Lectures in Instrumental analysis

Lecture 1

Instrumental Analysis

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Analytical Standards

To standardize an analytical method we use standards containing known mounts of analyte. The accuracy of a standardization, therefore, depends on the quality of the reagents and glassware used to prepare these standards. **A primary standard** must have a known stoichiometry, a known purity (or assay), and it must be stable during long-term storage. Because of the difficulty in stablishing the degree of hydration, even after drying, a hydrated reagent usually is not a primary standard. Reagents that do not meet these criteria are **secondary standards**. The concentration of a secondary standard must be determined relative to a primary standard.

Preparing Standard Solutions

It is often necessary to prepare a series of standards, each with a different concentration of analyte. We can prepare these standards in two ways. If the range of concentrations is limited to one or two orders of magnitude, then each solution is best prepared by transferring a known mass or volume of the pure standard to a volumetric flask and diluting to volume. When working with larger ranges of concentration, particularly those extending over more than three orders of magnitude, standards are best prepared by a serial dilution from a single stock solution.

Standardising analytical methods:

Standardization is defined as the process of determining the relationship between the signal and the amount of analyte in a sample. This relationship in equation:

$$S_{\text{total}} = k_A C_A + S_{\text{rcag}}$$

Where S_{total} is the signal, C_A is the analyte's concentration, k_A is the method's sensitivity for the analyte, and S_{reag} is the contribution to S_{total} from sources other than the sample. To standardize a method we must determine values for k_A and S_{reag}

Calibrating the Signal (S_{total})

The accuracy of our determination of k_A and S_{reag} depends on how accurately we can measure the signal, S_{total}. We measure signals using equipment, such as glassware and balances, and instrumentation, such as spectrophotometers and pH meters. To minimize determinate errors affecting the signal, we first calibrate our equipment and instrumentation. We accomplish the calibration by measuring S_{total} for a standard with a known response of S_{std}, adjusting S_{total} until

$$S_{\text{total}} = S_{\text{std}}$$

We also must calibrate our instruments. For example, we can evaluate a spectrophotometer's accuracy by measuring the absorbance of a carefully prepared solution of 60.06 mg/L $K_2Cr_2O_7$ in 0.0050 M H_2SO_4, using 0.0050 M H_2SO_4 as a reagent blank. An absorbance of 0.640 ± 0.010 absorbance units at a wavelength of 350.0 nm indicates that the spectrometer's signal is properly calibrated. Be sure to read and carefully follow the calibration instructions provided with any instrument you use.

Determining the Sensitivity (k_A)

The simplest way to determine the value of k_A in equation is by a single-point standardization in which we measure the signal for a standard, S_{std}, containing a known concentration of analyte, C_{std}.

$$k_A = \frac{S_{std}}{C_{std}}$$

Having determined the value for kA, we can calculate the concentration of analyte in any sample by measuring its signal, S_{samp}, and calculating C_A using the equation below.

$$C_A = \frac{S_{samp}}{k_A}$$

Types of Calibration methods

1- External standard method

Figure 1 shows a typical multiple-point external standardization. The volumetric flask on the left is a reagent blank and the remaining volumetric flasks contain increasing concentrations of Cu2+. Shown below the volumetric flasks is the resulting calibration curve. Because this is the most common method of

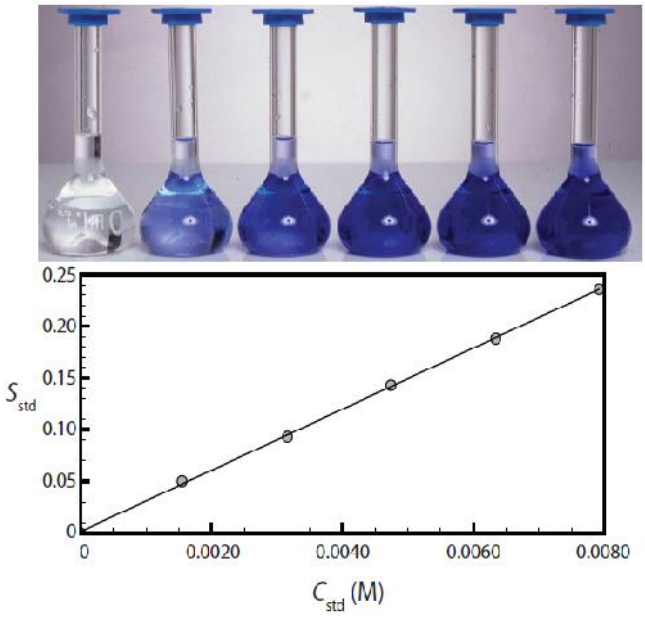

Figure 1 Shown at the top is a reagent blank (far left) and a set of five external standards for Cu2+ with concentrations increasing from left to right.

standardization the resulting relationship is called a normal calibration curve. This is the most desirable situation since the method's sensitivity remains constant throughout the nalyte's concentration range.

2- Standard Additions

This method is used in situations where sample matrix also contributes to the analytical signal, a situation known as the matrix effect, thus making it impossible to compare the analytical signal between sample and standard using the traditional calibration curve approach. This is known as the method of **standard additions**. A typical procedure involves preparing several solutions containing the same amount of unknown, but different amounts of standard. For example, five 25 mL volumetric flasks are each filled with 10 mL of the unknown. Then the standard is added in differing amounts, such as 0, 1, 2, 3, and 4 mL. The flasks are then diluted to the mark and mixed well.

3- Internal Standards

If our analyte is in a volatile solvent, then its concentration increases when we lose solvent to evaporation. The internal standard is a compound that is very similar, but not identical to the chemical species of interest in the samples.

Suppose we have a sample and a standard with identical concentrations of analyte and identical signals. If both experience the same proportional loss of solvent then their respective concentrations of analyte and signals continue to be identical. In effect, we can ignore evaporation if the samples and standards experience an equivalent loss of solvent. If an identical standard and sample lose different amounts of solvent, however, then their respective concentrations and signals will no longer be equal. In this case a simple external standardization or standard addition is not possible.

Instrumental chemical analysis

The aims of instrumental chemical analysis are the same as those of qualitative and quantitative chemical analysis; the difference is that instrumental techniques are used instead i.e. equipment which has been specially-designed to measure specific phenomena

Overview of Spectroscopy

The focus of this semester is on the interaction of ultraviolet, visible, and infrared radiation with matter. Because these techniques use optical materials to disperse and focus the radiation, they often are identified as optical spectroscopies. For convenience we will use the simpler term **spectroscopy** in place of optical spectroscopy; however, you should understand that we are considering only a limited part of a much broader area of analytical techniques.

Despite the difference in instrumentation, all spectroscopic techniques share several common features.

What is Electromagnetic Radiation?

Electromagnetic radiation—light—is a form of energy whose behaviour is described by the properties of both waves and particles. Some properties of electromagnetic radiation, such as its refraction when it passes from one medium to another are explained best by describing light as a wave. Other properties, such as absorption and emission, are better described by treating light as a particle. The exact nature of electromagnetic radiation remains unclear, as it has since the development of quantum mechanics in the first quarter of the 20^{th} century. Nevertheless, the dual models of wave and particle behaviour provide a useful description for electromagnetic radiation.

Wave Properties of Electromagnetic Radiation

Electromagnetic radiation consists of oscillating electric and magnetic fields that propagate through space along a linear path and with a constant velocity. In a vacuum electromagnetic radiation travels at the speed of light, *c*, which is 2.997 92 × 10^8 m/s (3.00 × 10^8 m/s, is accurate enough for most purposes).

The oscillations in the electric and magnetic fields are vertical to each other, and to the direction of the wave's propagation. Figure 2 shows an example of plane-polarized electromagnetic radiation, consisting of a single oscillating electric field and a single oscillating magnetic field.

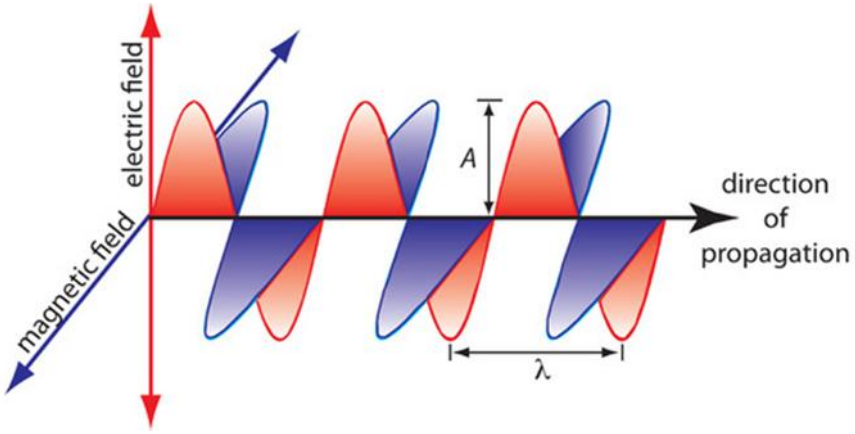

Figure 2: Plane-polarized electromagnetic radiation showing the oscillating electric field in red and the oscillating magnetic field in blue.

Particle Properties of Electromagnetic Radiation

When matter absorbs electromagnetic radiation it undergoes a change in energy. The interaction between matter and electromagnetic radiation is easiest to understand if we assume that radiation consists of a beam of energetic particles called photons. When a **photon** is absorbed by a sample it is "destroyed," and its energy acquired by the sample. The energy of a photon, is related to its frequency, wavelength, and wavenumber by the following equalities

$$E = h\nu = \frac{hc}{\lambda} = hc\bar{\nu}$$

Where h is Planck's constant, which has a value of 6.626×10^{-34}

Energy (E) / frequency (ν), Wavelength of the wave (λ), Wavenumbers ($\bar{\upsilon}$) which is the reciprocal of wavelength. Wavenumbers are frequently used to characterize infrared radiation, with the units given in cm^{-1}.

The Electromagnetic Spectrum

The frequency and wavelength of electromagnetic radiation vary over many orders of magnitude. For convenience, we divide electromagnetic radiation into different regions—the **electromagnetic spectrum**—based on the type of atomic or molecular transition that gives rise to the absorption or emission of photons (Figure 3). The limit between the regions of the electromagnetic spectrum are not rigid, and overlap between spectral regions is possible.

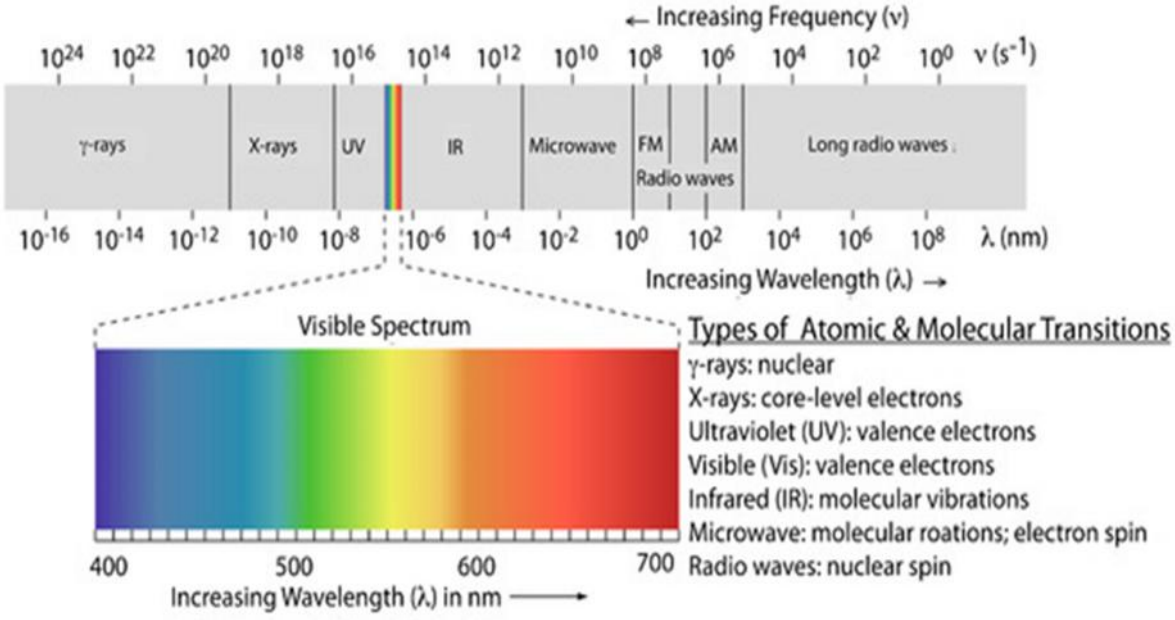

Figure 3: The electromagnetic spectrum showing the boundaries between different regions and the type of atomic or molecular transition responsible for the change in energy.

Photons as a Signal Source

Several characteristic properties of electromagnetic radiation have been defended, including its energy, velocity, amplitude, frequency, phase angle, polarization, and direction of propagation. A spectroscopic measurement is possible only if the photon's interaction with the sample leads to a change in one or more of these characteristic properties. We can divide spectroscopy into *two broad classes of techniques.*

(1) In one class of techniques there **is a transfer of energy** between the photon and the sample. Table 1 provides a list of several representative examples**.**

Table 1 Examples of Spectroscopic Techniques Involving an Exchange of Energy Between a Photon and the Sample

Type of Energy Transfer	Region of Electromagnetic Spectrum	Spectroscopic Technique[a]
absorption	γ-ray	Mossbauer spectroscopy
	X-ray	X-ray absorption spectroscopy
	UV/Vis	*UV/Vis spectroscopy* *atomic absorption spectroscopy*
	IR	*infrared spectroscopy* raman spectroscopy
	Microwave	microwave spectroscopy
	Radio wave	electron spin resonance spectroscopy nuclear magnetic resonance spectroscopy
emission (thermal excitation)	UV/Vis	*atomic emission spectroscopy*
photoluminescence	X-ray	X-ray fluorescence
	UV/Vis	*fluorescence spectroscopy* *phosphorescence spectroscopy* atomic fluorescence spectroscopy
chemiluminescence	UV/Vis	chemiluminescence spectroscopy

In absorption spectroscopy a photon is absorbed by an atom or molecule, which undergoes a transition from a lower-energy state to a higher-energy, or excited state (Figure 4). The type of transition depends on the photon's energy. The

electromagnetic spectrum in Figure 3, for example, shows that absorbing a photon of visible light raise one of the valence electrons of the atom or molecule to a higher-energy level. When a molecule absorbs infrared radiation, on the other hand, one of its chemical bonds experiences a change in vibrational energy.

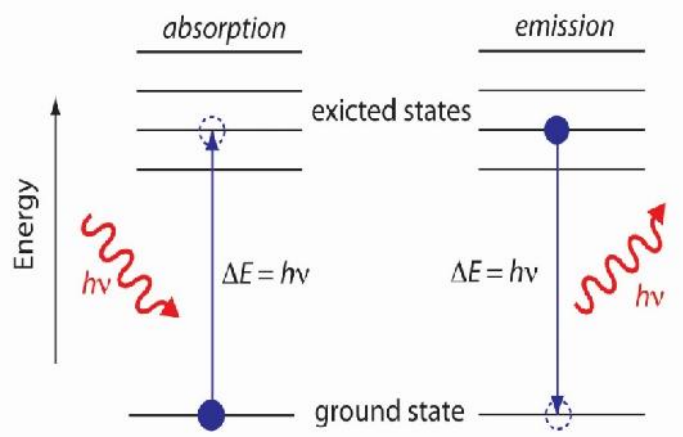

***Figure** 4: Energy diagram showing the absorption and emission of a photon by an atom or a molecule*

When it absorbs electromagnetic radiation the number of photons passing through a sample decreases. The measurement of this decrease in photons, which we call **absorbance**, is a useful analytical signal. Note that the each of the energy levels in Figure 10.4 has a well-defined value because they are quantized. Absorption occurs only when the photon's energy, $h\nu$, matches the difference in energy, ΔE, between two energy levels. **A plot of absorbance as a function of the photon's energy is called an absorbance spectrum.**

(2) In the **second broad class** of spectroscopic techniques, the electromagnetic radiation undergoes **a change in** amplitude (capacity), phase angle, polarization, or direction of propagation as a result of its refraction, reflection, scattering,

diffraction, or dispersion by the sample. Several representative spectroscopic techniques are listed in Table 2.

Table 2 Examples of Spectroscopic Techniques That Do Not Involve an Exchange of Energy Between a Photon and the Sample

Region of Electromagnetic Spectrum	Type of Interaction	Spectroscopic Technique[a]
X-ray	diffraction	X-ray diffraction
UV/Vis	refraction	refractometry
	scattering	*nephelometry* *turbidimetry*
	dispersion	optical rotary dispersion

[a]