When the pressure of the gas is low, only line spectra are emitted. But, if the pressure of a gas is high, band spectra and continuous spectra will be obtained.

The following are requirements of a radiation source.

a) It must be stable.

b) It must be of sufficient intensity for the transmitted energy to be detected at the end of the optical path.

c) It must supply continuous radiation over the entire wavelength region in which it is used

Reference: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no .396*

2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.167

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.29***

Name of topic/lesson – UV

2) MONOCHROMATORS.

All monochromators contain the following component parts;

- *An entrance slit*
- *A collimating lens*
- *A dispersing device (a prism or a grating)*
- *A focusing lens*
- *An exit slit*

Polychromatic radiation (radiation of more than one wavelength) enters the monochromator through the entrance slit. The beam is collimated, and then strikes the dispersing element at an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing element or the exit slit, radiation of only a particular wavelength leaves the monochromator through the exit slit.

The monochromator is used to disperse the radiation according to the wavelength. The essential elements of a monochromator are an entrance slit, a dispersing element and an exit slit. The entrance slit sharply defines the incoming beam of heterochromatic radiation. The dispersing element disperses the heterochromatic radiation into its component wavelengths whereas exit slit allows the nominal wavelength together with a band of wavelength s on either side of it. The position of the dispersing element is always adjusted by rotating it to vary the nominal wavelength passing through the exit slit.

The dispersing element may be a prism or grating. The prisms are generally made of glass, quartz or fused silica. Glass has the highest revolving power but it is not transparent to radiations having the wavelength between 2000 and 3000A^0 because glass absorbs strongly in this region. Quartz and fused silica prisms which are transparent throughout the entire UV range are widely used in UV spectrophotometers.

Fused silica prisms are little more transparent in the short wavelength region than quartz prisms and are used only when very intense radiation is required. The mirrors in the optical system are front surfaced because glass starts to absorb in the ultraviolet region.

Reference: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no .396*

2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.16

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.30***

Name of topic/lesson – UV

3).DETECTORS.

In order to detect radiation, three types of photosensitive devices are,

1. Photovoltaic cells or barrier- layer cell

2. Phototubes or photoemissive tubes

3. Photomultiplier tubes

1. BARRIER LAYER CELL2. PHOTOCELL

It consists of a high sensitive cathode in the form of a half cylinder of metal which is contained in an evacuated tube. The anode is also present in the tube which is fixed more or less along the axis of the tube. The inside surface of the photocell is coated with a light sensitive layer.

2. PHOTOCELL

It consists of a high sensitive cathode in the form of a half cylinder of metal which is contained in an evacuated tube. The anode is also present in the tube which is fixed more or less along the axis of the tube. The inside surface of the photocell is coated with a light sensitive layer.

3. PHOTOMULTIPLIER TUBE.

A photomultiplier tube is generally used as a detector in UV spectrophotometers. A photomultiplier tube is a combination of a photodiode and an electron multiplying amplifier. A photomultiplier tube consists of an evacuated tube which contains one photo- cathode and 9-16 electrodes known as dynodes. The surface of each dynode is of Be-Cu, Cs-Sb or similar material.

This cell is also known as photovoltaic cell. The barrier cell consists of a semiconductor, such as selenium, which is deposited on a strong metal base, such as iron. Then a very thin of silver or gold is sputtered over the surface of the semiconductor to act as a second collector electrode.

Reference: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no .392*

2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.167

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.31***

Name of topic/lesson – UV

SAMPLE CELLS.

The cells that are to contain samples for analysis should fulfil three main conditions:

1) They must be uniform in construction; the thickness must be constant and surface facing the incident light must be optically flat.

2) The material of construction should be inert to solvents.

3) They must transmit light of the wavelength used.

The most commonly used cells are made of quartz or fused silica. These are readily available even in matched pairs where sample cell is almost identical to the reference cell. A variety of sample cells available for UV region. The choice of sample cell is based on

a) the path length, shape, size

b) the transmission characteristics at the desired wavelength

c) the relative expense

MATCHED CELLS.

When double- beam instrumentation is used, two cells are needed, one for the reference and one of the sample. It is normal for the absorption by these cells to differ slightly.

RECORDING SYSTEM.

The signal from the photomultiplier tube is finally received by the recording system. The recording is done by recorder pen. The type of arrangement is only done in recording UV spectrophotometers.

Reference: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no .392*

2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.167

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.32-35***

Name of topic/lesson – UV

TYPES OF UV SPECTROPHOTOMETER (2)

1) SINGLE-BEAM INSRUMENT

2) DOUBLE- BEAM INSRUMENT

1) SINGLE-BEAM INSTRUMEN

In the single-beam system, UV radiation is given off by the source. A convex lens gathers the beam of radiation and focuses it on the inlet slit. The inlet slit permits light from the source to pass, but blocks out stray radiation. The light then reaches the monochromator, which splits it up according to wavelength. The exit slit is positioned to allow light of the required wavelength to pass through. Radiation at all other wavelengths is blocked out. The selected radiation passes through the sample cell to the detector, which measures the intensity of the radiation reaching it. By comparing the intensity of radiation before end after it passes through the sample, it is possible to measure how much radiation is absorbed by the sample at the particular wavelength used. The output of the detector is usually recorded on graph paper.

2) DOUBLE- BEAM INSRUMENT

Many modern photometers and spectrophotometers are based on a double- beam design. A double-beam-in-space instrument in which two beams are formed in space by a V-shape mirror called a beam splitter. One beam passes through the reference solution to a photo detector, and the second simultaneously traverses the sample to a second, matched detector. The two outputs are amplified, and their ratio is determined electronically or by a computer and displayed by the readout device. With manual instruments the measurement is a two step operation involving first the zero adjustment with a shutter in place between selector and beam splitter. In the second step, the shutter is opened and the transmittance or absorbance is displayed directly.

ADVANTAGES OF DOUBLE BEAM INSTRUMENTS

APPLICATIONS

Reference: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no .392,411*

2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.172

For the purpose of spectral analysis in order to relate chemical structure to electronic transitions, and for analytical situations in which mixture contribute interfering absorption, a method of manipulating the spectral data is called derivative spectroscopy. Derivative spectrophotometry involves the conversions of a normal spectrum to its first, second or higher derivative spectrum. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zero order, or D 0 spectrum.

(a) Simultaneous equation method

(b) Absorbance ratio method

(c)Derivative spectrophotometry

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.36***

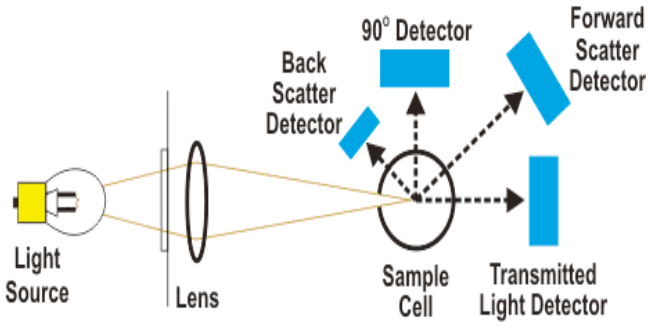
Name of topic/lesson – *Nephelometry and Turbidometry*

Introduction

- *When electromagnetic radiation (light) strikes a particle in solution, some of the light will be absorbed by the particle, some will be transmitted through the solution and some of the light will be scattered or reflected.*
- *The amount of light scattered is proportional to the concentration of insoluble particle. We will focus on the concept of light scatter*

-Scattered light may be measured by Turbidimetry & Nephelometry

In turbidimetry, the intensity of light transmitted through the medium, the unscattered light, is measured.



Reference*: 1.Pharmaceutical drug analysis by Ashutosh Kar,2nd edition,P.No. 283*

2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.389

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

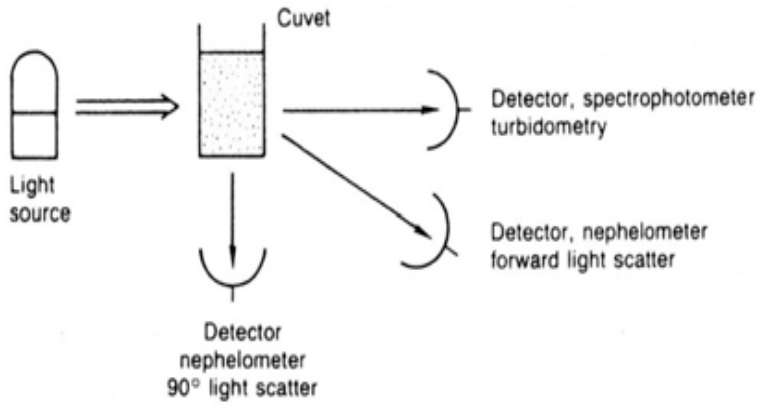
Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.37***

Name of topic/lesson – *Nephelometry and Turbidometry*

Turbidometric measurements are made at 180° from the incident light beam.

In Nephelometry, the intensity of the scattered light is measured, usually, but not necessarily, at right angles to the incident light beam

- *The two techniques differs only in the manner of measuring the scattered radiation.*



- *Light scattering is the physical phenomenon resulting from the interaction of light with a particles in solution. It Dependent on :*
- *Particle size*
- *Wavelength*
- *Distance of observation,*
- *Concentration of particles*
- *Mole.Wt. of particles*

Reference*: 1.Pharmaceutical drug analysis by Ashutosh Kar,2nd edition,P.No. 290*

2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.389

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.38***

Name of topic/lesson – *Nephelometry and Turbidometry.*

Instrumentation,

- *The basic instrument contains*
- *Light Source:Tungsten lamp,*

White light - nephelometers

- *Filters - Turbidimeter (blue filter or 530 nm)*

  Nephelometer (visible filter)
- *Sample cells*
- *Detectors (photometric)*
- ***CELLS***
- *cylindrical cells - flat faces to minimize reflections & multiple scatterings*

Applications.

1. *Analysis of water*

   clarity, conc. of ions
2. *Determination of CO_2*
3. *Determination of inorganic substances*

   Sulphate – barium chloride

   Ammonia – Nesslers reagent

   Phosphorus – Strychine molybedate
4. *Biochemical Analysis*
5. *Quantitative Analysis – (ppm level)*

Reference*: 1.Pharmaceutical drug analysis by Ashutosh Kar,2nd edition,P.No. 290*

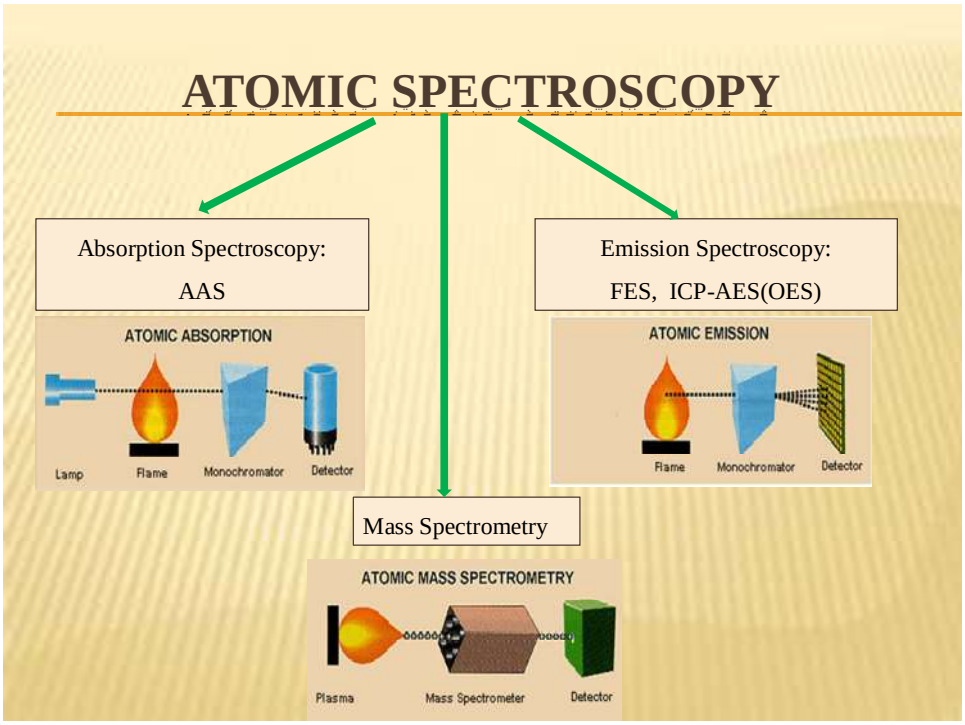
2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.389

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.39***

Name of topic/lesson – *Atomic Emission spectroscopy*

Atomic Emission spectroscopy



Class- Third Yr. B. Pharm **Subject-Pharmaceutical Analysis I**

Subject Incharge-Mr.P.R.Jadhav **Lecture Synopsis No.40**

Name of topic/lesson – *Atomic Emission spectroscopy*

ATOMIC SPECTROSCOPY

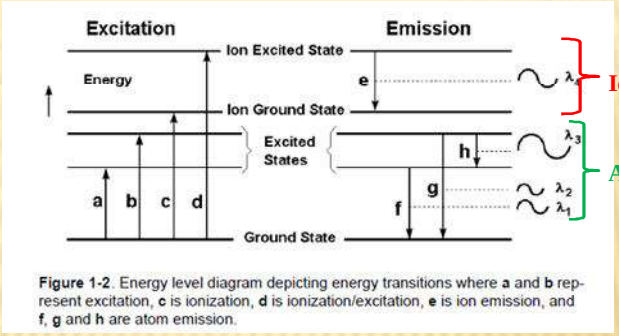


Figure 1-2. Energy level diagram depicting energy transitions where **a** and **b** represent excitation, **c** is ionization, **d** is ionization/excitation, **e** is ion emission, and **f**, **g** and **h** are atom emission.

$E = hc/\lambda$

E – energy difference between two levels;

h – Plank's constant, 6.626068 10^{-34} m^2kg/s;

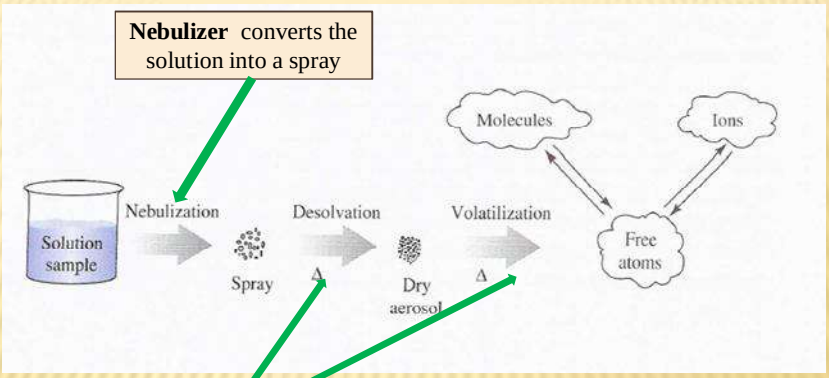
c – speed of light, 299 792 458 m/s;

λ – wavelenght, nm

After Boss. C.B. and Freden K.J. Concepts, Instrumentation and Techniques in Inductively Coupled Plasma Optical Emission Spectrometry. 1997

ATOMIC SPECTROSCOPY

Nebulizer converts the solution into a spray



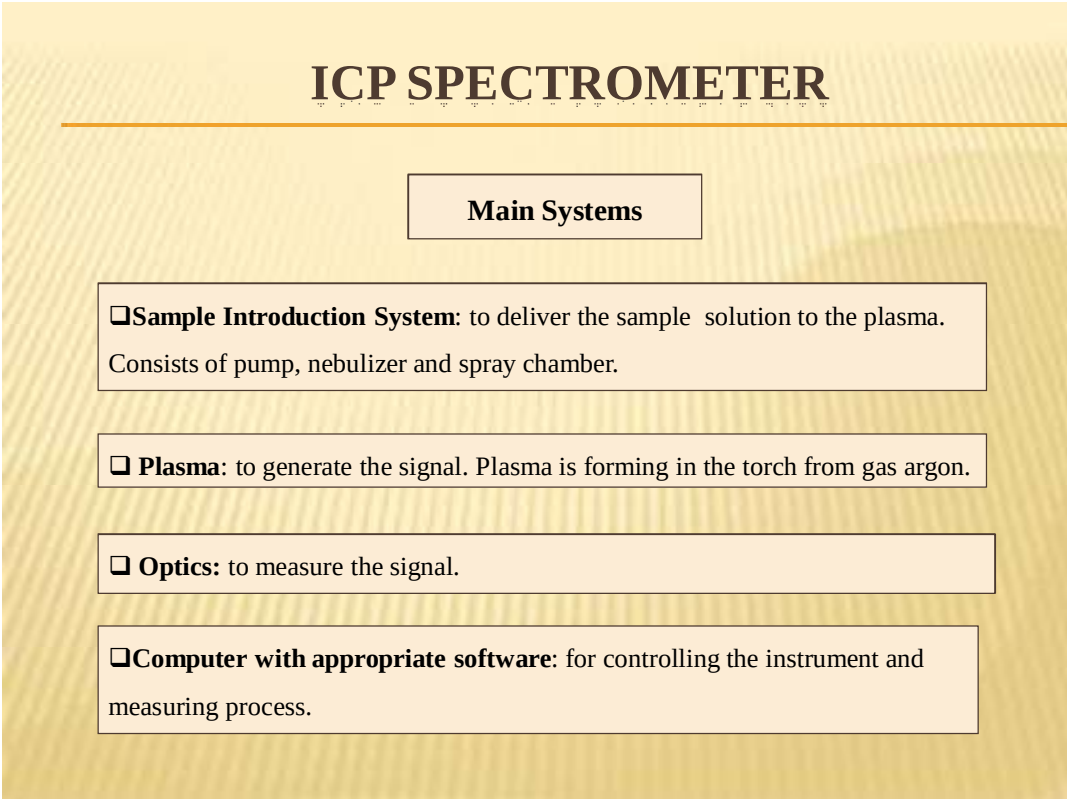
Flame (or Plasma) causes the solvent to evaporate, leaving dry aerosol particles, then volatilizes the particles, producing atomic, molecular and ionic species

After Skoog D. Fundamentals of Analytical Chemistry, 2004, p. 844

- Atomic emission spectroscopy measures the intensity of light emitted by atoms or ions of the elements of interest at specific wavelengths;

- Inductively Coupled Plasma spectrometers use emission spectroscopy to detect and quantify elements in a sample;
- ICP-AES uses the argon plasma (6000-10000º C) for atomization and excitation of the sample atoms;

ICP-AES determines approximately all of the elements except gases and some non-metals (C, N, F, O, H).



ICP-AES: Plasma

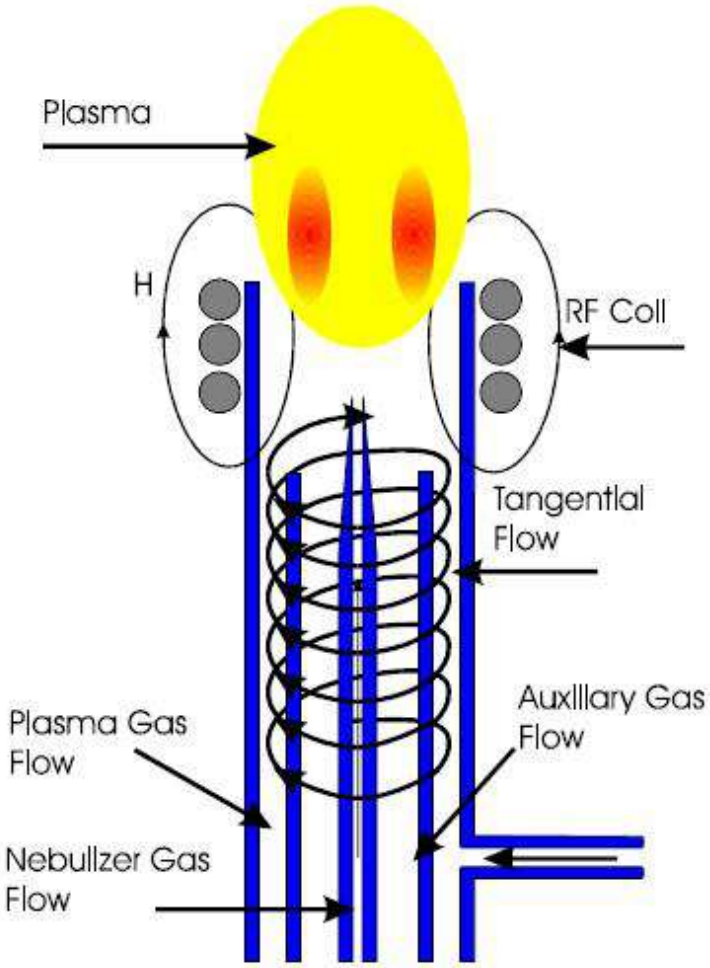
A **plasma** is a hot, partially ionized gas. It contains relatively high concentrations of ions and electrons.

Argon ions, once formed in a plasma, are capable of absorbing sufficient power from an external source to maintain the temperature at a level at which further ionization sustains the plasma indefinitely. The plasma temperature is about 10 000 K.

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.41***

Name of topic/lesson – *Atomic Emission spectroscopy*

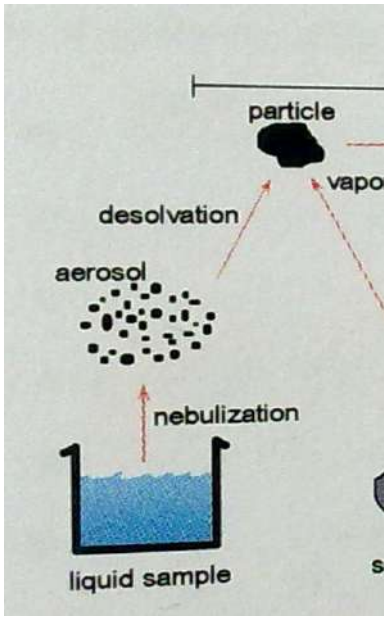


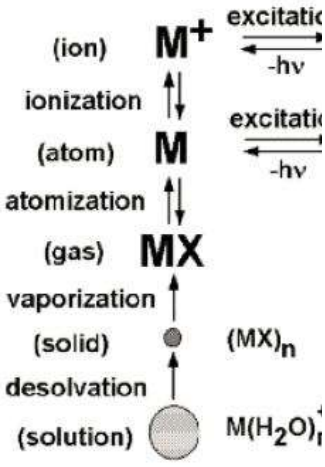
Inductively Coupled Plasma and its process

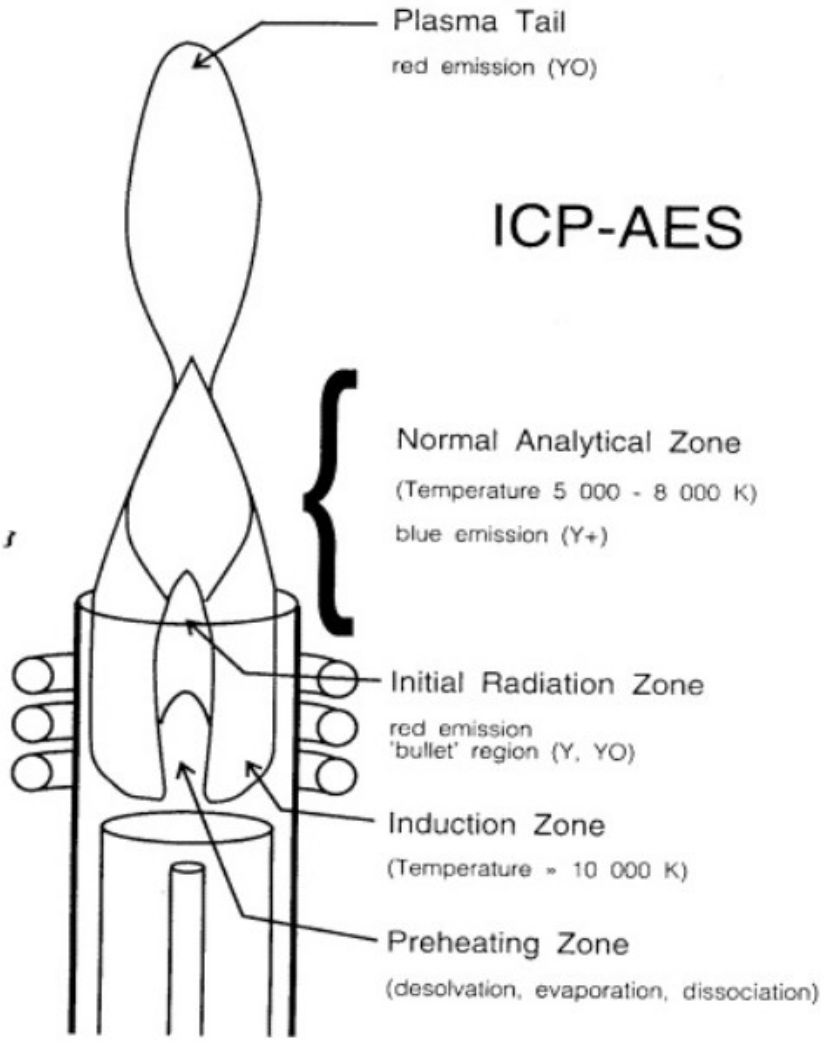
Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.42,43***

Name of topic/lesson – *Atomic Emission spectroscopy*







Application:

- **Clinical Analysis:** metals in biological fluids (blood, urine);
- **Environmental Analysis:** trace metals and other elements in waters, soils, plants, composts and sludges;
- **Pharmaceuticals:** traces of catalysts used; traces of poison metals (Cd, Pb etc);
- **Industry:** trace metal analysis in raw materials; noble metals determination.
- **Forensic science:** gunshot powder residue analysis, toxicological examination

  (e.g., thallium (Tl) determination)

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.44***

Name of topic/lesson – *Analytical Sample preparation techniques*

SAMPLE PREPARATION:

THE MEASUREMENT PROCESS

The purpose of an analytical study is to obtain information about some object or substance. The substance could be a solid, a liquid, a gas, or a biological material. The information to be obtained can be varied. It could be the chemical or physical composition, structural or surface properties, or a sequence of proteins in genetic material. Despite the sophisticated arsenal of analytical techniques available, it is not possible to find every bit of information of even a very small number of samples. For the most part, the
state of current instrumentation has not evolved to the point where we can take an instrument to an object and get all the necessary information. Although there is much interest in such noninvasive devices, most analysis is still done by taking a part (or portion) of the object under study (referred to as the sample) and analyzing it in the laboratory (or at the site). Some common steps involved in the process are shown in

The first step is sampling, where the sample is obtained from the object to be analyzed. This is collected such that it represents the original object. Sampling is done with variability within the object in mind. For example, while collecting samples for determination of Ca2þ in a lake, it should be kept in mind that its concentrations can vary depending on the location, the depth, and the time of year.
The next step is sample preservation. This is an important step, because there is usually a delay between sample collection and analysis. Sample preservation ensures that the sample retains its physical and chemical characteristics so that the analysis truly represents the object under study.

Qualitative and Quantitative Analysis

There is seldom a unique way to design a measurement process. Even an explicitly defined analysis can be approached in more than one ways. Different studies have di¤erent purposes, di¤erent financial constraints, and are carried out by sta¤ with di¤erent expertise and personal preferences. The most important step in a study design is the determination of the purpose, and at least a notion of the final results. It should yield data that provide useful information to solve the problem at hand.
The objective of an analytical measurement can be qualitative or quantitative. For example, the presence of pesticide in fish is a topic of concern. The questions may be: Are there pesticides in fish? If so, which ones? An

analysis designed to address these questions is a qualitative analysis, where the analyst screens for the presence of certain pesticides. The next obvious question is: How much pesticide is there? This type of analysis, quantitative analysis, not only addresses the presence of the pesticide, but also its concentration. The other important category is semiqualitative analysis.

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis I***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.45***

Name of topic/lesson – *Analytical Sample preparation techniques*

Analytes	*Sample Preparation*	*Instrumenta*
Organics	*Extraction, concentration, cleanup, derivatization*	*GC, HPLC, GC/MS, LC/MS*
Volatile organics	*Transfer to vapor phase, concentration*	*GC, GC-MS*
Metals	*Extraction, concentration, speciation*	*AA, GFAA, ICP, ICP/MS*
Metals	*Extraction, derivatization, concentration, speciation*	*UV-VIS molecular absorption spectrophotometry, ion chromatography*
Ions	*Extraction, concentration, derivatization*	*IC, UV-VIS*
DNA/RNA	*Cell lysis, extraction, PCR*	*Electrophoresis, UV-VIS, florescence*
Amino acids, fats carbohydrates	*Extraction, cleanup*	*GC, HPLC, electrophoresis*
Microstructures	*Etching, polishing, reactive ion techniques, ion bombardments, etc.*	*Microscopy, surface spectroscopy*

GC, gas chromatography; HPLC, high-performance liquid chromatography; MS, mass spectroscopy; AA, atomic absorption; GFAA, graphite furnace atomic absorption; ICP, inductively coupled plasma; UV-VIS, ultraviolet–visible molecular absorption spectroscopy; IC, ion chromatography.

The concern is not exactly how much is there but whether it is above or below a certain threshold level. The prostate specific antigen (PSA) test for the screening of prostate cancer is one such example. A PSA value of 4 ng/L (or higher) implies a higher risk of prostate cancer. The goal here is to determine if the PSA is higher or lower then 4 ng/L.

Once the goal of the analyses and target analytes have been identified, the methods available for doing the analysis have to be reviewed with an eye to accuracy, precision, cost, and other relevant constraints. The amount of labor, time required to perform the analysis, and degree of automation can also be important.

1.4. PRESERVATION OF SAMPLES

The sample must be representative of the object under investigation. Physical,chemical, and biological processes may be involved in changing the composition of a sample after it is collected. Physical processes that may degrade a sample are volatilization, di¤usion, and adsorption on surfaces.

Possible chemical changes include photochemical reactions, oxidation, and precipitation. Biological processes include biodegradation and enzymatic reactions. Once again, sample degradation becomes more of an issue at low analyte concentrations and in trace analysis.

The sample collected is exposed to conditions di¤erent from the original source. For example, analytes in a groundwater sample that have never been exposed to light can undergo significant photochemical reactions when exposed to sunlight. It is not possible to preserve the integrity of any sample indefinitely. Techniques should aim at preserving the sample at least until the analysis is completed. A practical approach is to run tests to see how long a sample can be held without degradation and then to complete the analysis within that time. Table 1.3 lists some typical preservation methods.

These methods keep the sample stable and do not interfere in the analysis. Common steps in sample preservation are the use of proper containers, temperature control, addition of preservatives, and the observance of recommended sample holding time. The holding time depends on the analyte of interest and the sample matrix. For example, most dissolved metals are stable for months, whereas Cr (VI) is stable for only 24 hours. Holding time can be determined experimentally by making up a spiked sample (or storing an actual sample) and analyzing it at fixed intervals to determine when it begins to degrade.

Name of topic/lesson – *Analytical Sample preparation techniques*

Volatilization

Analytes with high vapor pressures, such as volatile organics and dissolved gases (e.g., HCN, SO2) can easily be lost by evaporation. Filling sample containers to the brim so that they contain no empty space (headspace) is the most common method of minimizing volatilization. Solid samples can be topped with a liquid to eliminate headspace. The volatiles cannot equilibrate between the sample and the vapor phase (air) at the top of the container. The samples are often held at low temperature (4_C) to lower the vapor pressure. Agitation during sample handling should also be avoided. Freezing liquid samples causes phase separation and is not recommended.

Choice of Proper Containers:

The surface of the sample container may interact with the analyte. The surfaces can provide catalysts (e.g., metals) for reactions or just sites for irreversible adsorption. For example, metals can adsorb irreversibly on glass surfaces, so plastic containers are chosen for holding water samples to be analyzed for their metal content. These samples are also acidified with HNO3 to help keep the metal ions in solution. Organic molecules may also interact with polymeric container materials. Plasticizers such as phthalate esters can di¤use from the plastic into the sample, and the plastic can serve as a sorbent (or a membrane) for the organic molecules. Consequently, glass containers are suitable for organic analytes. Bottle caps should have Teflon liners to preclude contamination from the plastic cap.

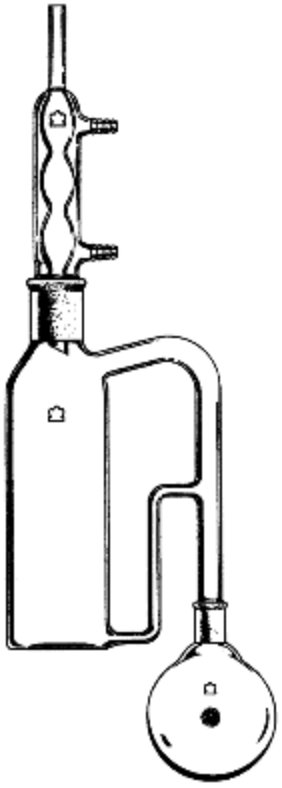
phases together in a separatory funnel (Figure 2.13a). Following mixing, the layers are allowed to separate. Flow from the bottom of the separatory funnel is controlled by a glass or Teflon stopcock and the top of the separatory funnel is sealed with a stopper. The stopper and stopcock must fit tightly

and be leakproof. Commonly, separatory funnels are globe, pear, or cylindrically shaped. They may be shaken mechanically, but are often shaken manually.With the stopcock closed, both phases are added to the separatory funnel. The stopper is added, and the funnel is inverted without shaking. The stop-cock is opened immediately to relieve excess pressure. When the funnel is inverted, the stem should be pointed away from yourself and others. The funnel should be held securely with the bulb of the separatory funnel in the palm of one hand, while the index finger of the same hand is placed over the stopper to prevent it from being blown from the funnel by pressure buildup during shaking. The other hand should be positioned to hold the stopcock end of the separatory funnel, and for opening and closing the stopcock.

The separatory funnel should be gently shaken for a few seconds, and frequently inverted and vented through the stopcock. When pressure buildsup less rapidly in the separatory funnel, the solvents should be shaken more vigorously for a longer period of time while venting the stopcock occasionally.

Name of topic/lesson – *Analytical Sample preparation techniques*

The organic solvent phase containing only weakly acidic and neutral compounds is sequentially back-extracted with an aqueous (pH 10) solution of sodium hydroxide (Figure 2.16, step 4). Neutral compounds remain in the organic solvent phase, while weak organic acids, ionized at this pH, will be extracted into the aqueous phase.



SOLID-PHASE EXTRACTION

The historical development of solid-phase extraction (SPE) has been traced by various authors [60,61]. After a long latency period (from biblical times to 1977) when the theoretical ''science'' of SPE was known but not frequently practiced, technological breakthroughs in sorbents and devices fueled the growth of SPE use that continues today. The modern era of SPE, which resulted in today's exponential growth in applications of this technique,began in 1977 when the Waters Corporation introduced commerciallyavailable, prepackaged disposable cartridges/columns containing bonded silica sorbents. The term solid-phase extraction was coined in 1982 by employees of the J.T. Baker Chemical Company. The most commonly cited benefits of SPE that led to early advances relative to LLE are reduced analysis time, reduced cost, and reduced labor (because SPE is faster and requires less manipulation); reduced organic solvent consumption and disposal [66–68], which results in reduced analyst exposure to organic solvents; and reduced potential for formation of emulsions

in SPE as described above, analysts performing SPE extraction and other analytical procedures must also be concerned with the potential for the analyte's association with particulate and colloidal matter contamination in the sample. Complex equilibria govern partitioning of organic analytes among the solution phase, colloidal material, and suspended particulate matter. Depending on the chemical nature of the analyte and the contamination, some of the analyte molecules can become sorbed to the contaminating particulate and/or colloidal matter in the sample. Analytes can adhere to biological particulates such as cellular debris or bind to colloidal proteins. Similarly, analytes can adhere to environmental particulates or associate with colloidal humic substances. If the sample is not filtered, particulates can partially or entirely elute from the sorbent, leading to both a dissolved and particulate result when the sample is analyzed [

Polar Sorbents

The earliest applications of chromatography, a term coined by Tswett in 1906, used polar sorbents to separate analytes dissolved in nonpolar solvents.Using light petroleum as the nonpolar mobile phase, Tswett separated a colored extract from leaves using column chromatography on a polar calcium carbonate column [78,79]. The alternate system, in which the sorbent is nonpolar while a polar solvent is used, was not used in chromatography until the late 1940s to early 1950s [80–83]. Howard and Martin [83] introduced the term reversed-phase to describe separation of fatty acids using solid-supported liquid para‰n or n-octane as nonpolar stationary phases that were eluted with polar aqueous solvents. At that time, these systems appeared to be ''reversed'' to the ''normal'' arrangement of polar stationary phases used with less polar eluents. Although reversed-phase applications outnumber normal-phase chromatographic applications today, the nomenclature still applies.

The most common polar sorbents used for normal-phase SPE are silica (SiO2)x, alumina (Al2O3), magnesium silicate (MgSiO3 or Florisil), and the bonded silica sorbents in which silica is reacted with highly polar functional groups to produce aminopropyl [(SiO2)xa(CH2)3NH2]-, cyanopropyl [(SiO2)xa(CH2)3CN]-, and diol [(SiO2)xa(CH2)3OCH2CH(OH)CH2(OH)]- modified silica sorbents