COMPUTER SIMULATION OF CAPILLARY ELECTROPHORESIS - ANALYSIS OF ORGANIC ACIDS

1. Qualitative analysis. Optimalization of organic acids mixture's separation

a) Run the program HPCESIM. Copy all basic parameters (Equipment, Capillary, Buffer, Sample, Inject and Detect) for electrophoretic separation of mixture of 4 organic acids into table 1.

Table 1

Voltage U (kV)	
Current I (μA)	
Thermostating temperature T (ºC)	
Capillary length to detector L (mm)	
ID (inner diameter of capillary) (μm)	
Separation mode (anionic/cationic)	
Composition of basic electrolyte	Carrier ion:
	counter ion:
pH of basic electrolyte	
I (ionic strength of basic electrolyte) (mmol/l)	
Analysed substances	
Injection volume V (nl)	
Type of detector	
Slit width (μm)	

b) Observe the influence of pH of basic electrolyte on the separation of organic acids (Buffer, pH)
Add glutamic acid to the original mixture of four organic acids (Sample, Add, arrows ↑↓, choose glutamic acid, Enter). Change the pH value of basic electrolyte from 4,0 - 5,0 – 6,0 – 7,0 (Buffer, pH) and observe changes of migration times and order of analyzed acids on the electropherogram (time ax is set using Detect, Interval). Copy migration times of analyzed substances (Sample, Time) in table 2. Draw a graphic representation of dependence of migration time (ax y) on pH value of basic electrolyte (ax x) and explain this dependence.

Table 2

Analyte	Migration time t (min)			
	pH 4,0	pH 5,0	pH 6,0	pH 7,0
formic acid				
acetic acid				
propanoic acid				
butyric acid				
glutamic acid				

After finishing the task, return to the original electropohoretic record by pressing Esc or File, Restart.

c) Observation of the voltage influence on the separation of organic acids
Change the value of working voltage (Equipment, Voltage) gradually from -5 kV to -30 kV and observe the changes on electropherogram and explain them. Copy the migration times of analyzed substances (Sample, Time) in the Table 3. Draw a graphic representation of dependence of migration time (ax y) on working voltage (ax x) and explain this dependence.

Table 3

Analyte	Migration time t (min)			
	-5 kV	-10 kV	-20 kV	-30 kV
formic acid				
acetic acid				
propanoic acid				
butyric acid				
glutamic acid				

d) Observe the dependence of effective mobility of analytes on their molar mass.
Write molar masses of analytes into table 4 and copy from computer their effective mobilities at pH 5.0 of basic electrolyte (Sample, μeff). Explain this dependence.

Table 4

Analyte	Molar mass M	effective mobility μ_{eff} (m^2/Vs)
formic acid		
acetic acid		
propanoic acid		
butyric acid		

e) Observe the dependence of effective mobility of analytes on pH value of basic electrolyte.
Change Ph values of basic electrolyte gradually from 4.0 – 5.0 – 6.0 – 7.0 (Buffer, pH) and observe changes of effective mobilities (Sample, μ_{eff}) and copy their values in table 5. Draw a graphic representation of dependence of analytes effective mobilities (ax y) on pH values (ax x) and explain this dependence.

Table 5

Analyte	effective mobility μ_{eff} (m^2/Vs)			
	pH 4,0	pH 5,0	pH 6,0	pH 7,0
formic acid				
acetic acid				
propanoic acid				
butyric acid				

Tell which working parameters (U, pH) are selective or non-selective and explain. What parameters influence effective mobility of analyte? Equations.

2. Quantitative analysis. Determination of unknown concentration of chosen organic acid in mixture

Observe the influence of the concentration of analyzed substance on the size (height, area) of the peak. Change the concentration from 10 μmol/l to 100 μmol/l (Sample, Concentration, number of analyte). Copy areas of peaks of analyzed substance (Sample, Area) in Table 6.

Table 6

	Area of peak A, mMs									
Analyte (μmol/l)	10	20	30	40	50	60	70	80	90	100

Draw a graphic representation of peak area of analyte (ax y) on concentration (ax x) and explain this dependence. Determine the concentration of the substance with peak area ..... mAUs using this dependence.
End the program (Files, Quit, Yes)

ELECTROPHORETIC SEPARATION OF SULPHONAMIDES OR ALKALOIDS

A) Electrophoretic separation of sulphonamides

Sulphonamides are the substances, which have an amphoteric character (they have in their molecule acidic and basic functional groups). The degree of ionization of these groups and therefore the mobility in direct current field depends on the acidity of the medium. The biggest differences in mobilities and therefore the best conditions for the separation are achieved in slightly alcalic medium. The suitable basic electrolytes for the separation are those with constant acidity (buffers) which prevents influence of ionization of separated substances during electrophoresis.

Standards:

1. Spofadazine
2. Sulphadiazine
3. Sulphaguanidine
4. Sulphacetamide
5. Sulphadimidine

B) Electrophoretic separation of alkaloids

Weak acids can ionize in water solutions by binding hydrogen protons. After this depending on their basicity and pH of the solution, there exist electroneutral non-ionized form B and cationic ionized form HB^+. Form HB^+ dominates in more acid solutions, form B dominates in more alkalic solutions. Only the form HB^+ is capable of electromigration. The degree of ionization and therefore the mobility in electric filed is determined by acidity of the medium.

Standards:

1. Papaverinium chloride
2. Pilokarpine chloride
3. Quinine sulphate
4. Ethylmorphine chloride
5. Atropine sulphate