Medical and General Chemistry Department

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ANALYTICAL CHEMISTRY

SUBMODULE 3

Part 3 Instrumental analysis

Student notebook for experimental chemistry

Kyiv-2021

Practical lesson 25

Optical methods. Problem solving and situational tasks. Laboratory work "Determination of analyte contents in the sample by the methods of visual colorimetry". Test control.

Control question for students out-of-class work

1. Classification of physical-chemical methods of the analysis: optical, electrochemical, chromatographic, kinetic.
2. Classification of optical methods of the analysis:
   - on investigated objects;
   - on the nature of interaction of electromagnetic radiation with substance;
   - on electromagnetic spectral range which use in analysis;
   - on the nature of energy jump.
3. The photometric analysis.
4. Molecular–absorption method: a principle, an origin of spectrum in UV, visible and IR of spectral range.
5. The fundamental law of light absorption.
6. Optimum conditions of photometric definition.
7. Colorimetry. Methods of colorimetry:
   1. The method of standard series;
   2. Method of color comparison;
   3. The method of dilution.

These questions are taken from the Test items for licensing examination "Krok 1. PHARMACY"

<u>The correct answer is first</u>

1. Why intensity of light which passes through a solution is less:
   A. With light absorption, dispersion, reflection.
   B. Only with light absorption by investigated substance.
   C. Only with light dispersion.
   D. Only with light reflection.
   E. Intensity of light does not change.
2. Optical methods of the analysis divide (in depending on character of interaction of substance with electromagnetic radiation) on:
   A. emissive and absorption;
   B. The kinetic;
   C. chromatographic;
   D. conductometric;
   E. voltamperemetric

3. The content of some substance is defined by spectrophotometric method. Someone plottes graphic dependence of absorbance from concentration. For calculation of the quantitative content is necessary to use:
   A. A method of calibration chart
   B. Comparison method.
   C. Method of molar or specific absorptivity.
   D. A method of indicators.
   E. Method of additives.
4. The content of some substance define spectrophotometric method, knowing specific absorptivity of substance by wavelength in maximum of absorption. For calculation of the quantitative content it is necessary to use:
   A. A method of indicators.
   B. A method of calibration chart.
   C. A comparison method.
   D. A method of additives.
   E. Method of molar or specific absorptivity
5. What substance in a solution can be determined in two ways - by the polarimetric or refractometric method?
   A. Ascorbic acid
   B. Sodium benzoate
   C. Benzoic acid
   D. Calcium gluconate
   E. Magnesium sulfate
6. During quantitative estimation of glucose by polarimetric method the following factor is measured:
   A. Angle of rotation of polarized beam plane
   B. Coefficient of light refraction
   C. Rate of polarized beam absorption by a solution
   D. Beam dispersion by a solution
   E. Optical density of a solution
7. Bouguer-Lambert-Beer law is the basis of molecular absorption analysis. According to this law, optical density of a solution is:
   A. Directly proportional to layer thickness and concentration of a substance
   B. Directly proportional to layer thickness and absorption coefficient
   C. Inversely proportional to layer thickness and concentration of a substance
   D. Directly proportional to concentration and inversely proportional to layer thickness
   E. Directly proportional to concentration and inversely proportional to absorption coefficient

Laboratory work

The colorimetric Determination of Iron in sampe by sulfosalicylic acid

The theoretical part.

Colorimetry is the simplest and oldest method. This method is based on visual color comparison of the test solution with the color of standard solutions containing known quantities of analyte.

The most widely used three methods of visual colorimetry:

- The method of standard series;
- Method of color comparison;
- The method of dilution.

The method of standard series (method of color scale):

Prepare a series of 10 ─ 12 standard solutions with different known, gradually increasing concentration (for example, 0,01; 0,02; 0,03; 0,04; 0,05 mg/ml) of the analyte which content must be defined in the tested sample.

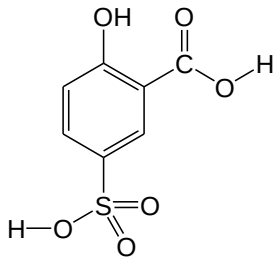
The intensity of color of each solution depends on the concentration of the analyte in the solution if the diameter of test tubes used and volume of solution is the same. If the intensity of color of the tested solution and one of the standard solutions is the same, the content of the analyte in these tubes are also the same. That is the concentration of the substance in the tested solution is equal to the concentration of the standard solution.

The method is simple in its implementation and does not require sophisticated equipment, but has low accuracy (error of definitions is 5 ─ 10%), which requires certain practical skills.

Comparison of the color intensity of test solution with color intensity of standard solution used in pharmaceutical analysis also for determining the maximum allowable content of certain impurities (iron, sulfates, heavy metals, etc.) in the investigated solutions of drugs. For example, to determine the impurity cations of lead (II) in pharmaceuticals to a certain amount of the analyzed drug added to an aqueous solution of a certain amount of dilute acetic acid and 2 drops of sodium sulfide Na_2S. The solution is stirred and compares the intensity of its color from the brownish color intensity of corresponding standard solution of lead acetate (II). Content impurity cations of lead (II) is considered acceptable if the color intensity of the test solution does not exceed the color intensity of the reference solution. The color should be compared with the diffuse light on a white background by placing test tubes on the sheet of white paper.

Principle

Sulfosalicylic acid has such structural formula:



In the pH 8 ~ 11 of the ammonia solution, trivalent iron and sulfosalicylic acid to form a stable yellow complex, its reaction formula:

$Fe^{3+} + 3SSal^{2-} = [Fe(SSal)_3]^{3-}$ (where $SSal^{2-}$ sulfosalicylic acid anion),

the maximum absorption wavelength of 420 nm, the color intensity is proportional to iron content.

Fe^{3+} at different pH may be forming with the sulfosalicylic acid composition and colors of several different complexes. In pH 1.8 ~ 2.5 of the solution, the formation of red purple [Fe(SSal)]; at pH 4 ~ 8 of the solution, the formation of brown $[Fe(SSal)_2)]^-$; at pH 8 ~ 11.5 of the ammonia solution in the formation of yellow $[Fe(SSal)_3)]^{3-}$; if the pH> 12, it can not form complexes generated iron hydroxide precipitation.

Ammonium hydroxide alkaline medium, divalent iron ion also with sulfosalicylic acid to form a yellow complex.

Reagents

Iron standard solution: dissolve 0.4317g ferric ammonium sulfate $[FeNH_4(SO_4)_2 \cdot 12H_2O]$ in 10 mL of 1:1 hydrochloric acid solution, moved into 500mL capacity bottle, water was diluted to scale. This solution iron content of 100 μg / mL. This solution diluted 10-fold, the concentration of iron standard solution ρ_{st} = 10μ g/mL - ***10 ml of solution iron content of 100 μg / mL. transfer into 100ml volumetric flask, add water till line and mix them thoroughly.***

Ammonium hydroxide (10%) ; sulfosalicylic acid 10%.

Preparation of a calibration scale

1. Transfer 0,5; 1,0; 1,5; 2,0; 2,5; 3,0; 3,5; 4,0; 4,5 and 5,0 ml of the standard iron(III)solution (concentration of Fe^{+3}-ion is 10 μg/ml) to a raw of test tubes N1-10.
2. Add water 9,5; 9,0; 8,5; 8,0; 7,5; 7,0; 6,5; 6,0; 5,5 and 5 ml to a raw of test tubes N1-10.
3. To each tube add 2 ml of 10 % solution of sulfosalicylic acid
4. To each tube add 1 ml of 10 % ammonium
5. Solutions shake.
6. After ≈ 5 minutes compare the color intensity of the analyzed solution and the standard solutions.

Calculation

1. Calculate the mass of Iron in each tube

$$m(Fe^{3+}) = \rho_{st} \cdot V_{st}$$

2. Calculate the mass concentration of Iron in each tube

$$\rho(Fe^{3+}) = \frac{\rho_{st} \cdot V_{st}}{V_{solution}}$$

$$V_{solution} = V_{st} + V(H_2O) + V(\text{sulfosalicylic acid}) + V(NH_3)$$

Determination of iron content in the sample.

1. To the sample add :
   A. 6ml of water
   B. 2 ml of 10 % solution of sulfosalicylic acid
   C. 2 ml of 10 % ammonium
2. Compere the volume of solution in yours test tube with the volume of solutions in a prepered **calibration scale** (raw of test tubes N1-10). If the volume of solution in yours test tube is less add water drop by drop to level the volume.
3. Solution shake
4. Compere the intensity of color in yours test tube with intensity of color in a prepered **calibration scale** (raw of test tubes N1-10).
5. Report the μg of iron(III) per litre of water .

Data for calibration scale prepearing

№	1	2	3	4	5	6	7	8	9	10
V_{st}, ml	0,5	1,0	1,5	2,0	2,5	3,0	3,5	4,0	4,5	5,0
$V(H_2O)$, ml	9,5	9	8,5	8,0	7,5	7,0	6,5	6,0	5,5	5,0
$m(Fe^{3+})$, μg										
$\rho(Fe^{3+})$, μg/l										

Observation: The intensity of the color of the analyzed solution has the same intensity of color of standard solution in the tube number .....

Conclusion:

Practical lesson 26

Spectrophotometry. Laboratory work "Spectrophotometric determination of substances in the sample". Problem solving and situational tasks. Writing control work and test.

Theoretical questions

Photocolorimetry and spectrophotometry.
Methods photocolorimetry, how to quantify:
- Method of comparing the optical densities of the standard and the solution is determined;
- Method of calibration graph;
- The method of determining the average value and the specific molar absorption coefficients;
- The method of supplementation.

Choosing the optimal conditions of photometric determinations.
Determination of the concentration of several substances in their joint presence (using the law of additivity optical densities).
Differential photometric analysis.
Extraction-photometric analysis.
Photometric titration.

TESTS AND REAL-LIFE SITUATIONS FOR SELF-ASSESSMENT

1. For definition of substances with high consentration use:
A. Spectrophotometry.
B. Multiwave spectrophotometry.
C. Nephelometry.
D. Refractometry.
E. Differential spectrophotometry.

2. The comparison solution in differntial spectrophotometry is a solution which contains:
A. The reagent solution practically identical on ionic strength with the investigated.
B. Water or other solvent.
C. Solution which contains all reagents.

D. A defined component with concentration close to concentration of an investigated solution.
E. A solution of defined component with unknown concentration.

3. For a choice of wavelength in photometric measurement build the graph of dependence of solution absorbance from wavelength. Choose such value of wavelength in which it is observed:

A. Absorption maximum;
B. Sharp maximum of absorption;
C. Flat minimum of absorption;
D. Sharp minimum of absorption;
E. Flat maximum of absorption.

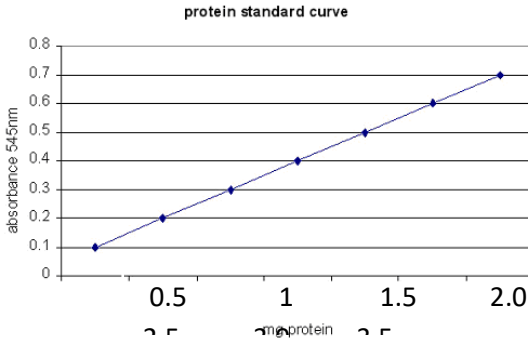
4. Optical methods of the analysis divide (in depending on character of interaction of substance with electromagnetic radiation) on:

A. voltamperemetric; B. The kinetic;
C. chromatographic; D. conductometric;
E. emissive and absorption

5. Molar absrbtivities monoethylamine at 785 and 728 cm^{-1} are equal $\sum_{785,1} = 1,67$ and $\sum_{728,1} = 0,0932$, and diethylamine are equal $\sum_{785,2} = 0,0446$ and $\sum_{728,2} = 1,17$. Calculate concentration (mol/L) mono- and diethylamine in technical triethilamine if values of measured absorbances in the same conditions at l = 1,0 cm are equal: $A_{785} = 0,525$, $A_{728} = 0,715$.
6. The sample (weight 0,0515 g) of sodium salicylate ($M(C_7H_5O_3Na) = 160,10$ g/mol) is dissolved in concentrated acetic acid and titrated by photometric method with indicator tropeolin 00 at λ=540 nm, using as titrant 0,1000 mol/L $HClO_4$ in concentrated acetic acid. Calculate contents (% w/w) of sodium salicylate in drug by results of titration:

V_{HClO4}, ml	0,0	1,0	1,2	1,6	2,0	2,4	2,6	2,8
A	0,020	0,040	0,040	0,050	0,070	0,110	0,150	0,230
V_{HClO4}, ml	3,0	3,1	3,2	3,4	3,6	4,0	4,4	
A	0,440	0,680	1,04	1,07	1,07	1,07	1,07	

7. Given the following standard curve, answer the following question



a. An unknown protein has an absorbance of 0.45, what would its concentration be in mg?

b. An unknown protein has an absorbance of 0.20, what would its concentration be in mg?

c. An unknown protein has a concentration of 1.60, what would its absorbance be?

8/ Molar absorptivity of copper (II) dithizon in CCl_4 at λ_{max} = 850 nanometers equal E = 4,52⊕10^4. Which contents (% w/w) of copper can be defined with dithizon if from sample of sample (weight 1,00 g) receive 25,00 mL of a solution dithizon in CCl_4 and measure the minimum absorbance 0,020 if thickness of a layer *l* = 5,0 cm.

ANSWERS ON THE SELF-ASSESSMENT

Tests: 1. E., 2. D., 3. E., 4. E.

Laboratory work

Spectrophotometric determination of the $K_2Cr_2O_7$ content in the sample

1. The theoretical part

Determination is based on the relationship between optical density and concentration of $K_2Cr_2O_7$ in solution. It is subordinated to the Bouguer - Lambert – Beer law.

To determine the concentration of the analyte in the solution necessary to meet a number of preparatory operations in the following sequence:

Wavelength Selection

record the absorption spectrum and select the optimum wavelength

Cuvettes Selection

$A = \varepsilon \cdot C \cdot l$

the optical density of the standard solution with an average concentration of analyte must be within the range of adsorbance 0.3 to 0.6;

Determination of the Relationship between Absorbance and Concentration

- Prepare standards of known concentration
- Measure absorbance at lmax
- Plot A vs. concentration

Obtain slope

- Use slope (and intercept) to determine the concentration of the analyte in the unknown

Measure the optical density of the standard solutions and build a calibration graph in coordinates "optical density" - "concentration detectable substance";

Measure the optical density of the test solution, the calibration graph to determine the concentration of the detectable substance.

The standard solution of $K_2Cr_2O_7$ contain 1mg/ml. To construct a calibration graph prepared a series of standard solutions according to Table 1.

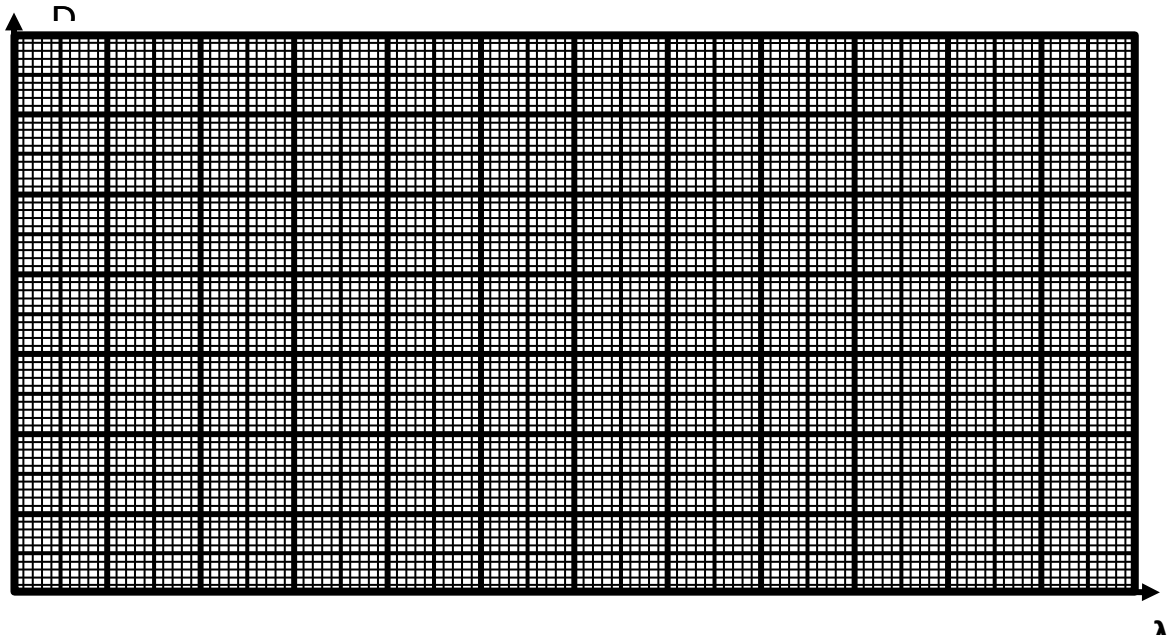
Preparation of a calibration scale

1. Transfer 0,5; 1,0; 2,0; 3,0; 4,0; 5,0; 6,0 and 7,0 ml of the standard $K_2Cr_2O_7$ solution (concentration is 1mg/ml) to a raw of test tubes N1-10.
2. Add water 9,5; 9,0; 8,5; 8,0; 7,5; 7,0; 6,5; 6,0; 5,5 and 5 ml to a raw of test tubes N1-10.
3. To each tube add 2 ml of 10 % solution of sulfosalicylic acid
4. To each tube add 1 ml of 10 % ammonium
5. Solutions shake.
6. After ≈ 5 minutes compare the color intensity of the analyzed solution and the standard solutions.

Table 1

№ standart. solution	Volume of solution from $\rho(K_2Cr_2O_7)$ 1mg/ml	Volume of solution H_2SO_4, ml	The volume of water	$\rho(K_2Cr_2O_7)$in standart solution, μg/ml
1	0,00	20	to100ml	0
2	1,00	20	to 100ml	1
3	2,00	20	to100ml	2
4	3,00	20	to 100ml	3
5	4,00	20	to100ml	4
6	5,00	20	to 100ml	5
7	6,00	20	to 100ml	6
8	7,00	20	to 100ml	7

To do this in a volumetric flask of 100 mL burette by transfer indicated in the table the number of milliliters of standard solution has ρ ($K_2Cr_2O_7$) = 1mg/ml and volumes are listed in the table of sulfuric acid, which is c (1/2 H2SO4) = 0.05 mol / L, and distilled water to the mark ring.



The absorption spectrum of $K_2Cr_2O_7$

Read the optical density of $K_2Cr_2O_7$ standard solution to number 6 in the wavelength range from 330nm to 580nm and recorded into Table 2 and construct the corresponding graph (absorption spectrum). Determine the wavelength of maximum absorption (λmax).

Table 2

λ, нм	330	340	345	350	355	360	365	370	375	380
A										
λ, нм	390	400	410	420	430	440	450	460	470	475
A										

Pick up the thickness of the cell so that the optical density of the solution at λmax number 6 was in the range of 0.5 to 0.8. Measure the optical density of standard solutions of $K_2Cr_2O_7$ № 2 - 8 at λmax. As a solution comparisons use standard solution number 1. The data record in Table 3.

Table 3

ρ($K_2Cr_2O_7$),mg/ml	0,01	0,02	0,03	0,04	0,05	0,06	0,07
A for λ=							

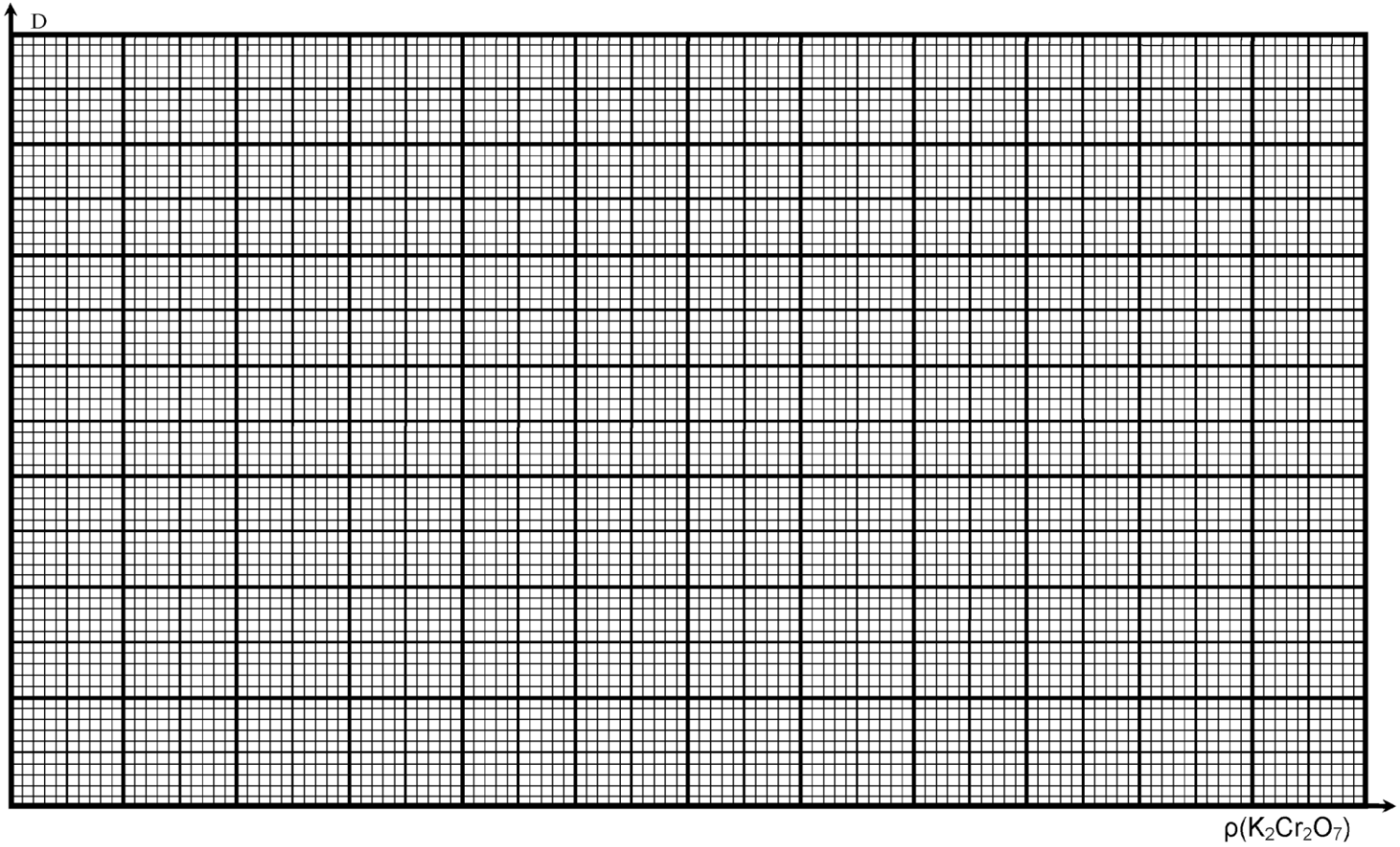
According to Table 3 construct the calibration graph, which is used to determine the concentration in the samples analyzed solutions.

A sample of the test solution containing $K_2Cr_2O_7$, poured into a cuvette, measure the absorbance at λmax. For calibration graph determine the concentration of $K_2Cr_2O_7$ solution in the sample analyzed.

The data obtained absorbance of the sample and calibration schedule found by $K_2Cr_2O_7$ concentration values are entered in Table 4.

Table 4

№ example	1	2	3
The optical density			
Density $K_2Cr_2O_7$, mg/ml			



Калібрувальний графік для $K_2Cr_2O_7$

Practical lesson 27

Electrochemical methods of analysis. Problem solving and situational tasks. Potentiometry. Potentiometric titration. Graphical methods of end-point detection. Laboratory work "Determination of the mass fraction of boric acid in the sample by potentiometric titration". Test control.

THE SELF-PREPARATION PROGRAM

1. Classification of electrochemical methods of analysis.
2. Electrogravimetry: the method theory (Faraday's laws), laws of electrolysis passing, possibilities of a method, condition of electrolysis (physical and chemical), usage.
3. Conductometry: the theory of a direct method and conductometric titration (specific and equivalent conductivity, their dependence on various factors), laws and the method possibilities, used reactions, application.
4. Coulometry: the theory of a method, a direct method and coulometric titration, features of application in the pharmaceutical analysis.
5. Potentiometry. Direct potentiometry: the theory of the quantitative analysis, methods of the quantitative analysis, application.
6. Potentiometric titration: an essence, reactions, titration curve, application.
7. Voltammetry: a method essence, electrodes, conditions analysis.
8. Qualitative polarographic analysis: polarographic wave, halfwave potential and factors of influence on it.
9. Quantitative polarographic analysis: Ilkovich equation and calculation of contents of substance by results polarography, application.
10. Amperometric titration: an essence, types of curves, application.

TESTS AND REAL-LIFE SITUATIONS FOR SELF-ASSESSMENT

1. Choose pair of electrodes for potentiometric definition of pH solutions (indicating electrode – reference electrode):

A. Glass - silver-chloride.
B. calomel – silver-chloride.
C. quinhydrone - antimonic.
D. mercury sulphat - silver-chloride.
E. Glass – antimonic.

2. For quantitative definition of iron by potentiometric method (oxidation-reduction reaction) as indicating electrode apply:

A. quinhydrone electrode.
B. silver-chloride electrode.
C. A platinum electrode.
D. mercury sulphate electrode.
E. A glass electrode.

3. The direct conductometry is successfully used for an estimation of solvent cleanliness. It is based on measurement of specific electric conductivity of solvents in special conductometric cells which basic element is:

A. silver electrode – a platinum electrode.
B. silver-chloride electrode.
C. quinhydrone electrode – antimonic electrode.
D. Two platinum electrodes.
E. A silver electrode.

4. Concentration of substance in an investigated preparation by polarographic analysis is defined on:

A. size of current force.
B. height and width polarographic waves.
C. In electromotive force.
D. size of potential allocation.
E. width of polarographic waves.

5. Polarographic method is widely used for the analysis inorganic cations and anions. Process of electro-reduction of investigated ions occurs on:

A. A silver electrode.
B. A platinum electrode.
C. Antimonic electrode.
D. calomel an electrode.
E. A mercury drop electrode.

6. What physical and chemical value can be defined by conductometric method?

A. Concentration of a solution
B. Degree and dissociation constant
C. All resulted values
D. Ionic product of water
E. Solubility product of slightly soluble electrolits

7. Sample of drug (weight 0,1260 g) which contains pyramidon (M=231,3 g/mol) is dissolved in waterless acetic acid, than ethylene dichloride is added and the prepeared solution has been titrated by 0,1 mol/L (K = 0,9109) $HClO_4$ solution in acetic acid. Plot titration curve and calculate content of pyramidon in gramme in drug:

V ($HClO_4$), mL	3,80	4,00	4,20	4,40	4,60	4,80	5,00	5,20
E, mV	355	360	382	523	579	597	611	620

6. The 100 mL solution of dissolved sample of sulphonamide contening drug is potentiometric titratied by 0,05 mol/L standard sodium nitrite solution. Calculate molarity of sulphonamide drug based on following results:

V, mL	15	15,05	15,10	15,15	15,20	15,25
E, mV	320	340	450	568	610	615

ANSWERS ON THE SELF-ASSESSMENT

Tests: 1. A, 2. C., 3. D., 4. A, 5. E., 6. C.

TEST QUESTION OF DATABASE "STEP 1"

Read the test questions with answers. To prove the correct answer, which is the first.

1. One of electrochemical methods of analysis is potentiometry. Potentiometry is a method of analysis based on the measurement (determination):

A. Potential of the indicator electrode.
B. Potential of the diffuse layer.
C. Zeta potential.
D. Redox system capacity.
E. Potential electrode.

2. To identify drugs by the polarographic method people define :

A. Half-wave potential.
B. Potential selection.
C. Potential schedule.
D. Limiting diffusion current.
E. Residual current.

3. Which an indicator electrode is suitable for potentiometric determination of ammonia and sodium hydroxide in solution:

A. Glass electrode.
B. Platinum electrode.
C. Silver electrode.
D. Chloric-silver electrode.
E. Zink electrode.

4. The content of acetic acid in the sample of solution is determined by potentiometric titration. Choose the indicator electrode:

A. glass electrode.
B. zinc electrode.
C. Silver Chlorid electrode.
D. mercury electrode.
E. calomel electrode.

5. One of electrochemical methods of analysis is polarography. The amount of analyte in the searching system in the polarographic analysis is defined by:

A. Height polarographic wave.
B. As a value of electromotive force.
C. The power supply.
D. Regulationof the polarographic wave.
E. Width of the polarographic wave.

6. Choose a glass which is used in the analysis for measuring the exact volume of the sample solution.

A. pipette
B. burette
C. volumetric flask
D. Measuring cylinder
E. Beaker

7. Choose the glass which is used in titrimetric analysis methods for measuring the volume of additional reagents.

A. Measuring cylinder
B. Volumetric flask
C. Burette
D. Pipette
E. Conical flask

8. Which of the physico-chemical analysis method based on measuring the electrical conductivity of the test solution.

A. Conductometry.
B. Coulometry.
C. Potentiometry.
D. Polarography.
E. Ampermetriya.

9. State the method based on measuring the amount of electricity consumed in the electrolysis of a certain number of detectable substances:

A. Coulometry.
B. Ampermetriy.
C. Potentiometry.
D. Polarography.
E. Conductometry.

10. Coulombmetry is based on measuring the amount of electricity which is spent on electrode reaction. Indicate the law which is the basis of the definition of coulombmetrycal substances:

A. Faraday's law.
B. Coulomb's law
C. Newton's law.
D. Stokes law
E. Law of Bouguer-Lambert-Beer.

11. Polarography – is both qualitative and quantitative analysis method. What is a quantitative characteristic of this method?

A. The value of the limiting diffusion current.
B. Electrode potential.
C. Half-wave potential.
D. Resistance of the solution.

E. The value of the electromotive force.

12. Potentiometric titration is used in cases if it is impossible to use visual indicators. During this titration is measured:

A. The potential of the indicator electrode.
B. The potential electrode.
C. Potential redox system.
D. Potential diffusion layer.
E. Zeta potential.

13. One of electrochemical methods of analysis is polarography. During the polarographic analysis of the substance is identified by:

A. Half-wave potentials.
B. As a value of electromotive force.
C. Height polarographic wave.
D. Position of polarographic wave.
E. Width of polarographic wave.

14. Choose a reference electrode, which can be applied in the potentiometric analysis of drug substance:

A. Silver Chlorid electrode.
B. The glass electrode.
C. Hinhidronnyy electrode.
D. Antimony electrode.
E. Zinc electrode.

15. What determines the height of the polarographic wave?

A. Renewable ion concentrations.
B. The composition of the electrolyte.
C. Capillary characteristics.
D. Capillary radius.
E. Capillary lengths.

16. One of electrochemical methods of analysis are potentiometry. Potentiometry - a method of analysis based on the measurement (determination) of:

A. Potential indicator electrode.
B. Potential diffuse layer.
C. Zeta potential.
D. Redox potential of the system.
E. Potential electrode.

17. To identify drugs by polarographic method defined:

A. Half-wave potential.
B. Potential selection.
C. Potential schedule.

D. Limiting diffusion current.
E. Residual current.

18. For potentiometric determination in solution containing ammonia and sodium hydroxide suitable indicator electrode:

A. glass electrode.
B. platinum electrode.
C. silver electrode.
D. silver chlorid electrode.
E. zinc electrode.

19. The concentration of acetic acid in the sample solution was determined by potentiometric titration. Select the indicator electrode:

A. glass electrode.
B. zinc electrode.silver chlorid electrode.
C. mercury electrode.
D. calomel electrode.

20. One of electrochemical methods of analysis is polarography. Amount of analyties in the studied system in the polarographic analysis is defined by:

A. Height of a polarographic wave.
B. As a value of electromotive force.
C. The power supply.
D. Regulation polarographic wave.
E. Width polarographic wave.

21. Add a reference electrode, which can be applied in the potentiometric studying drug substance:

A. Silver Chlorid electrode
B. The glass electrode.
C. *Quinhydrone* electrode.
D. Antimony electrode.
E. Zinc electrode.

22. What determines the height of the polarographic wave?

A. Renewable ion concentrations.
B. The composition of the electrolyte.
C. Capillary characteristics.
D. Capillary radius.
E. Capillary lengths.

Laboratory work

Determination of the mass fraction of boric acid in the sample by potentiometric titration

1. The theoretical part.

The potentiometric titrations invariably cover a broad-spectrum of chemical reactions that may be classified as follows :

- ✔ Neutralization reactions,
- ✔ Redox reactions,
- ✔ Precipitation reactions,
- ✔ Complexation reactions, and
- ✔ Potentiometric titrations in non-aqueous solvents.

The general principles which govern the above different types of reactions will be discussed briefly in the sections that follow :

Neutralization Reactions

The accuracy and precision with which the end-point can be determined potentiometrically solely depends upon the quantum of change in the observed e.m.f. in the vicinity of the equivalence point, which in turn entirely depends upon the strength and the concentration of acid and base employed.

Merits of the Method : It is found to be useful to titrate a mixture of acids having a significant difference in their strengths, for instance : HCl and CH_3COOH (alcoholic). In this case, the first-break in the titration curve signifies that the stronger of the two acids *i.e.*, HCl, gets neutralized ; whereas, the secondbreak represents the entire completion (*i.e.*, HCl + CH_3COOH).

In order to get fruitful and reproducible results it is quite necessary that the strengths between either the two acids or bases in question must vary by at least 105 to 1.

Demerits of the Method : The neutralization reactions often found to be giving unsatisfactory results in the following *two* instances. They are:

(*a*) when both the acid and the base are appreciably weak, and

(*b*) when either the acid or the base is very weak (*i.e.*, $K < 10^{-8}$) and also the prevailing solutions are dilute.

Note : In (*a*) above, an accuracy upto 1% is achievable in 0.1 M solution.

Choice of Electrodes :

Indicator Electrodes : Hydrogen, Glass or Antimony electrodes ;

Reference Electrode : Calomel electrode.

Redox Reactions

In this particular case the ratio of the concentrations of the oxidized and reduced forms of ionic species establishes the determining factor. Considering the following reaction,

Oxidised form + *n* electrons = Reduced form

The electrode potential E is given by the following expression:

$$E = E^0 + 0.0591/n \log[Ox]/[Red]$$

where, E^0 = Standard potential of the system.

In other words, the potential of the immersed indicator electrode is solely controlled and monitored by the ratio of the ionic concentrations in Eq. (*g*). Furthermore, in the course of either reduction of an oxidizing agent or *vice-versa i.e.* the said ratio, and hence the observed potential, undergoes an instant rapid change in the proximity of the end-point of the redox reaction.

Example : A typical example is that of titrations of Fe^{2+} with potassium permanganate or potassium dichromate or cerium (IV) sulphate.

Choice of Electrode : Indicator Electrode : Pt wire or foil.

The oxidizing agent is usually taken in the burette.

Precipitation Reactions

In this the determining factor mainly rests on the solubility product of the resulting nearly insoluble material generated in the course of a precipitation reaction and its ionic concentration at the equivalence point. It is, however, pertinent to mention here that the indicator electrode must readily come into equilibrium with one of the ions.

Example : Titration of Ag^+ with a halide (Cl^-, Br^- or I^-) or with SCN^- (thiocyanate ion).

Choice of Electrodes :

Reference Electrodes : Saturated Calomel Electrode (SCE) :

Silver-silver chloride Electrode ;

Indicator Electrodes : Silver wire or Platinum wire or gauze plated with silver and sealed into a glass-tube.

(It should readily come into equilibrium with one of the ions of the precipitate).

Salt-Bridge : For the determination of a halide the salt-bridge should be a saturated solution of potassium nitrate.

Note : Ion-selective electrode can also be employed.

Complexation Reaction

Complexation invariably occurs by the interaction of a sparingly soluble precipitate with an excess amount of the reagent, for instance : the classical example of titration between KCN and $AgNO_3$ as expressed by the following reactions :

$$KCN + AgNO_3 = AgCN + KNO_3$$

$$AgCN + KCN = K[Ag(CN)_2]$$

(Complex Ion)

The precipitate of AgCN is produced at first instance; consequently, the precipitate of AgCN initially produced gets dissolved by further addition of KCN to afford the complex ion $[Ag(CN)_2]^-$ and only a negligible quantum of Ag+ ions remain in the solution. Thus, the entire process from *ab initio* to the final stage of titration may be divided into *three* distinct portions, namely :

Upto end-point : Here, all the available CN^- ion has been virtually converted to the complex ion.

At this stage the ever increasing concentration reflects a gradually increasing concentration of Ag^+ ions, thereby slowly enhancing the potential of the Ag-electrode dipping in the solution,

At the end-point : It is usually visualized by a distinct and marked rise in potential, and

Beyond end-point : Further addition of $AgNO_3$ brings about only a gradual change in e.m.f. and AgCN gets precipitated. Ultimately, a second sudden change in potential may be visualized at this juncture when practically most of the CN^- ion gets precipitated as AgCN.

Choice of Electrodes :

Indicator Electrode : Silver electrode ;

Reference Electrodes : Colomel electrode ; Mercury-mercury (I) sulphate electrode.

Salt-Bridge : A saturated solution of KNO_3 or K_2SO_4 isolated from the reference electrode.

Potentiometric Titration in Non-Aqueous Solvents

The potentiometric technique has proved to be of great significance and utility for determining endpoints of titrations in a non-aqueous media. The mV scale rather than the pH scale of the potentiometer must be used for obvious reasons, namely :

(*i*) pH scale based upon buffers has no logical significance in a non-aqueous media, and

(*ii*) the potentials in non-aqueous media may exceed the pH scale.

The resulting titration curves are more or less emperical and afford a reasonably dependable and reproducible means of end-point detection.

Choice of Electrodes :

Indicator Electrodes : Glass electrode ;

Reference Electrode : Calomel electrode ;

Salt-Bridge : A saturated solution of KCl.

END-POINT DETERMINATION

In fact, there are several acceptable means to graph the potentiometric titration data generated from an actual titration in order to locate the exact (or nearest) end-point. The simplest and the most commonly used method is to plot the cell voltage E, millivolts (mV), versus the volume (ml) of titrant added. Ultimately, the end-point is determined from the point of maximum slope of the curve *i.e.*, the point of inflexion, as depicted in Figure 0. However, the degree of accuracy and precision with which this point of inflexion can be located from the plotted graph largely depends on the individual number of data points observed in the close proximities of the end-point.

Figure 0 gives rise to a **sigmoid-curve** (or **S-shaped curve**) obtained either by using an appropriate equipment (automatic titrators) that plots the graph automatically as the titration proceeds, or manually by plotting the raw experimental data. The central portion of the sigmoid curve, in fact is the critical zone where the point of inflexion

resides and this may be located by adopting any one of the following *three* procedures, namely :
(*i*) Method of parallel tangents,
(*ii*) Method of bisection, and
(*iii*) Method of circle fitting.

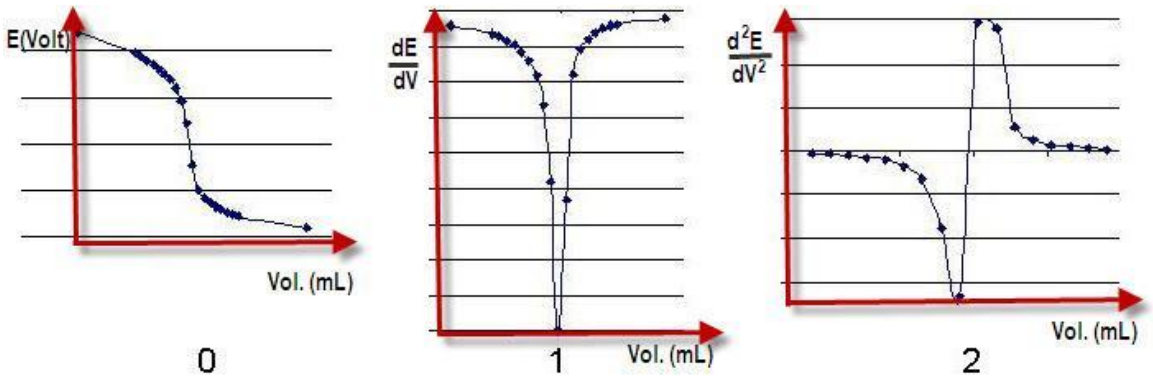


Figure 1 is obtained by plotting dE/dV against V which is termed as the **first derivative curve**. It gives a maximum at the point of inflexion of the titration curve *i.e.*, at the end-point.

Figure 2 is achieved by plotting the slope of the frst derivative curve against the volume of titrant added *i.e.*, by plotting d^2E/dV^2 Vs V and is known as the **second derivative curve**. Thus, the second derivative becomes zero at the point of inflexion and hence, affords a more exact measurement of the equivalence point.

The titration error (*i.e.*, difference between end-point and equivalence point) is found to be small when the potential change at the equivalence point is large. Invariably, in most of the reactions employed in potentiometric analysis, the titration error is normally quite small and hence may be neglected.

2. Procedure

1. Switch on pH-meter. Burette rinsed and filled with a working solution of KOH. 0,1000g of the sample of H_3BO_3 place in a clean glass.
2. Using graduated cylinder, measure 20 ml of glycerine and poured into a glass with sample.
3. Prepare a device that consists of Silver Chlorid electrode (reference electrode) and a glass electrode (measuring electrode) immersed in the water solution.
4. Dip the electrodes into analyzed solution and put glass on magnetic stirrer and switch on.
5. Read and record the pH in a table (row 1).
6. Add 1.0 ml of titrant and after pH is stabilized read and record the pH in a table (row 2).
7. Calculate the ratio $\Delta pH/\Delta V$ and record in a table.
8. Add 0,5 ml of titrant and after pH is stabilized read and record the pH in a table (row 3).

9. Calculate the ratio ΔpH/ΔV and record in a table.
10. Reiterate 8 and 9 till the ratio ΔpH/ΔV begins to increase.
11. Add 0.1 ml of titrant and after pH is stabilized read and record the pH in a table.
12. Calculate the ratio ΔpH/ΔV and record in a table.
13. Reiterate 11 and 12 till the ratio ΔpH/ΔV begins to decrease.
14. Add 0,5 ml of titrant three times more and after pH is stabilized read and record the pH in table.
15. Calculate the ratios ΔpH/ΔV and record in a table.

Evaluation table.

№	V(KOH), ml	ΔV, ml	pH	Δ pH	ΔpH/ΔV
1.	0	—		—	—
2.	1,0	1,0			
3.	1,5	0,5			
4.	2,0	0,5			
5.					
6.					
7.					
8.					
9.					
10.					
11.					
12.					
13.					
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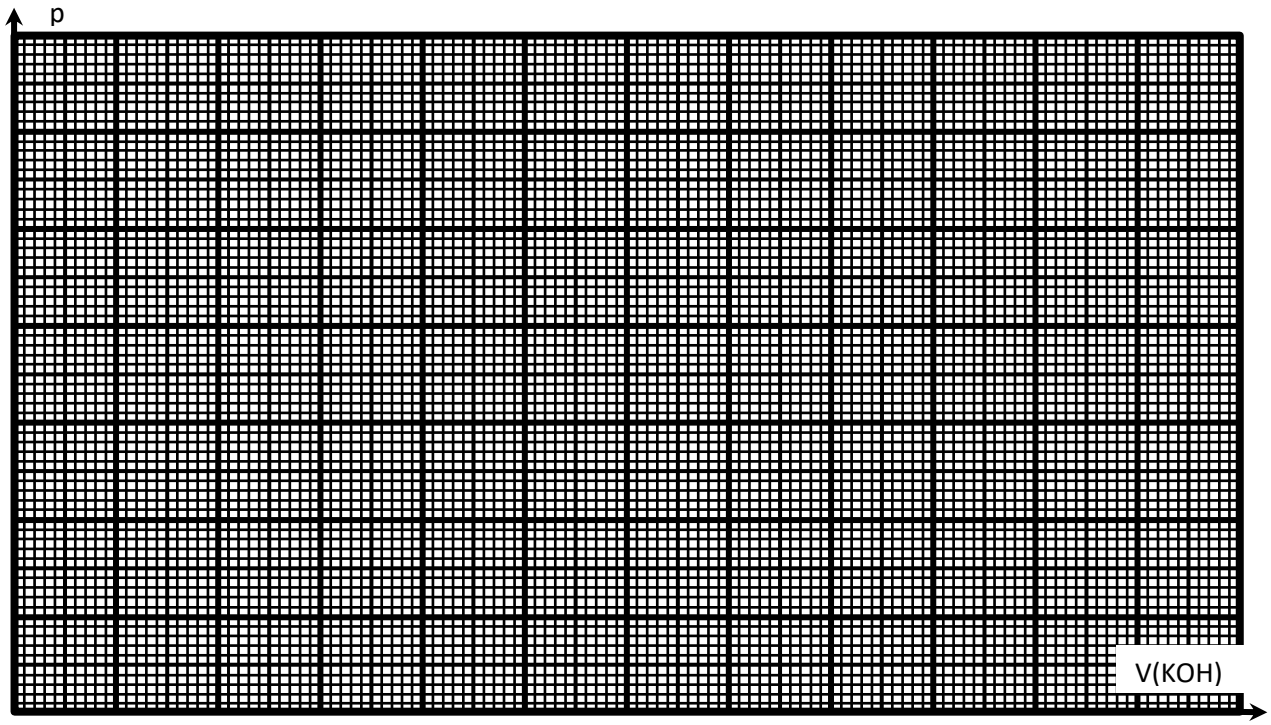
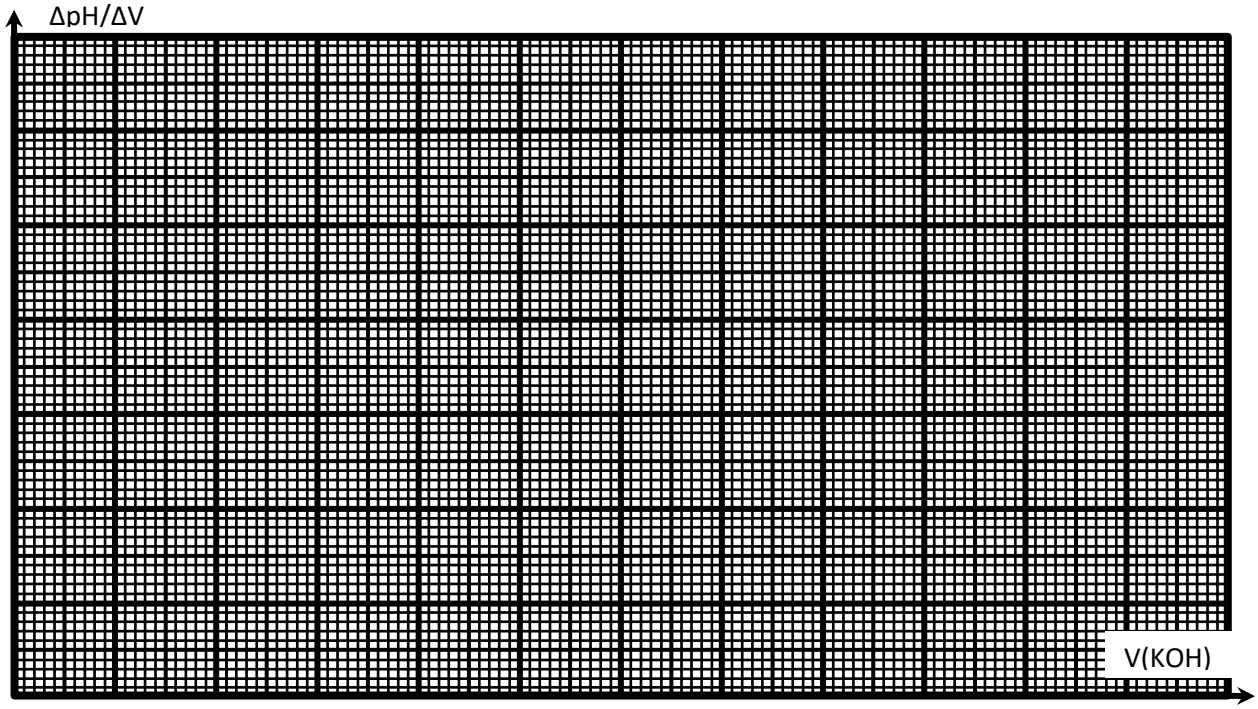


Titration curve - the dependence of E on V (KOH);



Differential titration curve - the dependence of ΔpH / ΔV to V (KOH)

3. Evaluation

Mass of sample ..... g

$M(H_3BO_3)$ = …….. g / mol.

c (KOH) = …….. mol / L

According to data from the table build two graphs:

a) the integral titration curves - f (pH)= V (titrant);

b) the differential titration curves - f(ΔpH /ΔV) = V (titrant).

At each of the graphs determine the equivalence point and the volume of the titrant corresponde to it.

$V_{eq(int)}(KOH)$=

$V_{eq(diff)}(KOH)$=

$V(KOH) = [V_{eq(int)}(KOH) + V_{eq(diff)}(KOH)]/2$=

Then calculate the mass fraction of H_3BO_3 in the sample using the formula:

$$\omega(H_3BO_3) = \frac{c(KOH)\, V(KOH)\, M(H_3BO_3)}{1000 \cdot m(sample)} \cdot 100\% =$$

Practical lesson 28

Chromatographic methods of analysis. Adsorptional and ion-exchange chromatography. Problem solving. Laboratory work "Quantitative determination of alkaline-earth cations in solution by ion exchange chromatography". Test control.

THE SELF-PREPARATION PROGRAM

1. Chromatogram and its characteristics: retention time, adjusted time, peak width on half of height.
2. Chromatographic process: the theory of theoretical plates and the kinetic theory.
3. Characteristics of division efficiency: separation degree, resolution, number of theoretical plates, the height equivalent to a theoretical plate.
4. Ion-exchange chromatography.

5. Paper and thin-layer chromatography: an essence, kinds, the qualitative and quantitative analysis, application.
6. High-performance liquid chromatography: an essence, equipment, detector systems, the qualitative and quantitative analysis; application.

TESTS AND REAL-LIFE SITUATIONS FOR SELF-ASSESSMENT

1. In a liquid chromatography apply such stationary and mobile phases:
A) Firm, liquid;
B) Firm, gaseous;
C) Firm or liquid, liquid;
D) Firm or liquid, gaseous;
E) Liquid, liquid.

2. High-performance liquid chromatography differs from a classical variant of a liquid chromatography all specified signs, except:
A) the liquid mobile phase is applied;
B) the smaller size of sorbent grains is applied in HPLC;
C) not only superficial but also volume-porous sorbents are applied in HPLC;
D) pumps of high pressures are used in HPLC;
E) high-sensitivity detectors are applied in HPLC.

3. How is called chromatography if on it stationary and mobile phases is liquid:
A) Gas-liquid;
B) the Liquid-liquid;
C) liquid-adsorbtion;
D) thin-layer;
E) ionic-exchange.

4. Liquid adsorption chromatography is divided on normal-phase and reverse-phase chromatography. Which combination of stationare/mobile phases is applied in is normal-phase:
A) the Polar sorbent, nonpolar mobile phase;
B) the Polar sorbent, nonpolar gas mobile phase;
C) nonpolar sorbent, a polar mobile phase;
D) nonpolar sorbent, a polar gas mobile phase;
E) nonpolar liquid sorbent, a polar gas mobile phase.

ANSWERS ON THE SELF-ASSESSMENT

Tests: 1. E, 2. D, 3. B, 4. A.

Questions for the theoretical training

Learning Objectives:

Upon completion of this module you should be able to
1. Explain the basic principles, operation and application of IC.
2. Differentiate between IC and other chromatographic methods.
3. Explain the chemical basis for stationary phase effects and mobile phase effects.

4. Predict retention order given the relative dominance between stationary phase effects and mobile phase effects.
5. Differentiate between stationary phases used in anion exchange and cation exchange.
6. Explain the basis for the common IC detection methods.
7. Describe the general process of analyzing a sample by IC.
8. Explain the rationale for using a suppressor cartridge and how it works.
9. Predict the effects of overloading, eluting too quickly, eluting too slowly, baseline drift.

Ion Exchange Chromatography

Ion chromatography is used for water chemistry analysis. Ion chromatographs are able to measure concentrations of major anions, such as fluoride, chloride, nitrate, nitrite, and sulfate, as well as major cations such as lithium, sodium, ammonium, potassium, calcium, and magnesium in the parts-per-billion (ppb) range. Concentrations of organic acids can also be measured through ion chromatography. The most popular method for the purification of proteins and other charged molecules is ion exchange chromatography.

How Does Ion Chromatography Work?

Ion chromatography, a form of liquid chromatography, measures concentrations of ionic species by separating them based on their interaction with a resin. Ionic species separate differently depending on species type and size.

In cation exchange chromatography positively charged molecules are attracted to a negatively charged solid support. Conversely, in anion exchange chromatography, negatively charged molecules are attracted to a positively charged solid support.

Sample solutions pass through a pressurized chromatographic column where ions are absorbed by column constituents. As an ion extraction liquid, known as eluent, runs through the column, the absorbed ions begin separating from the column. The retention time of different species determines the ionic concentrations in the sample.

Mechanism

To optimize binding of all charged molecules, the mobile phase is generally a low to medium conductivity (i.e., low to medium salt concentration) solution. The adsorption of the molecules to the solid support is driven by the ionic interaction between the oppositely charged ionic groups in the sample molecule and in the functional ligand on the support. The strength of the interaction is determined by the number and location of the charges on the molecule and on the functional group. By increasing the salt concentration (generally by using a linear salt gradient) the molecules with the weakest ionic interactions start to elute from the column first. Molecules that have a stronger ionic interaction require a higher salt concentration and elute later in the gradient. The binding capacities of ion exchange resins are generally quite high. This is of major importance in process scale chromatography, but is not critical for analytical scale separations.

Buffer pH

As a rule, the pH of the mobile phase buffer must be between the pI (isoelectric point) or pKa (acid dissociation constant) of the charged molecule and the pKa of the charged group on the solid support.

For example, in cation exchange chromatography, using a functional group on the solid support with a pKa of 1.2, a sample molecule with a pI of 8.2 may be run in a mobile phase buffer of pH 6.0.

In anion exchange chromatography a molecule with a pI of 6.8 may be run in a mobile phase buffer at pH 8.0 when the pKa of the solid support is 10.3.

Salt Gradients

As in most other modes of chromatography (SEC being the exception) a protein sample is injected onto the column under conditions where it will be strongly retained. A gradient of linearly increasing salt concentration is then applied to elute the sample components from the column. An alternative to using a linear gradient is to use a step gradient. This requires less complicated equipment and can be very effective to elute different fractions if the appropriate concentrations of salt are known, usually from linear gradient experiments.

Varying pH

Many chromatographers also use changes in pH to affect a separation.

In cation exchange chromatography, raising the pH of the mobile phase buffer will cause the molecule to become less protonated and hence less positively charged. The result is that the protein no longer can form a ionic interaction with the negatively charged solid support, which ultimately results in the molecule to elute from the column.

In anion exchange chromatography, lowering the pH of the mobile phase buffer will cause the molecule to become more protonated and hence more positively (and less negatively) charged. The result is that the protein no longer can form a ionic interaction with the positively charged solid support which causes the molecule to elute from the column.

Applications

Some typical applications of ion chromatography include:

- Drinking water analysis for pollution and other constituents
- Determination of water chemistries in aquatic ecosystems
- Determination of sugar and salt content in foods
- Isolation of select proteins

How to - Sample Collection, Preparation and Concerns

Liquid Samples:

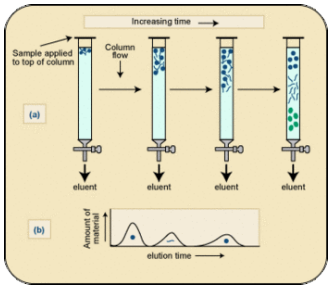
Liquid samples should be filtered prior to evaluation with an ion chromatograph to remove sediment and other particulate matter as well as to limit the potential for microbial alteration before the sample is run. Aqueous samples should be collected using a sterile syringe or bottle rinsed three times with sample water and then filtered

through 0.45um (or smaller) filters. The collection vial should likewise be rinsed three times with filtrate before being filled brim-full of sample filtrate. Samples should be stored cold until they can be processed. The minimum sample required for analysis is approximately 5mL, with no maximum limits.

Solid samples and Organic Liquids

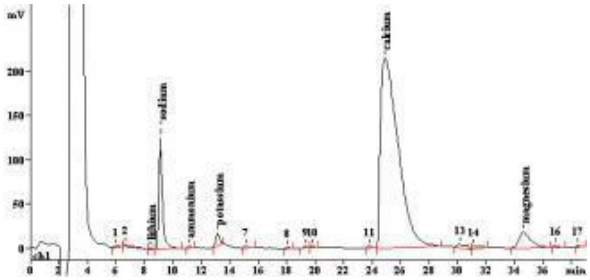
Solid samples can be extracted with water or acid (cations) to remove ions from the sample surface. Liquid samples must also be filtered and stored cold until analysis can be performed. The minimum sample required for a solid sample is approximately 2-3 cm^2 for solids, with no maximum limits.

Data Output and Analysis

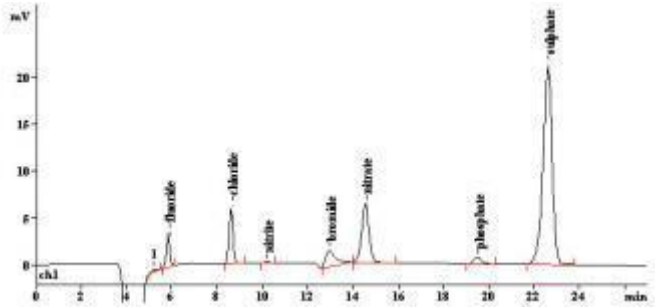


This schematic diagram 1 of an ion chromatography run depicts how elution time correlates to output peak data.

The diagram 1 shows how an ion chromatograph works to output data. Each peak represents a separate ion from the sample solution. The elution time, or time it takes for the ion to move through the column, varies for each ion species as they elute from the column separately as the pH and/or ionic strength of the eluent is increased. The concentration of ions moving through the column at a particular time is represented by the height and the breadth of the peaks and can be correlated to the concentration of a particular species in the sample solution.



This ion chromatograph 1 displays data from a cation analysis of glacial waters. Each peak represents the concentration of each cation.



This ion chromatograph 2 displays data from an anion analysis of glacial waters. Each peak represents the concentration of each anion.

The graphs display typical data output from an ion chromatography run. The graph 1 shows cation concentrations and the graph 2 depicts anion concentrations from dilute glacial waters. Ion concentrations can be calculated using the area under each peak, where a larger area correlates with a higher concentration of a particular ion species. Most ion chromatography machines provide software that calculates this area, which users can convert to ppm or other quantity using calibration standard solutions.

Laboratory work
Quantitative determination of calcium chloride in solution by ion exchange chromatography.

Calcium chloride solution is passed through a column cationite:

$$RH_m + Ca^{2+} \rightleftarrows RH_{(m-2)}Ca + 2H^+$$

Number of hydrogen ions removed off adsorbent is equivalent to content of Cl^- ions in analyzed solution. As a result the acid HCl is formed that can be titrated by NaOH solution with phenolphthalein indicator.

Activities

1. Place the sample of $CaCl_2$ solution into the column containing a cationite (cation exchanger) in H–form. At first remove some of the distilled water covering the cationite (the water is needed to avoid contact of the cationite with air) in the column.
2. Turn the stopcock (tap) in the column to let the liquid drop from the colunm into the flask. Make sure the cationite (cation exchanger) is always covered by the liquid.
3. Rinse the flask containing the analyzed solutions 2 – 3 times with small amounts of distilled water and pass these portions through column again.
4. Rinsing waters have to be collected in the same flask where all eluate was collected.
5. Add some (15 – 20 ml) of distilled water into the column and collect the eluate again in the same flask.
6. On finishing the experiment close the tap and put some more distilled water into the column.
7. Add 2 – 3 drops of phenolphthalein to the collected eluate and titrate it with natrium hydroxide solution c (NaOH) = 0,1 mol/l until a slightly pink color appear.

Evaluation

1. Determine the **volumes of titrant obtained from a titration** and put them in the table.
2. Calculate the average volume of titrant delivered in the titration

$$V_{av.} = \frac{V_1 + V_2 + V_3}{3} mL = \_\_\_\_\_ = \_\_\_\_\_ mL$$

3. Convert $V_{av.}$ in mL of NaOH solution into liters dividing by 1000:
   1 L = 1000 mL =1· 10^{-3} mL
   V_{av}(NaOH) mL · 1L / 1000 ml = V_{av}(NaOH) ml · 10^{-3} L =_____ · 10^{-3} L

Evaluation table

Molarity of **NaOH** solution _____ mol/L

M($\frac{1}{2}$ Ca^{2+}) = $\frac{1}{2}$ ·M(Ca^{2+}) = g/mol

NaOH *titration of eluate*		
NaOH volume	**NaOH average volume**	m(Ca^{2+})
Trial 1 _____ mL	_____mL	_____g
Trial 2 _____ mL		
Trial 3 _____ mL		

Mass of Ca^{2+} (in grams) in the analyzed solution must be calculated according to the formula:

$$m(Ca^{2+})=c(NaOH)\cdot V_{ev}(NaOH)\cdot M(\tfrac{1}{2}Ca^{2+}).$$

m(Ca^{2+})=

Practical lesson 29

Separation of mixture components by thin-layer or paper chromatography. Situational tasks. Laboratory works: "Identification of amino acids in the mixture using paper chromatography", "Separation and identification of constituents in the mixture using thin layer chromatography". Test control.

THE SELF-PREPARATION PROGRAM

1. Theoretical bases of chromatographic methods.
2. Classification of chromatographic methods on modular condition of phases, on way of moving of phases, on sorption mechanism, on performance techniques.
3. Chromatogram and its characteristics: retention time, adjusted time, peak width on half of height.
4. Chromatographic process: the theory of theoretical plates and the kinetic theory.

5. Characteristics of division efficiency: separation degree, resolution, number of theoretical plates, the height equivalent to a theoretical plate.
6. Theoretical bases of chromatography.
7. The main parts of chromatographic equipment.
8. Gas chromatography (at constant temperature and with temperature programming); the qualitative and quantitative analysis; application.

TESTS AND REAL-LIFE SITUATIONS FOR SELF-ASSESSMENT

1. For separation substance in mix we use chromatography. This method is based on redistribution of substance between:

A. Liquid and firm phases.
B. Two liquid phases.
C. Stationary and mobile phase.
D. Liquid and gas phases.
E. Gas and firm phases.

2. Quantitive analysis in gas chromatography is based on dependence:

A. height (area) of chromatographic peak and substance concentration.
B. Retention time and substance concentration.
C.retention volume and substance concentration.
D. Peak width and substance concentration.
E. the height equivalent to a theoretical plate and the substance's concentration.

3. At definition of residual quantity of solvent intelligent use:

A. volatilization method
B. liquid chromatography
C. extraction-photometric analysis
D. gas chromatography
E. thin layer chromatography

4. Menthol is fugitive substance. Which physical-chemical method you may use for its definition:

A. differential spectrophotometry;
B. thin-layer chromatography;
C. liquid chromatography;
D. gas chromatography;
E. multiwave spectrophotometry.

ANSWERS ON THE SELF-ASSESSMENT

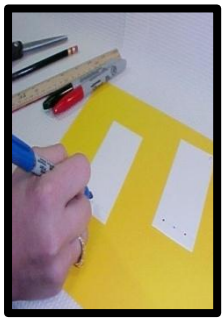
Tests: 1. C., 2. A, 3. D., 4. D.

Paper or Thin Layer Chromatography

Some technical terms: The substances (**solutes**) to be analysed must dissolve in the **solvent**, which is called the **mobile phase** because it moves. The paper or thin layer of material on which the separation takes place is called the **stationary or immobile phase** because it doesn't move.

- Modified Papers – acid or base washed filter paper, glass fiber type paper.
- Hydrophilic Papers – Papers modified with methanol, formamide, glycol, glycerol etc.
- Hydrophobic papers – acetylation of OH groups leads to hydrophobic nature, hence can be used for reverse phase chromatography.
- Impregnation of silica, alumna, or ion exchange resins can also be made.

PREPARATION OF PAPER



Cut the paper into desired shape and size depending upon work to be carried out.

The starting line is marked on the paper with an ordinary pencil 5cm from the bottom edge.

On the staring line marks are made 2cm apart from each other.

Preparation of the solution

Choice of suitable solvent for making solution is very important. Pure solutions can be applied direct on the paper but solids are always dissolved in small quantity of a suitable solvent.

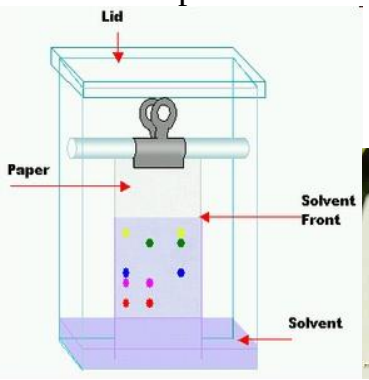
Biological tissues are treated with suitable solvents and their extracts obtained. Proteins can be precipitated with alcohol and salts can be removed by treatment with ion exchange resin.

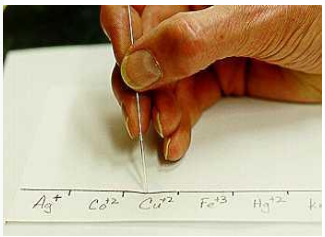
APPLICATION OF SAMPLE

The sample to be applied is dissolved in the mobile phase and applied as a small spot on the origin line, using capillary tube or micropipette.

very low concentration is used to avoid larger zone

- The spot is dried on the filter paper and is placed in developing chamber.





Choice of the Solvent

The commonly employed solvents are the polar solvents, but the choice depends on the nature of the substance to be separated.

If pure solvents do not give satisfactory separation, a mixture of solvents of suitable polarity may be applied.

MOBILE PHASE

- Pure solvents, buffer solutions or mixture of solvents
- Examples- **Hydrophilic mobile phase**
- Isopropanol: ammonia:water 9:1:2
- Methanol : water 4:1
- N-butanol : glacial acetic acid : water 4:1:5

Hydrophobic mobile phases

- dimethyl ether: cyclohexane
- kerosene : 70% isopropanol

CHROMATOGRAPHIC CHAMBER

The chromatographic chamber are made up of many materials like glass, plastic or stainless steel. Glass tanks are preferred most. They are available in various dimensional size depending upon paper length and development type.

The chamber atmosphere should be saturated with solvent vapor.

DEVELOPMENT TECHNIQUE

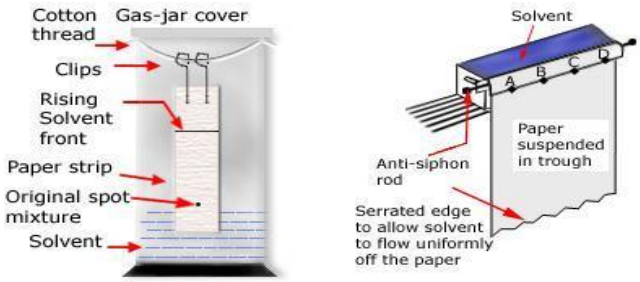
Paper is flexible when compared to glass plate used in TLC, several types of development are possible which increases the ease of operation.

The paper is dipped in solvent in such a manner that the spots will not dip completely into the solvent.

The solvent will rise up and it is allowed to run 2/3rd of paper height for better and efficient result.

Different types of development tech. are

1) ASCENDING DEVELOPMENT (go up)



- Like conventional type, the solvent flows against gravity. The spots are kept at the bottom portion of paper and kept in a chamber with mobile phase solvent at the bottom.

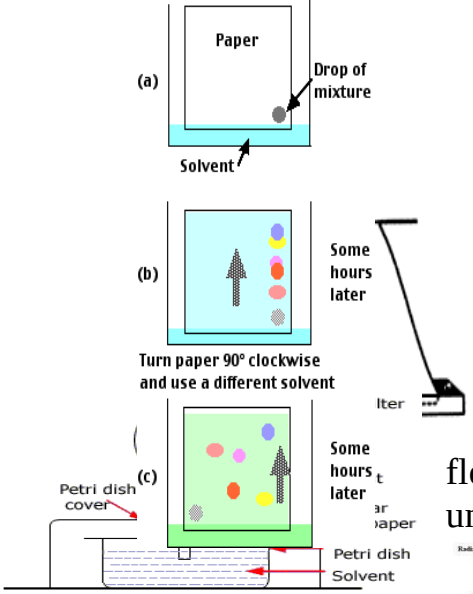
2) DESCENDING TYPE (a downward slope)

- This is carried out in a special chamber where the solvent holder is at the top. The spot is kept at the top and the solvent flows down the paper.
- In this method solvent moves from top to bottom so it is called descending chromatography.

ADVANTAGE IS THAT, DEVELOPMENT IS FASTER

3)ASCENDING – DESCENDING DEVELOPMENT

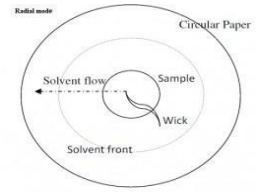
A hybrid of above two technique is called ascending-descending chromatography.



Only length of separation increased, first ascending takes place followed by descending

4)CIRCULAR / RADIAL DEVELOPMENT

Spot is kept at the centre of a circular paper. The solvent flows through a wick at the centre & spreads in all directions uniformly.



5)TWO DIMENSIONAL DEVELOPMENT

In this method the paper is developed in one direction and after development, the paper is developed in the second direction allowing more compounds to be separated into individual spots. In the second direction, either same solvent/different solvent system can be used for development.

DRYING OF CHROMATOGRAM

After the solvent has moved a certain distance for certain time the chromatogram is taken out from the tank & position of the solvent front is marked with a pencil.

They are dried by cold or hot air depending on volatility of solvents. A simple hair dryer is a convenient device to dry chromatograms.

DETECTING / VISUALISING AGENTS

If the substance are colored they are visually detected easily.

But for colorless substance, Physical and chemical methods are used to detect the spot.

(a) Non specific methods (Physical methods)

E.g. iodine chamber method,

UV chamber for fluorescent compounds – at 254 or at 365nm.

(b) Specific methods (Chemical methods) or Spraying method - examples,

- Ferric chloride - Phenolic comp. & tannins
- Ninhydrin in acetone - Amino acids
- Dragendroff's reagents - Alkaloids
- 3,5 dinitro benzoic acid -Cardiac glycosides

It is possible to analyse colourless mixture if the components can be made coloured e.g. protein can be broken down into **amino acids** and **coloured purple** by a chemical **reagent called Ninhydrin** and many **colourless organic molecules fluoresce** when **ultra-violet light is shone on them**. These are called **locating agents**.

Following detecting tech. can also be categorized as

1) Destructive techniques

Specific spray reagents, samples destroyed before detection e.g. – ninhydrin reagent

2) Non-destructive techniques

- For radio active materials - Geiger Muller counter
- uv chamber, iodine chamber

QUANTITATIVE ESTIMATIONS

The method can be divided into two main groups

1. Direct techniques-

2. Indirect techniques-

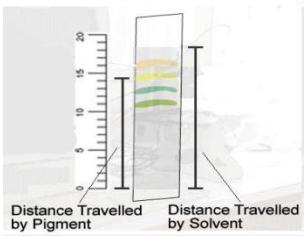
Direct Measurement Method

- Comparison of visible spots
  A rough quantitative measurements
  Component in a mixture can be carried out by comparing the intensity and size of the spot with a standard substance.
- Photo densitometry
  The method is used with the chromatograms of colored compound, instrument which measures quantitatively the density of the spots.
- Fluorimetry
  The compound to be determined by fluorimetry must be fluorescent or convertible into fluorescent derivatives.
- Radiotracer Method
  The compound containing radioactive element is labeled and treated with locating reagent. Using Geiger Muller counter.
- Polarographic & Conductometric methods
  Used to measure the amount of material in the spot

Indirect Measurement Method

In this technique, the spots are cut into portions and eluted with solvents. This solution can be analyzed by any techniques of analysis like spectrophotometry, electrochemical methods, etc.

Rf VALUE (Retardation Factor)



In paper chromatography the results are represented by Rf value which represent the movement or migration of solute relative to the solvent front.

The distance a substance moves, compared to the distance the solvent front moves (top of grey area on 2nd diagram) is called the reference or **R_f value** and has a value of 0.0 (not moved - no good), to 1.0 (too soluble - no good either), but **R_f** ratio values between 0.1 and 0.9 can be useful for analysis and identification.

R_f = distance moved by dissolved substance (solute) / distance moved by solvent.

Factors affecting Rf VALUE

- The temperature
- The purity of the solvents used
- The quality of the paper, adsorbents & impurities present n the adsorbents
- Chamber saturation techniques, method of drying & development
- The distance travelled by the solute & solvent
- Chemical reaction between the substances being partitioned.
- pH of the solution

R_x VALUE

- In many cases it has been observed that the solvent front is run off the end of the paper. $\mathbf{R_x}$ value is thus used,
- It is the ratio of distance travelled by the sample and the distance travelled by the standard. $\mathbf{R_x}$ value is always closer to 1.

Sources of Error

1. Error during application of the spots

- Apply minimum volume of the concentrated solution in order to avoid diffusion through the paper which leads to poor separation
- Spots should be approximately of the same diameter.

2. Development

- Improper adjustment of the paper in the tank leads to this error so the paper should be held vertically.
- Do chamber saturation

3. Detection

- The spraying methods affect the final result

APPLICATIONS

- Separation of mixtures of drugs
- Separation of carbohydrates, vitamins, antibiotics, proteins, etc.
- Identification of drugs
- Identification of impurities
- Analysis of metabolites of drugs in blood , urine ….

ADVANTAGES OF P.C

Simple ,rapid ,inexpensive ,excellent resolving power

PRECAUTIONS IN P.C

Establishing the vapor solvent equilibrium

Stability of solvent mixture is first ensured

Laboratory work 6

SEPARATION OF AMINO ACIDS MIXTURE USING PARTITION CHROMATOGRAPHY ON PAPER

Activities

1. Mark the line by a pencil (starting line) at 2-3 cm from the top of paper and then place a drop of the sample of amino acids mixture on this line. Let the paper dry for several minutes.
2. Place 3-4 ml of the solvent (mixture of phenol with water) into the chromatographic chamber. Then the end of the paper having the drop should be placed in the solvent. Make sure the solvent doesn't touch the spot on the paper. Close up the chromatographic chamber.
3. As soon as the solvent rises as high as 16-18 cm up on the paper, take out the paper from the chamber and fix the line the solvent reached by pencil (finishing line) and let the paper dry.
4. Treat the paper of ninhydrin solution, and then dry it at 50-60 °C.
5. Measure the traversed path by each amino acid, i.e. the distance from the starting line to the center of the spot on the chromatogram (X_1), and the distance passed by the solvent (X_2).
6. .Calculate the coefficient Rf for each of the amino acids using the formula: $R_f = \frac{X_1}{X_0}$

Rf(i)=X_i/X_0

Rf(1)=X_1/X_0=

Rf(2)=X_2/X_0=

7. Put down these results into the table 1.

Table 1

Distance passed by the amino acid X_1, cm	Distance passed by the solvent X_0, cm	R_f

8. Compare calculated Rf with the tabular data (Table 2) and then identify the amino acids in sample.

Table 2

Amino acid	R_f	***Amino acid***	R_f
Asparagine	0,07	Arginine	0,41

Glutamine	0.16	Tyrozine	0,52
Cysteine	0,19	Alanine	0,55
Glycine	0,03	Leucine	0.79
Methionine	0,30		

Conclusion
Sample of the amino acids consist of _____

TEST QUESTION DATABASE "STEP 1"

Read the test questions with answers. To prove the correct answer, which is the first.

1.Chromatographic separation can be done in two ways: planar (on plates) and column. In column (flow) chromatographic methods of analysis of the substance is determined by:

A. The area under each chromatographic peak.
B. Width of the chromatographic peak.
C. Retention time.
D. Retention volume.
E. Height equivalent to a theoretical plate.

2. The quantitative analysis of gas chromatography based on relationship of analytes *concentration and*:

A. The heights of the chromatographic peak and area under chromatographic peak.
B. Retention time.
C. Retention volume.
D. Width of the chromatographic peak.
E. Heights equivalent to a theoretical plate.

3. Rf values are:

A. An indication of the mobility of the substance.
B. Absolute characteristic of the substance.
C. An indication of the solubility of substances in the liquid phase.
D. An indicator of sorption capacity of the solid phase.
E. An indicator of sorption capacity of the substance.

4. The phenomenon of adsorption chromatographic methods used in the analysis. Activity sorbent (capacity) is characterized by the number of electrolyte absorbed by a unit mass or unit volume of sorbent. In which case the capacity of adsorbent is maximum?

A. *0.02 g of adsorbent absorbs 0.003 mol Na ions*
B. 0.01 g of adsorbent absorbs 0.1 mmol Na ions
C. 0.05 g of adsorbent absorbs 0.2 mmol Na ions
D. 0.08 g of adsorbent absorbs 0.08 mmol Na ions
E. 0.06 g of adsorbent absorbs 0.0002 mol Na ions

5. Ion-exchange chromatography is one of the quantitative analysis methods. What process is used in the method ion-exchange chromatography?

A. The ionic interaction between the oppositely charged ionic groups in the sample molecule and in the functional ligand on the support.
B. Adsorption of ions on the surface discrabes by the Paneth – Faience rule.
C. Redox processes .
D. Reaction formation and dissolution of precipitates on the support
E. The formation of chelate compounds on the support

6. Determination of thermally unstable impurities in the low concentration carry out using:
Higheffective liquid chromatography

A. Gas chromatography
B. Paper chromatography
C. Ion-exchange chromatography
D. Thin-layer chromatography

7. What is Rf of novocaine if the distance of spot from the starting line is 3 cm, and the distance of the front solvents -10cm?

A. 0.3
B. 0.4
C. 0, 5
D. 0, 6
E. 0.7

8. What method of chromatographic analysis in which as a sorbent uses the ionits.
A. Ion-exchange
B. Gas
C. Paper
D. Thin-layer
E. Gel-filtration

9. To identify drug-using tonkosha Rehoboam chromatography using parameter:
A. Rf
B. N
C. E, mV
D. I, A
E. K_p

10. For separating mixtures of substances used chromatographic method.
Chromatography - a method of analysis based on the redistribution of matter between:

Moving and stationary phases
Solid and gas phases
Liquid and solid phases
The two liquid phases are not mixed together
Liquid and gas phases
11. Should reveal that alcohol is in an aqueous solution by gas-liquid chromatography. What size are used to identify substances by gas-liquid chromatography?
Options retention.
Height chromatographic peak.
Height equivalent to a theoretical plate.
The area of the chromatographic peak.
The number of theoretical plates.
12. In the method of paper chromatography studies zakinchuyet Xia:
When the solvent line "finish"
After 5 minutes after the start of the experiment
In moving spots on vidstan10sm. the line "start"
After 10 min. after the start of the experiment
In moving spots to the line "finish"

13. The basis of the quantitative analysis of gas chromatography is the relationship:
The heights of the chromatographic peak and its area of concentration of the substance.
Retention time on the concentration of the substance.
Retention volume on the concentration of the substance.
Width of the chromatographic peak concentration.
Heights of equivalent to a theoretical plate number of substances.
14. Pharmacist-analyst conducts analysis of the drug by thin-layer chromatography. In this article the pharmacopeia value of Rf. Rf values are:
An indication of the mobility of the substance.
Absolute characteristic of the substance.
An indication of the solubility of substances in the liquid phase.
An indicator of sorption capacity of the solid phase.
An indicator of sorption capacity of the substance.

15. Should reveal that alcohol is in an aqueous solution by gas-liquid chromatography. What size are used to identify substances by gas-liquid chromatography?
Options retention.
Height chromatographic peak.
Height equivalent to a theoretical plate.
The area of the chromatographic peak.
The number of theoretical plates.
16. The basis of the quantitative analysis of gas chromatography is the relationship:
Heights of chromatographic peak and its area of concentration of substances.
Retention time on the concentration of the substance.

Retention volume on the concentration of the substance.
Width of the chromatographic peak concentration.
Heights, equivalent to a theoretical plate, depending of number of substances.

17. Partition chromatography is based primarily on:
Different solubility of components in the two liquids that do not mix
Adsorption various components of the mixture on the selected adsorbent
Ion exchange using yonitiv different nature
Deposition of mixture components on the selected adsorbent
Distribution of mixing between the moving liquid and solid stationary phases

18. It is known that menthol is highly volatile substances. Which physical and chemical methods of analysis can be used to quantify the content of menthol in drops Zelenin that are difficult drug:
gas chromatography
thin layer chromatography
liquid chromatography
differential spectrophotometry
Multiwave spectrophotometry
19. The basis of the qualitative analysis of the gas and liquid chromatography is so dependent on the nature of the substance:
retention time or volume
peak width at the base of chromatograms
half-width of the chromatographic peak
peak width at half height
height or area of the chromatographic peak

20. In order to quantify the substances by gas chromatography using the calibration graph. Calibration graph for chromatographic determination of substances - a relationship:
Square chromatographic peak on the concentration of the substance.
Time of supporting the volume of injected sample.
The heights of the chromatographic peak retention time.
The heights of the chromatographic peak in the chromatogram of the distance.
Peak heights of supporting the distance.
21. In determining the content of residual solvents in drug substances most efficiently apply:
By gas chromatography
The method of liquid chromatography
Extraction-photometric analysis
The method of direct and indirect stripping
The method of thin layer chromatography

Practical lesson 30

Methods for determining the qualitative and quantitative content of analyte based on results of Electrochemical and Chromatographic methods. Problem solving and situational tasks. Writing control work and test.

Example of the control work

Test

1 Molar absorbtivities of compounds exhibiting charge transfer absorption are

A.small
B.moderate
C.large
D.none of these

2 Molar absorbtivity is the measure of the

A.amount of light absorbed per unit length
B.amount of light absorbed per unit concentration
C.amount of light reflected and absorbed per unit concentration
D.None of the above

3 Which of the following relationships between absorbance and %transmittance is incorrect?

A.$A = \log_{10} 100 / \%T$
B.$A = 2 - \log_{10} \%T$
C.$A = \log_{10} 1 / T$
D.All are correct

4 Why is it generally preferable to use absorbance as a measure of absorption rather than % transmittance?

A.Because %T cannot be measured as accurately as absorbance
B.Because %T is dependant on the power of the incident radiation
C.Because absorbance is proportional to the concentration of the analyte, whereas %T is not
D.none of the above

5 In the past, IR spectra had to be aquired one wavelength at a time, which took a long time. Today quick spectra is due to the

A.the Fourier Transfer Algorithm allows us to scan all frequencies at once
B.light is faster today that it used to be
C.absence of broad spectrum of wavelenth
D.none of the above

6 Which of the following is not an IR vibrational mode?

A.Stretching
B.Scissoring
C.Rocking
D.Rolling

7 Which of the following will oscillate the fastest?

A.A large mass on a weak spring
B.A large mass on a stiff spring
C.A small mass on a stiff spring
D.A small mass on a weak spring

8 In the equation, A = ε bc, what quantity is represented by "ε"?

A.Absorbtivity
B.Molar absorbtivity
C.Path length
D.None of these

9 Why must the voltage supplied to a tungsten lamp be very stable?

A.Because if it wasn't, the lamp would bum out
B.Because amount of energy the lamp emits is proportional to the fourth power of the operating voltage
C.Because the lamp will only function at a specific voltage
D.All of the above

10 Where does a carbonyl (C=0) stretch appear in an IR spectrum?

A.1740-1720
B.1870-1650
C.3640-3250
D.160-110

11 Which of the following components of a monochromator is the dispersing element?

A.The collimating lens
B.The entrance slit
C.The diffraction grating
D.None of these

12 A simple harmonic oscillator may absorb energy

A.at anytime.
B.when the frequencies match exactly
C.when the amplitudes are the same.
D.at no time.

13 UV-Vis spectroscopy of organic compounds is usually concerned with which electronic transition(s)?

A. $\sigma \rightarrow \sigma^*$
B. $n \rightarrow \sigma^*$
C. $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$
D. none of these

14 Vibrational spectroscopy is

A. a large mass on a weak spring
B. a flashlight through a prism and shake it
C. a class of spectroscopic techniques which analyzes molecular motions
D. an Infrared spectroscopy

15 Why are rotational transitions of little use to a spectroscopist?

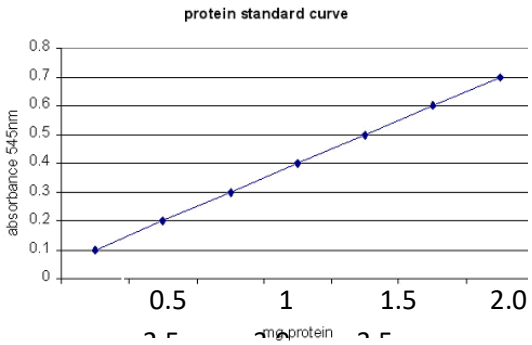
A. Because the energy required to induce a rotational transition is so small that it cannot be measured
B. Because rotational transitions are extremely rare
C. Because, in liquids and solids, spectral lines corresponding to rotational transitions are broadened as the result of molecular collisions and other interactions
D. All of the above

16 Beer's Law states that

A. absorbance is proportional to both the path length and concentration of the absorbing species
B. absorbance is proportional to the log of the concentration of the absorbing species
C. absorbance is equal to P_0 / P
D. none of the above

Written test

1.Given the following standard curve, answer the following question



d. An unknown protein has an absorbance of 0.45, what would its concentration be in mg?
e. An unknown protein has an absorbance of 0.20, what would its concentration be in mg?
f. An unknown protein has a concentration of 1.60, what would its absorbance be?

6. Molar absorptivity of copper (II) dithizon in CCl_4 at λ_{max} = 850 nanometers equal E = $4{,}52 \oplus 10^4$. Which contents (% w/w) of copper can be defined with dithizon if from sample of sample (weight 1,00 g) receive 25,00 mL of a solution dithizon in CCl_4 and measure the minimum absorbance 0,020 if thickness of a layer l = 5,0 cm.
7. Calculate the coefficient Rf for each of the amino acids and compare calculated Rf with the tabular data and then identify the amino acids in sample if

Distance passed by the amino acid X_1, cm	Distance passed by the solvent X_0, cm	R_f
7,91	10,01	
3,94	10,01	

Amino acid	R_f	*Amino acid*	R_f
Asparagine	0,07	Arginine	0,41
Glutamine	0.16	Tyrozine	0,52
Cysteine	0,19	Alanine	0,55
Glycine	0,03	Leucine	0.79
Methionine	0,30		

Sample of the amino acids consist of _____

Oral part

1. Mass spectroscopy
2. What property does paper chromatography make use of to separate its components?