



# INSTRUMENTAL (PHYSICAL AND PHYSICAL-CHEMICAL METHODS OF ANALYSIS



# INSTRUMENTAL (PHYSICAL AND PHYSICAL-CHEMICAL METHODS OF ANALYSIS

*Physical and physical-chemical methods of analysis* are based on the dependence of measured physical properties of substances on their qualitative and quantitative composition. Since the physical properties of substances are measured using various equipment (“instruments”), these methods of analysis are also called instrumental methods.

# CLASSIFICATION OF PHYSICAL AND PHYSICAL-CHEMICAL METHODS OF ANALYSIS.

- *Optical methods* are based on measuring the optical properties of substances.
- *Chromatographic methods* are based on the ability of various substances for selective sorption.
- *Electrochemical methods* are based on measuring the electrochemical properties of systems.
- *Radiometric methods* are based on measuring the radioactive properties of substances.
- *Thermal methods* are based on measuring the heating effects of corresponding processes.
- *Mass spectrometric methods* are based on the analysis of ionized fragments of substances.

# THE ADVANTAGES OF PHYSICAL AND PHYSICAL-CHEMICAL METHODS

- Low limit of detection ( $1\text{--}10^{-9}$  mcg) and low limit concentration (up to  $\sim 10^{-12}$  g/ml) of determined substance.
- High sensitivity, formally determined by the tangent of angle slope of the corresponding calibration curve, which actually reflects the dependence of the measured physical parameter (usually plotted on the Y-axis) on concentration or amount of determined substance (usually plotted on the X-axis).
- High selectivity (selectability) of methods. Often, it is possible to determine the constituent components directly in the analyzed mixtures, without their separation and without isolation of individual components.
- Rapid analysis, the possibility of automation and computerization.

# THE DISADVANTAGES OF PHYSICAL AND PHYSICAL-CHEMICAL METHODS

- Sometimes the reproducibility of results is worse than the reproducibility of classical chemical methods of quantitative analysis, such as gravimetry and titrimetry.
- Determination errors of physical and physicochemical methods of analysis are often about  $\pm 5\%$  (and in some cases up to  $\pm 20\%$ ), while in classical chemical analysis (gravimetry, titrimetry) they usually do not exceed  $\pm(0.1-0.5)\%$ .
- Complexity and high cost of the equipment used.

## OPTICAL METHODS OF ANALYSIS

*Optical methods of analysis* are based on measuring the optical properties of a substance (emission, absorption, scattering, reflection, refraction, light polarization), which occur as a result of interaction between electromagnetic radiation and substance.

# CLASSIFICATION OF OPTICAL METHODS OF ANALYSIS

1. According to studied objects: atomic and molecular spectral analysis
2. According to the nature of the interaction between electromagnetic radiation and a substance.
3. According to the electromagnetic spectrum range used.
4. According to the nature of energy transitions.

## CLASSIFICATION ACCORDING TO THE NATURE OF THE INTERACTION BETWEEN ELECTROMAGNETIC RADIATION AND A SUBSTANCE.

1. **Atomic absorption analysis.** The method is based on measuring monochromatic radiation absorption by determined substance atoms in the gas phase after substance atomization.
2. **Emission spectral analysis.** The method is based on measuring the light intensity emitted by a substance (commonly by atoms or ions) during its energy excitation, for example, in electric discharge plasma
3. **Flame photometry.** The method is based on using the gas flame as radiation energy excitation source.
4. **Molecular absorption analysis.** The method is based on measuring light absorption by molecules or ions of analyzed substance. This method is the most widely used.
5. **Luminescent analysis.** The method is based on measuring luminescence radiation intensity, i.e., substance radiation under the influence of various types of excitation.
6. **Spectral analysis using Raman scattering effect (Raman effect).** The method is based on measuring radiation intensity under the influence of Raman scattering.



## CLASSIFICATION ACCORDING TO THE NATURE OF THE INTERACTION BETWEEN ELECTROMAGNETIC RADIATION AND A SUBSTANCE.

7. ***Nephelometric analysis.*** The method is based on measuring light scattering by particles of a disperse system (medium).
8. ***Turbidimetric analysis.*** The method is based on measuring radiation intensity decrease during its transmission through a disperse medium.
9. ***Refractometric analysis.*** The method is based on measuring refractive indices of substances.
10. ***Interferometric analysis.*** Based on the study of light interference phenomenon.
11. ***Polarimetric analysis.*** Based on measuring the value of optical rotation (the rotation angle of the light polarization plane by optically active substances).

# CLASSIFICATION ACCORDING TO THE ELECTROMAGNETIC SPECTRUM RANGE USED

- ***Spectroscopy (spectrophotometry) in the UV-V-IR spectrum range***, i.e., in the near-ultraviolet (UV) range within the wavelength interval of 200-400 nm and in the visible range within the wavelength interval of 400-760 nm.
- ***Infrared spectroscopy*** studying the electromagnetic spectrum range of 0.76-1000  $\mu\text{m}$  ( $1 \mu\text{m} = 10^{-6} \text{ m}$ ).
- ***The following methods*** are less commonly used in analytics: X-ray spectroscopy that studies X-ray spectra; microwave spectroscopy that studies electromagnetic radiation with wavelengths from  $10^{-1}$  to 10 cm.

# CLASSIFICATION ACCORDING TO THE NATURE OF ENERGY TRANSITIONS

- ***Electronic spectra (mainly in the UV-V-IR range)*** occur as a result of changing the electronic state energy of particles (atoms, ions, radicals, molecules, crystals).
- ***Vibrational spectra.*** These spectra include IR range and Raman spectra. Vibrational spectra occur as a result of vibrational state energy change of particles (two- and polyatomic ions, radicals, molecules, and also liquid and solid phases).
- ***Rotational spectra.*** These spectra include far-infrared, and microwave ranges of electromagnetic radiation. Rotational spectra occur as a result of rotational state energy change of molecules, two- and polyatomic ions, radicals.

## PRINCIPAL LIGHT ABSORPTION LAW (PRINCIPAL LAW OF PHOTOMETRY)

*Photometric measurements and calculations* are based on *two light absorption laws* (two laws of photometry), which characterize the dependence of monochromatic (at a constant wavelength) radiation absorption on the absorbing layer thickness and concentration of light-absorbing particles.

*The principal law of light absorption* is valid only for monochromatic light absorption at constant wavelength  $\lambda = \text{const.}$

# THE FIRST LAW OF PHOTOMETRY

*The first law of light absorption (Bouguer-Lambert law) :*

*each thin layer of constant thickness in a homogeneous medium absorbs an equal fraction of incident light flux or a fraction of light flux absorbed by a homogeneous medium is directly proportional to the absorbing layer thickness:*

$$\Delta I/I = k l \cdot l$$

where  $\Delta I$  is the absorbed part of incident light  $I$ ;  $l$  is absorbing layer thickness;  $k l$  is the proportionality coefficient.

## THE SECOND LAW OF PHOTOMETRY

*The second law of light absorption (Bouguer-Beer law) :*

*a fraction of light flux absorbed by a given thin layer in a homogeneous medium is proportional to the number of light-absorbing particles per volume unit, i.e., concentration:*

$$\Delta I/I = k_2 \cdot C$$

where  $C$  is concentration;  $k_2$  is a proportionality factor.

## BOTH LAWS OF LIGHT ABSORPTION ARE COMBINED INTO BOUGUER—LAMBERT—BEER- BERNARD CONSOLIDATED PRINCIPAL LAW OF LIGHT ABSORPTION.

*Bouguer—Lambert—Beer- Bernard consolidated principal law of light absorption:*

*the intensity of light absorption by solutions of substances is proportional to their concentration  $C$  and the thickness of the absorbing layer  $l$*

$$I = I_0 e^{-kcl}$$

or in a logarithmic form:

$$A = \varepsilon Cl$$

where  $I_0$  is the intensity of monochromatic radiation (light flux) incident on the given light-absorbing medium;  $I$  is the intensity of the radiation (light flux) transferred through this medium ( $I < I_0$ );  $k$  is light absorption coefficient (factor);  $C$  is the concentration of light-absorbing particles in the given medium;  $l$  is light absorbing layer length;  $e$  is base of the natural logarithms;  $A$  is optical density (absorbance), which is also called extinction;  $\varepsilon = k/2.3$  is extinction coefficient (factor), or the coefficient of extinction (absorptivity), which is also often called absorption coefficient (factor).

## THE MOLAR EXTINCTION COEFFICIENT ( $\epsilon$ )

*The molar extinction coefficient ( $\epsilon$ )* is measured in units of  $\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ . Numerically, molar extinction coefficient is equal to the optical density of the solution with a concentration of the dissolved light-absorbing substance of  $C = 1 \text{ mol/L}$  and the absorbing layer thickness of  $l = 1 \text{ cm}$ .



# LIGHT TRANSMISSION

$$T = \frac{I}{I_0} \cdot 100\%$$

$$\frac{1}{T} = \frac{I_0}{I} \cdot \frac{1}{100\%}, \quad -\lg T = \lg \frac{I_0}{I} - 2 = A - 2,$$

## SPECIFIC EXTINCTION COEFFICIENT (INDEX)

**E** is the specific extinction coefficient (index), which is often called the «specific absorption coefficient (index)», but the latter term is not quite correct. In a pharmacopoeial analysis, specific extinction coefficient is used more often than the molar extinction coefficient.

**The specific extinction coefficient** is numerically equal to the optical density of the solution with a concentration of  $W = 1 \text{ g/100 ml}$  and the absorbing layer thickness of  $l = 1 \text{ cm}$ .

$$A = E W l$$

molar and specific extinction coefficients (indices) are related by equation:

$$\epsilon = E \frac{cM}{10c} = E \frac{M}{10}$$

# THE LAW (OR THE RULE) OF OPTICAL DENSITY ADDITIVITY IS TRUE: OPTICAL DENSITY A OF A MIXTURE OF SUBSTANCES

At a constant wavelength of the incident light, the optical density of the solution, containing a mixture of several light absorbing substances, non-interacting between themselves, the law (or the rule) of optical density additivity is true: optical density  $A$  of a mixture of substances, which comply with the basic law of light absorption and do not interact with each other chemically, is equal to the sum of optical densities  $A$  of the components (at  $\lambda = \text{const}$  and  $l = \text{const}$ ):

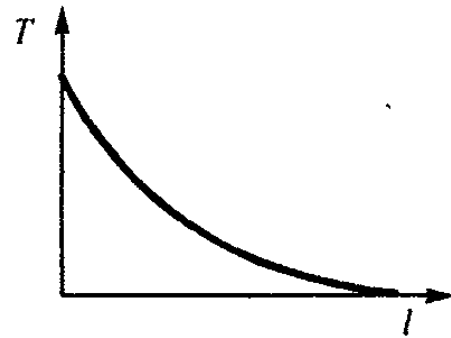
$$A = \sum_i A_i = l \sum_i \epsilon_i c_i$$

## THE VALUE OF TRUE EXTINCTION COEFFICIENT IS OBTAINED IF THE FOLLOWING CONDITIONS ARE MET:

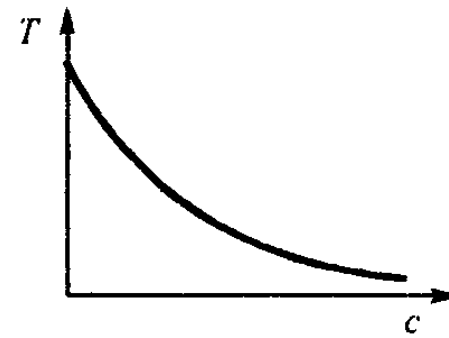
- the light flux passing through solution must be strictly monochromatic;
- at a given wavelength  $\lambda$ , at which the optical density is measured, particles of only one type (one composition) absorb light;
- the true concentration of light absorbing particles is known.

# GRAPHICAL PRESENTATION OF TRANSMISSION CHANGE AND OPTICAL DENSITY A DEPENDING ON ABSORBING LAYER THICKNESS $l$ AND CONCENTRATION OF SOLUTION $c$

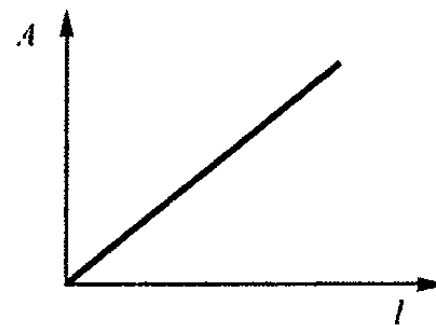
The linear dependence of optical density on concentration for many substances is observed only within a certain range of solution concentrations change.



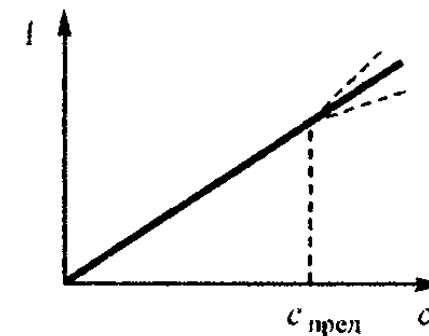
$\lambda = \text{const}, c = \text{const}$



$\lambda = \text{const}, l = \text{const}$



$\lambda = \text{const}, c = \text{const}$



$\lambda = \text{const}, l = \text{const}$

# THE APPARENT DEVIATIONS FROM THE BASIC LIGHT ABSORPTION LAW

- *The apparent deviations from the basic light absorption law*, both positive (the curve is above the straight line) and negative (the curve is under the straight line).
- The medium acidity and the presence of other substances affect the value of limit concentration.
- Apparent deviations from the principal law of light absorption can be caused by physical-chemical or instrumental reasons.

## PHYSICAL-CHEMICAL REASONS

With increasing the concentration of the solution, after a certain limit concentration, association, polymerization, complexation and other processes become noticeable. In this case, the number of light-absorbing particles decreases, since part of them is involved in chemical interactions leading to decrease in optical density of the solution and appearance of *negative deviations* from the straight line in the chart of solution optical density dependence on its concentration.

## PHYSICAL-CHEMICAL REASONS

- *Positive deviations* are caused by the fact that new particles formed as a result of various interactions with increasing solution concentration may have higher light absorption at a given wavelength, which leads to an increase in total light absorption of the solution and its optical density.
- Sometimes more *complex deviations* from the linear dependence of optical density of the solution on its concentration are observed, for example, in the form of convex or concave curves, which are caused by specificity of chemical interactions in the solution with a change in concentration of dissolved substances.



## INSTRUMENTAL REASONS

They are mainly caused by insufficiently strict monochromaticity of the light flux (usually resulting in negative deviations), the effect of diffuse light, non-linearity of emission receiver operation (photovoltaic cells, photo-multipliers, etc.) in spectral devices at very low and very high emission intensity, i.e., at very high (more than 1.5) and at very low (less than 0.1) optical densities of solution through which the light flux passes into the emission receiver.

# THE METHODS OF ABSORPTION ANALYSIS

- Colorimetry,
- Photoelectrocolorimetry
- Spectrophotometry

# COLORIMETRY

- **Colorimetry** is the simplest and oldest method, based on visual comparison of liquids color.
- In the majority of cases (although not always) in colorimetry, the strict satisfiability of the principal law of light absorption is not required.
- During colorimetric measurements, simple devices are used: glass colorimetric test tubes, glass cylinders with valves, colorimeters, photometers.
- Colorimetry is used in pharmacy to determine liquid color, the content of lead impurities and other heavy metals, less often to determine the pH of solutions according to color of the corresponding acid-base indicators.
- Three colorimetric methods are the most widely used: method of standard types (scale method), method of color smoothing, and dilution method, which is sometimes attributed to the method of color smoothing.

## METHOD OF STANDARD TYPES

The color intensity of colored liquid dosage forms and solutions must meet the certain requirements, which provide for visual comparison with color of standard solutions (a series of 10-12 standard solutions with different, known, gradually increasing concentration of the same determined substance ). The test liquid and reference solutions are taken in equal amounts, placed in identical glass tubes and compared in reflected light on a white matte background. The color intensity of the test liquid should not exceed the color intensity of the corresponding standard solution (mentioned in normative documents), although the color shade can be slightly different. Liquids are considered colorless if their color does not differ from water or the corresponding pure solvent in case of non-aqueous solutions.

## METHOD OF STANDARD TYPES

- The method is simple in its implementation, it does not require complex equipment, however, it requires a certain skill and has low accuracy (the determination error is about 5-10%), therefore, it can be used only for approximate evaluation of determined substance concentration in the analyzed solution.
- In a pharmacopoeial analysis, a variant of this method is widely and systematically used to determine colors of liquids in order to evaluate color indices of liquid dosage forms and solutions containing colored pharmaceutical preparations during quality control. Color test is obligatory for colored liquids containing drug products.

## METHOD OF STANDARD TYPES

$$A_1 = \varepsilon C_1 l, \quad A_2 = \varepsilon C_2 l,$$

$$A_1 = A_2,$$

$$\varepsilon C_1 l = \varepsilon C_2 l$$

$$C_1 = C_2$$

# METHOD OF COLOR SMOOTHING

Smoothing of color intensities of two liquids can be performed by various methods.

**First method.** The color intensity of the analyzed solution containing the determined colored substance is visually smoothed with color intensity of the reference solution containing all the same components as the analyzed solution, except for the determined substance. Known quantities of the determined substance are gradually added to the reference solution until the color intensities of the reference solution and the analyzed solution become equal, which is usually evaluated visually. If the equal color intensity of both solutions is reached, the concentration of colored determinate in these solutions is considered equal. Knowing the amount of the determined substance contained in the reference solution, the concentration of the determined substance in the analyzed solution is calculated.

## METHOD OF COLOR SMOOTHING

*Second method.* For visual smoothing of color intensity of two liquids, the absorbing layer thickness of the compared analyzed and standard solutions are changed until the color intensity of both solutions matches. In this case, satisfiability of the principal law of light absorption is required.

$$A = \epsilon Cl = \epsilon C_x l_x$$

$$C_x = Cl/l_x$$



## METHOD OF COLOR SMOOTHING

*The third method.* Equalization of the light absorption intensity of two liquids can be carried out visually by means of photometers in which color equalization is carried out without sacrificing the change in thickness of absorbing layer but by overlapping a part of one of the light fluxes.

The method of color smoothing is not very accurate; the error in determining the concentration of solutions is about  $\pm(5-10)\%$ .

## DILUTION METHOD

- *Dilution method* is, in essence, also color intensity equalization of analyzed and standard solutions by diluting one or the other solution with the solvent.
- In this method, satisfiability of the principal law of light absorption is not required.
- The method is not very accurate; as in previous cases, the determination error is about  $\pm(5—10)\%$ .