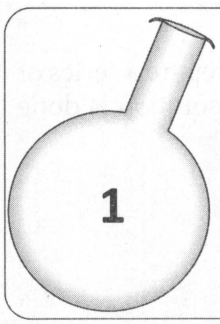

1 Development of Standard Curve by UV-spectrophotometry

In Pharmacy, standard curves are mostly used to determine the concentration of substances. It shows the relationship between concentration of a substance and its response. The response might be optical density, luminescence, fluorescence, radioactivity or something else. In a standard curve (Fig. 1.1), known concentrations are plotted on the x axis, and assay measurements (responses) on the y axis. Most of the drugs show a quantitative relationship with its *UV* absorbance. Hence concentration vs. absorbance type standard curves are abundantly used in Pharmacy.

Principle

Lambert-Beer law indicates that the concentration of a substance [C] can be determined from its absorbance [A], provided the molar extinction coefficient [ϵ] of a compound and its light path (l) through the solution are known. The formula is:

$$A = \epsilon Cl$$

Thus, concentration can be calculated as, $C = \frac{A}{\epsilon l}$

Steps for Making a Standard Curve

- Weighing of the substance for which standard curve is desired.
- Making of the stock solution.
- Making a series of standard solutions.
- Measurement by *UV*-spectrophotometry.

Weighing the Substance

The choice of balance is crucial. The error from weighing increases with decreasing amounts of weighed substance. Hence a quantity greater or equivalent to the minimum weighable quantity of the balance should be taken.

Making of the Stock Solution

It is desirable to dissolve the drug in a single *UV* transparent solvent. But for poorly soluble substances, a binary solvent may be required. Usually, a poorly soluble drug is dissolved in a little amount of methanol and the solution is made up to volume with water.

affect absorbance, accurate results cannot be obtained. The timing of reading absorbance, temperature at which we keep the materials and all other physical factors should also be similar.

Before recording the absorbance, baseline correction must be done. In UV/VIS spectrophotometers, the most frequently used light sources are tungsten-halogen lamps for visible light, roughly 350 to 750 nm, and a small deuterium arc lamp for UV light (190 to 350 nm). Regions where the energy of the source is weak and the sensitivity of the detector is low are areas where increased noise is likely to occur. Regions from 190 to 215 nm and 300 to 350 nm are typically much noisier than other regions (even if no sample is present in the sample compartment, there may be some absorbance displayed in the monitor). These effects are due entirely to the combined responses of the lamp, detector, and other components in the instrument. Modern spectrophotometers have the facility to compensate for these energy variations, which is known as baseline correction. For poorly soluble drug, methanol/ethanol is used as co-solvent. In such cases too, baseline correction is a must.

For most of the analytes, Lambert-Beer law is valid within a certain concentration range only. Hence absorbances of unknown sample should be taken within that range, to have an accurate estimate of concentrations.

The ideal value of regression coefficient is 1. Hence, the closer the value to 1, the stronger is the relationship. In this case, the r^2 vale is 0.9761—which indicates it is not a very perfect relationship. A repeat experiment is recommended in such cases.

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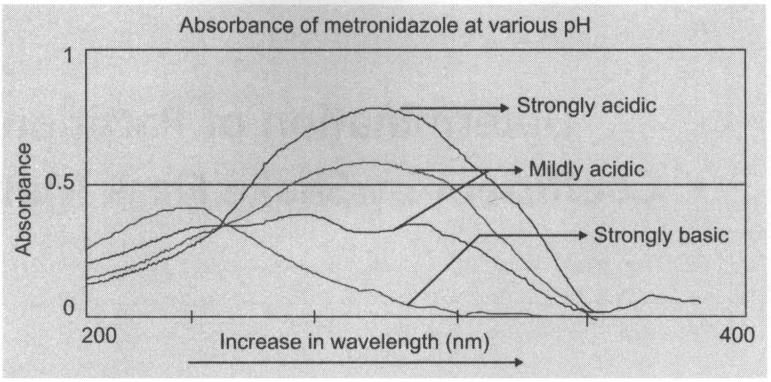

Fig. 4.1: Absorbances of metronidazole at various pH

A number of methods have been investigated for the determination of pK_a. In the spectrophotometric method, a series of solution is made for an ionisable moiety having the same concentration but different pHs. Absorption spectra of these solutions show a pH-dependent absorption profile. However, spectrophotometric method can only be applied for those compounds which have a chromophore at close proximity of the ionization centre. Only those drugs that show a pH-dependent shift of absorption can be determined in this process. Metronidazole is such a drug, whose absorbance changes with pH of the media.

Metronidazole is a weak base. It should be completely ionized in strongly acidic media and completely unionized in strongly basic media. At different pH, the extent of ionization is different and the drug shows different absorbance. The ratio of theses absorbances are used for pK_a determination.

pK_a values are temperature dependent in a non-linear and unpredictable way. Samples measured by potentiometry are held at a constant temperature using a water jacket and thermostatic water bath. Spectroscopic values are measured at ambient temperature. No pK_a value should ever be quoted without the temperature.

Note: When pH equals pK_a the ionization is 50%. So for demonstration purpose, values of A_i and A_u should be carefully chosen so that these pHs are sufficiently removed from the pK_a values. Spectrophotometric technique can be applied for determination of common drugs like amiloride hydrochloride, atenolol, propranolol hydrochloride and gliclazide.

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6 Determination of Particle Size by Gravity Sedimentation Method

The method of determining the particle size distribution by Andreasen's pipette is described in ISO 13317. This method can be used to determine the particle size distribution in powder materials which are dispersible in liquid or are already present in a suspension/slurry.

Objective

To determine the average particle size of sand using Andreasen's pipette.

Principle

The experiment is performed in Andreasen's pipette and the average particle size (diameter) of sand is determined by Stoke's law equation which is based on the relationship among drag force, gravitational force and buoyancy force when a spherical small particle falls freely with constant terminal settling velocity. This settling velocity varies with the size or mass of particle. Under certain fixed parameters, average diameter of particles vary with the settling velocity. In Andreasen's pipette, the distribution of the particle size in a powder material can be directly measured using Stoke's law equation. In a suspension, particles are allowed to settle under gravity and samples are withdrawn during sedimentation by means of a calibrated pipette at predetermined intervals of times. The tip of the pipette is kept at a known depth below the surface (h). At any time, t, the collected sample contains only those particles with Stokes' diameters less than those particles settling at rate h/t. The cumulative undersize distribution by mass of the powder is obtained directly by weighing the residue after removal of the suspending medium from each sample withdrawn.

Materials

Sand particles, sodium hexametaphosphate.

Equipment

Andreasen's pipette, weighing balance, with an accuracy >/= 0.1 mg, aluminum dishes/petridishes, wide-mouthed weighing bottles/beakers, ovens, stopwatch.

Description of Andreasen's pipette

Andreasen's pipette (Fig. 6.1) is a cylindrical glass vessel in which a 10 ml pipette with a stopper is mounted centrally. A graduated scale (0–20 cm) is marked on the side wall of the glass vessel (sedimentation chamber), of ~5 cm internal diameter.