OPTICAL METHODS. PHOTOELECTROCOLORIMETRY, SPECTROPHOTOMETRY.

PHOTOCOLORIMETRY

Photocolorimetry is based on measuring the intensity of non- monochromatic light flux passing through the analyzed solution by means of photovoltaic cells of photocolorimeters and photoelectric colorimeters. The light flux from the emission source (usually an incandescent lamp) passes through the light filter, transparent to emission at specified wavelength only, through the cell with an analyzed solution and onto the photovoltaic cell which converts the light energy to into photocurrent recorded by the relevant device. The higher light absorption of the analyzed solution (i.e., the higher its optical density), the lower is light flux energy incident on a photovoltaic cell.

PHOTOELECTRIC COLORIMETERS

- Photoelectric colorimeters are equipped with several light niters mat nave a maximum light transmission at different wavelengths.
- Various designs of photoelectric colorimeters, intended for operation in near UV and visible (mainly) region of the spectrum, are developed. Light filters (more often this is a glass of various composition and color) are transparent for emission with width in some tens of nanometers, about 20 to 50 nm.

PHOTOELECTRIC COLORIMETERS

- Photoelectric colorimeters with one or two photoelectric cells are the most widespread. Photoelectric colorimeters with one photovoltaic cell provide for measuring the energy of a single beam light flux. Devices with two photovoltaic cells measure the energy of two light fluxes (two-beam scheme) one of which passes through the analyzed solution while the other — through the comparison solution («zero» solution).
- Photoelectric colorimeters allow to measure optical density or transmission of a solution with only a few light filters; therefore, they do not allow obtaining a continuous absorption spectrum in a certain spectral range.

PHOTOCOLORIMETRY

The concentration of a determined substance in the analyzed solution is calculated either using the principal law of light absorption with a previously established concentration range of its satisfiability for a given light filter and the absorbing layer thickness or using the calibration curve method. In the latter case, the strict satisfiability of the principal law of light absorption is not required.

PHOTOCOLORIMETRY

- The relative error of photocolorimetric determination of concentration usually does not exceed ±3%.
- The method has a relatively high sensitivity, reproducibility, and selectivity; measurement of optical density or transmittance is simple, and relatively simple equipment is used. However, non-monochromaticity of recorded light flux slightly decreases the accuracy and reproducibility of analytical measurements.
- Photoelectrocolorimetry is widely used in analytical practice, for example, in the analysis of such drug products as diethylstilbestrol, levomycetine, menthol, novocaine, pilocarpine hydrochloride, rutin, streptomycin, ethacridine lactate and many others.

SPECTROPHOTOMETRY

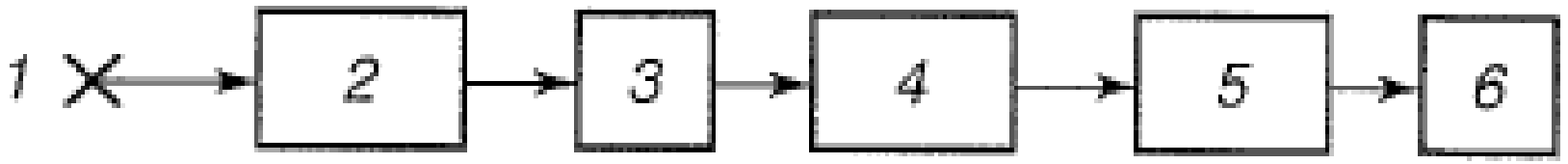
Spectrophotometry is the most often used and the most advanced among the other methods of absorption molecular analysis, is based on the use of special spectral devices — spectrophotometers, which allow recording light fluxes in a wide range of wavelengths from -185 nm to 1100 nm, i.e., in the UV, visible and near-infrared ranges of the spectrum, and to provide a high degree of light monochromaticity (0.2-5 nm) passing through the analyzed medium.

SPECTROPHOTOMETERS

In most spectrophotometers used in the analytical practice, monochromatization of the light flux is performed by using dispersing (decomposing light into a spectrum) elements — prisms or diffraction gratings. Various designs of spectrophotometers based on a single-beam or double-beam (double-channel) scheme have been developed.

BASIC BLOCK SCHEME OF SPECTROPHOTOMETER

The light from the radiation source 1 enters monochromator 2, in which it decomposes into a spectrum. Then, monochromated light flux passes through a cell holder 3, in which cells with analyzed solution and comparison solution ("zero" solution) are installed. After passing through cells with solutions, the light flux falls on photovoltaic cells of the emission receiver 4 in which the energy of light flux is converted to current, amplified by the amplifier module 5, then the amplified electrical signal is recorded by the recorder module 6as either a spectral curve or counting device readings.



SPECTROPHOTOMETERS

- Incandescent lamps are used as the emission source when operating in the visible region of the spectrum, in which, they provide continuous light flux (unlike linear provided by a mercury lamp) as well as hydrogen or deuterium lamps for operating in the UV region of the spectrum (200-350 nm).
- Prisms or diffraction gratings are commonly used to decompose light beam into a spectrum in a monochromator. For visible and near-infrared region operation, glass prisms and glass condensers (lenses) and cells are used. For UV region -200-400 nm operation, it is necessary to use auartzontics (prisms, optical condensers, cells) because glass absorbs UV rays.

SPECTROPHOTOMETERS

- When using spectrophotometers operating by the single ray principle, a cell with a comparison solution (zero solution) and a cell with an analyzed solution are alternatively installed into the light flux in the cell holder. Both cells are simultaneously installed into the cell holder of spectrophotometers operating by the two-ray principle: the cell with zero solution — into the comparison channel, the cell with an analyzed solution — into the measuring channel.
- Both cells (with zero solution and analyzed solution) must be exactly identical, with equal absorbing layer thickness. At absorbing layer thickness of l = 1 cm, the allowable deviation must not exceed $\Delta l = \pm 0.005$ cm at temperature $(20 \pm 1)^0$C. Both cells, filled with pure solvent, must have the same optical density at the same wavelength.

SPECTROPHOTOMETRY

- Spectrophotometric methods are more accurate and sensitive than photoelectrocolorimetric ones, they allow to analyze multicomponent systems without separation of components, to determine substances not absorbing in the visible spectrum (but having absorption bands in the UV range). The relative errors of spectrophotometric determinations do not exceed ±2%.
- Among all photometric methods, spectrophotometry is the most frequently used method for analyzing various objects of inorganic and organic nature.

SPECTROPHOTOMETRY

Various techniques of spectrophotometry:

- Direct
- Differential
- Derivative spectrophotometry
- Spectrophotometric titration

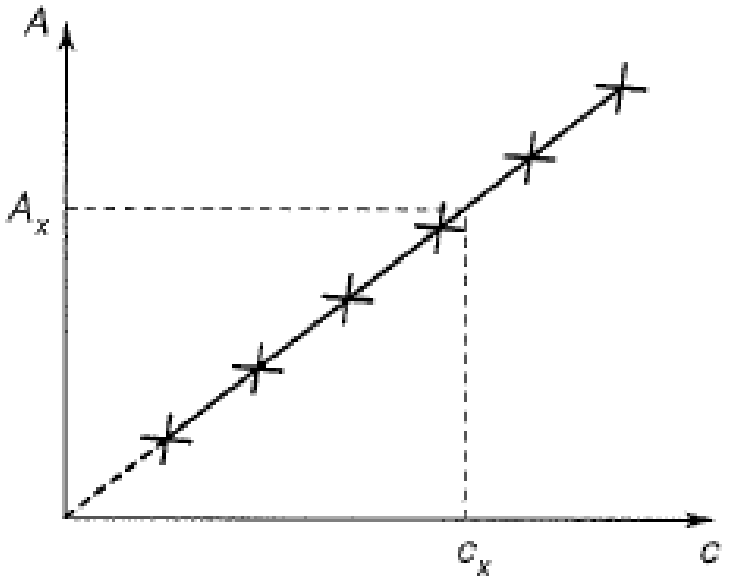
During spectrophotometric measurements, the concentration of a determined substance in the analyzed solution is calculated, as in photoelectric colorimetry, using the ***basic law of light absorption or calibration graphs.***

QUANTITATIVE PHOTOMETRIC ANALYSIS

Conditions for photometric determination.

- Selection of analytical wavelength. An analytical wavelength is a wave-
- Selection of measured solution concentration and absorbing layer thickness.
- Use of comparison solution.

CALCULATING THE CONCENTRATION OF A DETERMINED SUBSTANCE



Calibration graph method (calibration curve method). By results of optical density A measurements of five-six standard solutions with different precisely known concentration c at analytical wavelength, plot the calibration curve in A—c coordinates. Optical density Ax of the analyzed solution is measured under the same conditions as the optical density of standard solutions (cell, analytical wavelength, comparison solution). Based on calculated value Ax, concentration cv of determined substance is found using the calibration curve.

CALCULATING THE CONCENTRATION OF A DETERMINED SUBSTANCE

Single standard method. This method is applicable if the basic law of light absorption is satisfied. The essence of the method is as follows. A standard (standard solution) with a precisely known concentration of determinate c(st) is prepared, and its optical density A(st) measured at analytical wavelength vs. the comparison solution. Then, in the same cell and under the same conditions, measure optical density A(x) of an analyzed solution with unknown concentration c(x) of determinate.

$$A(\text{st}) = \varepsilon c(\text{st}) l,$$

$$A(\text{x}) = \varepsilon c(\text{x}) l,$$

$$c(\text{x}) = \frac{A(\text{x})}{A(\text{st})}\, c(\text{st}).$$

CALCULATING THE CONCENTRATION OF A DETERMINED SUBSTANCE

Calculation of concentration according to the molar or specific extinction coefficient. The method is applicable if the basic law of light absorption is satisfied. The numerical value of molar or specific extinction coefficient must be known. If it is unknown, then it is necessary to determine the mean value of E or E by experiment, carrying out photometric measurements of the optical density of standard solution with a precisely known concentration of determinate at analytical wavelength.

$$A(x) = \varepsilon c(x) l,\ c(x) = A(x)/\varepsilon l$$

$$A(x) = EW(x)1,\ W(x) = A(x)/El,$$

CALCULATING THE CONCENTRATION OF A DETERMINED SUBSTANCE

$$A_1 = \varepsilon c(x) l,$$

$$A_2 = \varepsilon [c(x) + c] l,$$

$$\frac{A_1}{A_2} = \frac{c(x)}{c(x)+c}; \quad A_1 c(x) + A_1 c = A_2 c(x),$$

$$c(x)(A_2 - A_1) = A_1 c,$$

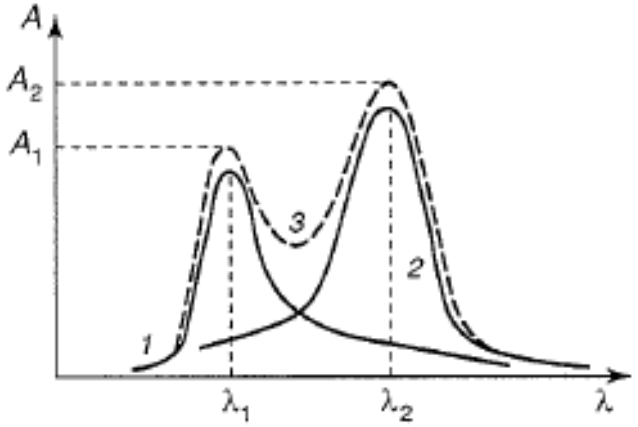
$$c(x) = \frac{A_1}{A_2 - A_1} c.$$

Standard addition method. The method is applicable if the basic law of light absorption is satisfied.

Two solutions are prepared: the first solution is the analyzed solution with unknown concentration c(x) of determinate and the second is the analyzed solution with the precisely known added quantity (standard additive) of determinate so that its concentration in the latter solution is equal to c(x) + c, where c is the precisely known increase in concentration due to standard additive.

CALCULATING THE CONCENTRATION OF A DETERMINED SUBSTANCE

Determination of concentration of several substances if present simultaneously. The method is based on the optical density additivity law in compliance with the basic light absorption law.



The absorption spectrum of two substances in their joint presence: 1 — absorption band of component 1,2 — absorption band of component 2; 3 — total absorption spectrum of solution

$$A_1 = \varepsilon(1)_{\lambda_1} c_1 l + \varepsilon(2)_{\lambda_1} c_2 l,$$

$$A_2 = \varepsilon(1)_{\lambda_2} c_1 l + \varepsilon(2)_{\lambda_2} c_2 l,$$

$$c_1 = \frac{\varepsilon(2)_{\lambda_2} A_1 - \varepsilon(2)_{\lambda_1} A_2}{[\varepsilon(2)_{\lambda_2} \varepsilon(1)_{\lambda_1} - \varepsilon(2)_{\lambda_1} \varepsilon(1)_{\lambda_2}]l},$$

$$c_2 = \frac{\varepsilon(1)_{\lambda_1} A_2 - \varepsilon(1)_{\lambda_2} A_1}{[\varepsilon(1)_{\lambda_1} \varepsilon(2)_{\lambda_2} - \varepsilon(1)_{\lambda_2} \varepsilon(2)_{\lambda_1}]l}.$$

DIFFERENTIAL PHOTOMETRIC ANALYSIS

- ***Differential spectrophotometry(photometry).*** If the light absorption of analyzed solution is measured relative to a comparison medium (comparison solution, membrane, optical wedge) whose optical density A is significantly more than zero (for example, A = 0.1-1.0), such a spectrophotometric method is called differential spectrophotometry or differential photometric analysis.
- One of the main advantages of differential spectrophotometry consists in reducing the error of spectrophotometric measurements. Therefore sometimes differential spectrophotometry is called ***precise spectrophotometry***.

THE ESSENCE OF THE METHOD

- A series (five to ten) of reference solutions with different, precisely predetermined concentrations of determined substance (c_0, c_1, c_2,c_j is prepared. First, at a selected wavelength, it is necessary to place into both channels of spectrophotometer matched cells with the same standard solution (determinate concentration is equal to c_0), against which the following measurements will be carried out, and install the optical density scale in position A = 0.
- Then, at the same constant analytical wavelength, optical density A.(*i* =1, 2,..., n) of each standard solution and optical density Ax of an analyzed solution is measured relative to a standard solution with concentration c_0 and own optical density A_0 (relative to pure solvent), then concentration cx of determinate in analyzed solution is calculated by the following methods.

CALCULATION METHOD

$$A_x = \varepsilon l (c_x - c_0),$$

$$c_x - c_0 = A_x / \varepsilon l,$$

$$c_x = c_0 + A_x / \varepsilon l,$$

$$F = 1 / \varepsilon l,$$

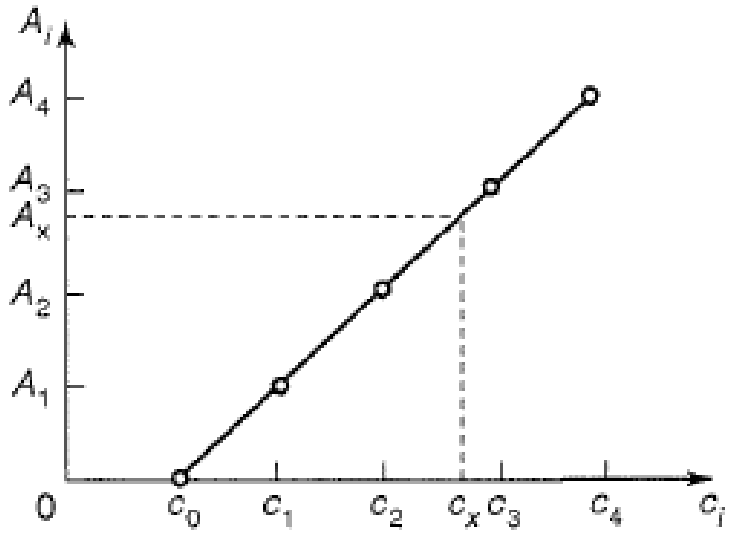
$$c_x = c_0 + F A_x.$$

$$A_i = \varepsilon l (c_i - c_0),$$

$$F = 1 / \varepsilon l = (c_i - c_0) / A_i$$

$$\overline{F} = \frac{1}{n} \sum_{i=1}^{i=n} \frac{c_i - c_0}{A_i},$$

THE METHOD OF THE CALIBRATED CURVE

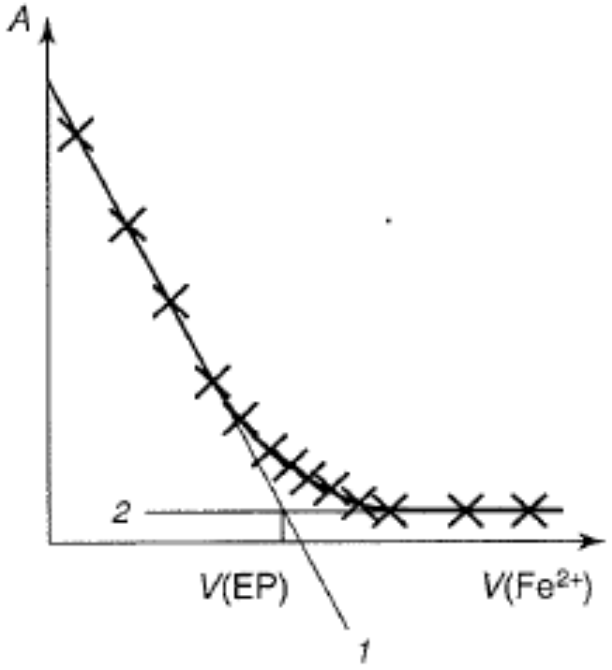


The method of the calibrated curve. From the obtained experimental values A*i* the calibration curve is constructed plotting the known concentration values of standard solutions c-t on the abscissa and on the ordinate — the values of standard solution optical densities A*i*, measured relatively standard solution with concentration c_0. According to this curve, with a knowledge of the measured value Ax, concentration cx of analyzed solution can be found.

CONCEPT OF PHOTOMETRIC TITRATION

The method is based on the determination of the end of titration by the sharp change in light absorption of a titrated solution at the equivalent point or near it. Titration is carried out measuring in sequence the light absorption of titrated solution when adding to it titrant at an analytical wavelength corresponding to the peak in the absorption spectrum of either titrated substance or titrant or product of titrimetric reaction (titration without indicator) or added indicator (color-indicator titration).

CONCEPT OF PHOTOMETRIC TITRATION



Based on the results of measuring the light absorption of a titrated solution, the titration curve is plotted in optical density A — a volume of added titrant V(T) coordinates. The sharp break of the titration curve corresponding to the end of titration is rarely observed. More often, the end of titration is found by extrapolation of the titration curve linear portions. The crossing point of extrapolating straight lines represents the end of the titration.

CONCEPT OF PHOTOMETRIC TITRATION

The method exhibits selectivity, higher sensitivity compared to other titrimetric methods, higher accuracy than direct photometry of solutions with constant concentration allows to use reactions with not sufficiently high equilibrium constants at a temperature of titration and low-stability reaction products, allows to carry out determination at a wavelength at which other components of a solution also absorbed (because the end of titration is determined by the change in light absorption rather than by the absolute value of optical density of solution).