CHEMISTRY: ART, SCIENCE, FUN



PRACTICAL EXAMINATION PROBLEMS

JULY 18, 2007 MOSCOW, RUSSIA

General Directions

- **safety rules** follow them as in the Preparatory problems described, no eating or drinking is allowed in the lab.
- violating safety rules you get one warning, offend again: you are out.
- **problem booklet** 12 pages (incl. cover sheet and Periodic table of elements) with 2 problems. Start with problem 1.
- **time** 5 hours; 30 minutes warning before the end.
- answer sheets: 5 pages (incl. cover sheet).
- your name and student code write it on every answer sheet.
- **answers** only in the appropriate places of the answer sheets, nothing else will be marked. Relevant calculations have to be shown.
- use only the pen and calculator provided.
- **results** the number of significant figures in numerical answers must conform to the rules of evaluation of experimental error. Mistakes will result in penalty points even if your experimental technique is flawless.
- **burette** read it as accurately as possible.
- more chemicals needed? Ask your lab assistant. No penalty for this.
- Extra sample to be analyzed or broken column a penalty of 10 marks.
- questions concerning safety, apparatus, chemicals, organization, toilet break: ask your lab assistant.
- **chemical waste** put it only in the designated containers.
- official English-language version available on request for clarification only. Ask your lab assistant.
- **after the stop signal** put your answer sheets and spectra in the envelope (don't seal), deliver them to your lab assistant. Keep the problem booklet together with the pen and calculator.
- You must stop your work immediately after the stop signal has been given. A delay of 5 minutes will result in zero points for the current task.
- During the Practical examination some of your glassware and plastics may have to be used more than once. Clean it carefully.

List of Chemicals

Reagent	Quantity	Placed in	Labeled
	Task 1		
Eluent 1	100 mL	Amber glass bottle*	Eluent 1
Eluent 1	1 mL	Plastic microtube	Eluent 1
Eluent 2	50 mL	Amber glass bottle*	Eluent 2
Eluent 2	1 mL	Plastic microtube	Eluent 2
Eluent 3	50 mL	Amber glass bottle*	Eluent 3
Eluent 3	1 mL	Plastic microtube	Eluent 3
0.5 M Carbonate buffer solution, pH 9.5	10 mL	Glass vial	NaHCO ₃
0.5 M Tris-HCl buffer solution, pH 8.5	10 mL	Glass vial	Tris-HCl
Mixture of amino acids to be analyzed**	1.2 mL	Plastic microtube	A number between 301 and 600
Ellmann reagent: 0.2 M Phosphate buffer solution containing 10 mM EDTA and 3 mM 5,5'-Dithiobis(2-nitrobenzoic acid), pH 7.0	10 mL	Glass vial	DTNB
Pauli's reagent: solution of sodium 4-diazonium- benzenesulfonate in 0.1 M aqueous HCl	1 ml	Plastic microtube	Pauli
Sodium hydroxide, 10% aqueous solution	10 mL	Glass vial	NaOH 10%
8-Hydroxyquinoline, 5.2 mM solution in ethanol/n-butanol (9:1) mixture	5 ml	Glass vial	8-HQ
Sodium hypobromite, 0.24 M solution in 10% aqueous NaOH	1.2 ml	Plastic microtube	NaBrO
2,4,6–Trinitrobenzenesulfonic acid, 3.4 mM aqueous solution	1 mL	Plastic microtube	TNBS
8 M Aqueous urea solution	1 mL	Plastic microtube	Urea
•	Task 2		•
HCl, standard solution, ~1 M (see exact value on the label)	40 mL	Amber glass vial	HCl <and concentration="" exact=""></and>
NaOH (to be standardized)	200 mL	Amber glass vial	NaOH
Powdery sample to be analyzed**	0.5 - 1 g	150 mL beaker covered	<number of<="" td=""></number>
		with watch glass	workplace>
H ₂ O distilled	400 mL	Plastic wash bottle	H_2O
H ₂ O distilled (shared between 2 students)	30 mL	Glass drop bottle	H ₂ O
H ₂ O distilled (for common use)	5 L	Bottle with tubing and clamp on top of the bench	H ₂ O
NaH ₂ PO ₄ , 15% solution (shared between 2 students)	20 mL	Glass drop bottle	NaH ₂ PO ₄ 15%
Bromocresol Green, 0.5% solution in 20% ethanol (shared among 3-4 students in a raw)	30 mL	Glass drop bottle	Bromcresol green
Thymolphthalein, 0.5% solution in ethanol (shared among 3-4 students in a raw)	30 mL	Glass drop bottle	Thymolphtalein
K ₂ C ₂ O ₄ , 15% solution (shared between 2 students)	50 mL	Amber glass vial	K ₂ C ₂ O ₄ 15%

^{*}Fixed on the top shelf (do not try to remove), with connected tubing and clamp **10 marks penalty for an extra portion of the sample

Components of Eluents 1 to 3

Eluent 1: 0.1 M aqueous sodium citrate, 50 mM sodium chloride, 40 mM thiodiglycol, 1 mM caprylic acid, 0.1% Brij-35; pH 4.9.

Eluent 2: 0.2 M aqueous sodium phosphate, 0.1% Brij-35; pH 7.0.

Eluent 3: 0.2 M aqueous sodium hydroxide.

Apparatus and Suppliers

Marker 1	Item	Quantity
Chromatography column with ion-exchange resin	Test tube rack	1
Laboratory stand with white covering Double clamp for burette 1 Double clamp for burette 1 1 25 mL Burette 1 100 mL flask labeled "Waste" 100 mL Volumetric flask 2 Syringe with needle Graduated test tubes for collecting fractions and preparing mixtures 50 96-well plate Fipettor (micropipette) with fixed volume of 0.1 mL Disposable tips (in blue plastic cup) Spectrophotometric cuvettes labeled "A1", "B1", "A2", "B2", "A3", "B3" in cuvette holder 10 mL Graduated plastic pipettes 10 mL Graduated plastic pipettes 11 Pipettor filler 12 3-Way Bulb 13 Glass rod 14 Filter funnel Small funnel 10 mL Amber glass vials for combined fractions (peaks) 10 mL Measuring cylinder labeled "K2-C2-Q1 15%" (shared between 2 students) 10 mL Measuring cylinder (shared between 2 students) 10 mL Measuring cylinder (shared between 2 students) 10 mL Measuring cylinder (shared between 2 students) 11 120 mL Measuring cylinder (shared between 2 students) 12 13 14 15 15 16 17 17 18 18 18 18 19 19 19 10 10 10 10 10 10 10	Laboratory stand	1
Double clamp for burette Ring for funnel 25 mL Burette 100 mL flask labeled "Waste" 1100 mL Volumetric flask 2 2 100 mL Frlenmeyer flask 2 2 Syringe with needle Graduated test tubes for collecting fractions and preparing mixtures 50 96-well plate 1 1 Disposable tips (in blue plastic cup) Spectrophotometric cuvettes labeled "A1", "B1", "A2", "B2", "A3", "B3" in cuvette holder 10 mL Glass pipette 1 1 Pipetter filler 1 3-Way Bulb Glass rod 1 1 Filter funnel Small funnel 1 1 Small funnel 1 1 Om L Measuring cylinder labeled "K2C2O4 15%" (shared between 2 students) 1 1 1 0 mL Measuring cylinder (shared between 2 students) 1 1 1 10 mL Measuring cylinder (shared among 3-4 students in a row) 1 2 3 filters per student 1 4 4 8 1	Chromatography column with ion-exchange resin	1
Ring for funnel 25 mL Burette 100 mL Glask labeled "Waste" 100 mL Volumetric flask 2 100 mL Erlenmeyer flask 2 Syringe with needle Graduated test tubes for collecting fractions and preparing mixtures 50 65-well plate 1 Pipettor (micropipette) with fixed volume of 0.1 mL Disposable tips (in blue plastic cup) Spectrophotometric cuvettes labeled "A1", "B1", "A2", "B2", "A3", "B3" in cuvette holder 10 mL Graduated plastic pipettes 10 mL Glass pipette 11 Pipette filler 11 3-Way Bulb 11 Glass rod Filter funnel Small funnel 10 mL Measuring cylinder labeled "K2C2Q415%" (shared between 2 students) 10 mL Measuring cylinder (shared between 2 students) 10 mL Measuring cylinder (shared between 2 students) 11 10 mL Measuring cylinder (shared between 2 students) 11 110 mL Measuring cylinder (shared between 3 -4 students in a row) 11 12 13 filters per student be used at your bench "SP") Marker Marker 1 lall Ruler	Laboratory stand with white covering	1
25 mL Burette 100 mL flask labeled "Waste" 1 100 mL Volumetric flask 2 Syringe with needle Graduated test tubes for collecting fractions and preparing mixtures 96-well plate Pipettor (micropipette) with fixed volume of 0.1 mL Disposable tips (in blue plastic cup) 20 Spectrophotometric cuvettes labeled "A1", "B1", "A2", "B2", "A3", "B3" in cuvette holder 10 mL Graduated plastic pipettes 10 mL Graduated plastic pipettes 11 Pipettor filler 11 3-Way Bulb 11 Glass rod 11 Filter funnel 12 Small funnel 13 Small funnel 14 Go mL Amber glass vials for combined fractions (peaks) 10 mL Measuring cylinder labeled "K ₂ C ₂ O ₄ 15%" (shared between 2 students) 11 Do mL Measuring cylinder (shared between 2 students) 12 Do mL Measuring cylinder (shared between 2 students) 11 Do mL Measuring cylinder (shared among 3-4 students in a row) 11 Plastic plate with filters*** (shared among 3-4 students in a row) 12 Plastic plate with filters*** (shared among 3-4 students in a row) 13 Platic plate (for common use in a fume hood) 14 Platic plate (for common use in a fume hood) 15 Plates of plate sper hood 15 Plates of plates per hood 16 Plates per hood 17 Plates of plate (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker 11 Plates of the spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP")	Double clamp for burette	1
100 mL flask labeled "Waste" 1 100 mL Volumetric flask 2 2 100 mL Frlenmeyer flask 2 2 100 mL Erlenmeyer flask 2 2 100 mL Erlenmeyer flask 2 2 100 mL Frlenmeyer flask 2 2 100 mL Frlenmeyer flask 2 1 1 1 1 1 1 1 1 1	Ring for funnel	1
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	25 mL Burette	1
100 mL Erlenmeyer flask 2	100 mL flask labeled "Waste"	1
Syringe with needle Graduated test tubes for collecting fractions and preparing mixtures 96-well plate Pipettor (micropipette) with fixed volume of 0.1 mL Pipettor (micropipette) with fixed volume of 0.1 mL Disposable tips (in blue plastic cup) Spectrophotometric cuvettes labeled "A1", "B1", "A2", "B2", "A3", "B3" in cuvette holder 10 mL Graduated plastic pipettes 3 10 mL Glass pipette 1 Pipette filler 1 3-Way Bulb 1 Glass rod 1 Filter funnel 1 Small funnel 1 Mauber glass vials for combined fractions (peaks) 10 mL Measuring cylinder labeled "K ₂ C ₂ O ₄ 15%" (shared between 2 students) 1 10 mL Measuring cylinder (shared between 2 students) 1 100 mL Measuring cylinder labeled "H ₂ O" (shared among 3-4 students in a row) 1 Plastic plate with filters*** (shared among 3-4 students in a row) 1 Plastic plate with filters*** (shared among 3-4 students in a row) 4 Filter protection tips (for common use in a fume hood) 8 Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker 1 Ruler	100 mL Volumetric flask	2
Graduated test tubes for collecting fractions and preparing mixtures 96-well plate Pipettor (micropipette) with fixed volume of 0.1 mL Disposable tips (in blue plastic cup) Spectrophotometric cuvettes labeled "A1", "B1", "A2", "B2", "A3", "B3" in cuvette holder 10 mL Graduated plastic pipettes 11 ml. Glass pipette 12 pipette filler 13-Way Bulb 14 glass rod 15 pitter funnel 16 mL Amber glass vials for combined fractions (peaks) 10 mL Measuring cylinder labeled "K2C2O4 15%" (shared between 2 students) 10 mL Measuring cylinder (shared between 2 students) 10 mL Measuring cylinder labeled "H2O" (shared among 3-4 students in a row) 17 plastic plate with filters*** (shared among 3-4 students in a row) 18 plastic plate (for common use in a fume hood) Rubber protection tips (for common use a fume hood) Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker Ruler	100 mL Erlenmeyer flask	2
96-well plate Pipettor (micropipette) with fixed volume of 0.1 mL Disposable tips (in blue plastic cup) Spectrophotometric cuvettes labeled "A1", "B1", "A2", "B2", "A3", "B3" in cuvette holder 6 10 mL Graduated plastic pipettes 3 10 mL Glass pipette 1 Pipette filler 1 3-Way Bulb 1 Glass rod Filter funnel 1 Small funnel 1 60 mL Amber glass vials for combined fractions (peaks) 10 mL Measuring cylinder labeled "K2C2Q415%" (shared between 2 students) 1 10 mL Measuring cylinder (shared between 2 students) 1 10 mL Measuring cylinder (shared between 2 students) 1 100 mL Measuring cylinder abeled "H2O" (shared among 3-4 students in a row) 1 Plastic plate with filters*** (shared among 3-4 students in a row) 1 Plastic plate with filters*** (shared among 3-4 students in a row) 4 Rubber protection tips (for common use in a fume hood) 8 Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker 1 Ruler 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Syringe with needle	1
Pipettor (micropipette) with fixed volume of 0.1 mL Disposable tips (in blue plastic cup) Spectrophotometric cuvettes labeled "A1", "B1", "A2", "B2", "A3", "B3" in cuvette holder 10 mL Graduated plastic pipettes 1 mL Pipette filler 3-Way Bulb Glass rod Filter funnel Small funnel 1 mL Small funnel 1 mL Measuring cylinder labeled "K2C2O4 15%" (shared between 2 students) 1 mL Measuring cylinder (shared between 2 students) 1 mL Measuring cylinder labeled "H2O" (shared among 3-4 students in a row) Heating plate (for common use in a fume hood) Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker Ruler	Graduated test tubes for collecting fractions and preparing mixtures	50
Disposable tips (in blue plastic cup) Spectrophotometric cuvettes labeled "A1", "B1", "A2", "B2", "A3", "B3" in cuvette holder 10 mL Graduated plastic pipettes 1 mL Glass pipette 1 pipette filler 3-Way Bulb Glass rod Filter funnel Small funnel 1 mL Amber glass vials for combined fractions (peaks) 10 mL Measuring cylinder labeled "K2C2O4 15%" (shared between 2 students) 10 mL Measuring cylinder (shared between 2 students) 10 mL Measuring cylinder 100 mL Measuring cylinder labeled "H2O" (shared among 3-4 students in a row) 1 plastic plate with filters*** (shared among 3-4 students in a row) Rubber protection tips (for common use in a fume hood) Rubber protection tips (for common use a fume hood) Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker 1 Ruler	96-well plate	1
Spectrophotometric cuvettes labeled "A1", "B1", "A2", "B2", "A3", "B3" in cuvette holder610 mL Graduated plastic pipettes310 mL Glass pipette1Pipette filler13-Way Bulb1Glass rod1Filter funnel1Small funnel160 mL Amber glass vials for combined fractions (peaks)310 mL Measuring cylinder labeled "K2C2O415%" (shared between 2 students)110 mL Measuring cylinder (shared between 2 students)150 mL Measuring cylinder1100 mL Measuring cylinder1190 mL Measuring cylinder labeled "H2O" (shared among 3-4 students in a row)1Plastic plate with filters*** (shared among 3-4 students in a row)3 filters per studentHeating plate (for common use in a fume hood)6 plates per hoodRubber protection tips (for common use a fume hood)6 pairs per hoodSpectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP")1Marker1Ruler1	Pipettor (micropipette) with fixed volume of 0.1 mL	1
10 mL Graduated plastic pipettes 3 10 mL Glass pipette 1 Pipette filler 1 3-Way Bulb 1 Glass rod 1 Filter funnel 1 Small funnel 1 60 mL Amber glass vials for combined fractions (peaks) 3 10 mL Measuring cylinder labeled "K ₂ C ₂ O ₄ 15%" (shared between 2 students) 1 10 mL Measuring cylinder (shared between 2 students) 1 50 mL Measuring cylinder 1 100 mL Measuring cylinder labeled "H ₂ O" (shared among 3-4 students in a row) 1 Plastic plate with filters*** (shared among 3-4 students in a row) 3 filters per student Heating plate (for common use in a fume hood) 6 plates per hood Rubber protection tips (for common use a fume hood) 6 pairs per hood Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") 1 Marker 1 Ruler 1		20
10 mL Glass pipette Pipette filler 13-Way Bulb 1 Glass rod 1 Filter funnel 1 Small funnel 1	Spectrophotometric cuvettes labeled "A1", "B1", "A2", "B2", "A3", "B3" in cuvette holder	6
Pipette filler 1 3-Way Bulb 1 Glass rod 1 Filter funnel 1 Small funnel 1 60 mL Amber glass vials for combined fractions (peaks) 3 10 mL Measuring cylinder labeled "K ₂ C ₂ O ₄ 15%" (shared between 2 students) 1 10 mL Measuring cylinder (shared between 2 students) 1 10 mL Measuring cylinder (shared between 2 students) 1 100 mL Measuring cylinder labeled "H ₂ O" (shared among 3-4 students in a row) 1 Plastic plate with filters*** (shared among 3-4 students in a row) 3 filters per student Heating plate (for common use in a fume hood) 6 plates per hood Rubber protection tips (for common use a fume hood) 6 pairs per hood Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker 1 Ruler 1	10 mL Graduated plastic pipettes	3
3-Way Bulb Glass rod Filter funnel Small funnel 1 Small funnel 1 60 mL Amber glass vials for combined fractions (peaks) 10 mL Measuring cylinder labeled "K ₂ C ₂ O ₄ 15%" (shared between 2 students) 1 10 mL Measuring cylinder (shared between 2 students) 1 100 mL Measuring cylinder labeled "H ₂ O" (shared among 3-4 students in a row) 1 Plastic plate with filters*** (shared among 3-4 students in a row) 1 Plastic plate with filters*** (shared among 3-4 students in a row) 4 Rubber protection tips (for common use in a fume hood) 5 Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker 1 Ruler 1		1
Glass rod 1 Filter funnel 1 Small funnel 1 60 mL Amber glass vials for combined fractions (peaks) 3 10 mL Measuring cylinder labeled "K ₂ C ₂ O ₄ 15%" (shared between 2 students) 1 10 mL Measuring cylinder (shared between 2 students) 1 50 mL Measuring cylinder (shared between 2 students) 1 100 mL Measuring cylinder a 1 100 mL Measuring cylinder labeled "H ₂ O" (shared among 3-4 students in a row) 1 Plastic plate with filters*** (shared among 3-4 students in a row) 3 filters per student Heating plate (for common use in a fume hood) 6 plates per hood Rubber protection tips (for common use a fume hood) 6 plates per hood Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker 1 Ruler 1	Pipette filler	1
Filter funnel Small funnel 1 60 mL Amber glass vials for combined fractions (peaks) 10 mL Measuring cylinder labeled "K ₂ C ₂ O ₄ 15%" (shared between 2 students) 10 mL Measuring cylinder (shared between 2 students) 10 mL Measuring cylinder (shared between 2 students) 100 mL Measuring cylinder 100 mL Measuring cylinder labeled "H ₂ O" (shared among 3-4 students in a row) Plastic plate with filters*** (shared among 3-4 students in a row) Heating plate (for common use in a fume hood) Rubber protection tips (for common use a fume hood) Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker Ruler		1
Small funnel160 mL Amber glass vials for combined fractions (peaks)310 mL Measuring cylinder labeled "K2C2O415%" (shared between 2 students)110 mL Measuring cylinder (shared between 2 students)150 mL Measuring cylinder1100 mL Measuring cylinder labeled "H2O" (shared among 3-4 students in a row)1Plastic plate with filters*** (shared among 3-4 students in a row)3 filters per studentHeating plate (for common use in a fume hood)6 plates per hoodRubber protection tips (for common use a fume hood)6 pairs per hoodSpectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP")1Marker1Ruler1		1
60 mL Amber glass vials for combined fractions (peaks) 10 mL Measuring cylinder labeled "K ₂ C ₂ O ₄ 15%" (shared between 2 students) 10 mL Measuring cylinder (shared between 2 students) 10 mL Measuring cylinder 100 mL Measuring cylinder 100 mL Measuring cylinder labeled "H ₂ O" (shared among 3-4 students in a row) 1 Plastic plate with filters*** (shared among 3-4 students in a row) 1 Heating plate (for common use in a fume hood) Rubber protection tips (for common use a fume hood) Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker 1 Ruler		1
10 mL Measuring cylinder labeled "K ₂ C ₂ O ₄ 15%" (shared between 2 students) 10 mL Measuring cylinder (shared between 2 students) 10 mL Measuring cylinder 100 mL Measuring cylinder labeled "H ₂ O" (shared among 3-4 students in a row) 11 labeled "H ₂ O" (shared among 3-4 students in a row) 12 lastic plate with filters*** (shared among 3-4 students in a row) 13 filters per student 14 leating plate (for common use in a fume hood) 15 labeled "H ₂ O" (shared among 3-4 students in a row) 16 plates per hood 17 labeled "H ₂ O" (shared among 3-4 students in a row) 18 labeled "H ₂ O" (shared among 3-4 students in a row) 19 lastic plate with filters*** (shared among 3-4 students in a row) 10 mL Measuring cylinder (shared among 3-4 students in a row) 10 mL Measuring cylinder (shared among 3-4 students in a row) 10 mL Measuring cylinder (shared among 3-4 students in a row) 10 mL Measuring cylinder (shared among 3-4 students in a row) 10 mL Measuring cylinder (shared among 3-4 students in a row) 10 mL Measuring cylinder (shared among 3-4 students in a row) 11 measuring cylinder (shared among 3-4 students in a row) 12 measuring cylinder (shared among 3-4 students in a row) 13 filters per student (shared among 3-4 students in a row) 14 measuring cylinder (shared among 3-4 students in a row) 15 measuring cylinder (shared among 3-4 students in a row) 16 plates per hood 17 measuring cylinder (shared among 3-4 students in a row) 18 measuring cylinder (shared among 3-4 students in a row) 19 plates per student (shared among 3-4 students in a row) 10 plates per student (shared among 3-4 students in a row) 10 plates per student (shared among 3-4 students in a row) 10 plates per student (shared among 3-4 students in a row) 10 plates per student (shared among 3-4 students in a row) 10 plates per student (shared among 3-4 students in a row) 11 plates per student (shared among 3-4 students in a row) 12 plates per student (shared among 3-4 students in a row) 13 plates per student (shared among 3-4 stud	Small funnel	1
10 mL Measuring cylinder (shared between 2 students) 150 mL Measuring cylinder 100 mL Measuring cylinder labeled "H ₂ O" (shared among 3-4 students in a row) 1 Plastic plate with filters*** (shared among 3-4 students in a row) 3 filters per student Heating plate (for common use in a fume hood) 6 plates per hood Rubber protection tips (for common use a fume hood) 6 pairs per hood Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker 1 Ruler		3
50 mL Measuring cylinder 100 mL Measuring cylinder labeled "H ₂ O" (shared among 3-4 students in a row) 1 Plastic plate with filters*** (shared among 3-4 students in a row) 3 filters per student Heating plate (for common use in a fume hood) 6 plates per hood Rubber protection tips (for common use a fume hood) 6 pairs per hood Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker 1 Ruler 1		1
100 mL Measuring cylinder labeled "H ₂ O" (shared among 3-4 students in a row) Plastic plate with filters*** (shared among 3-4 students in a row) Heating plate (for common use in a fume hood) Rubber protection tips (for common use a fume hood) Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker Ruler 1 1 1		1
Plastic plate with filters*** (shared among 3-4 students in a row) Heating plate (for common use in a fume hood) Rubber protection tips (for common use a fume hood) Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker Ruler 1 1	E 3	1
Heating plate (for common use in a fume hood) Rubber protection tips (for common use a fume hood) Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker Ruler 6 plates per hood 6 pairs per hood 1 1		1
Rubber protection tips (for common use a fume hood) Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker Ruler 1 1		
Spectrophotometer (shared by a group of students; see the number of the spectrophotometer to be used at your bench "SP") Marker 1 Ruler 1		
to be used at your bench "SP") Marker 1 Ruler 1		6 pairs per hood
Marker 1 Ruler 1		
Ruler 1	to be used at your bench "SP")	
	Marker	1
White sheet of paper 1	Ruler	1
***If needed, ask your lab assistant for extra filters		1

^{***}If needed, ask your lab assistant for extra filters.

Safety regulations, S-phrases, R-phrases

salety regulations	y > pintases, it pintases
Disodium hydrogen phosphate	R:36/37/38 S:26-36
Ethylenediaminetetraacetic acid, disodium salt	R:36/37/38 S:26-36/37/39
Tris-HCl	R:36/37/38 S:26-36
Arginine	R:36 S:26
Cysteine	R:22
Histidine	S:22-24/25
Hydrochloric acid	R:34-37 S:26-36-45
Sodium 4-diazoniumbenzenesulfonate	R:1-37/37 S:26-36
Sodium hydroxide	R:34-35 S:26-36-37/39-45
8-Hydroxyquinoline	R:22-36/37/38 S:26-36/37
Ethanol	R:11 S:7-16
Butanol-1	R:10-22-37/38-41-67 S:7/9-13-26-37/39-46
Sodium hypobromite	R31-34 S:26-36-45
5,5'-Dithiobis(2-nitrobenzoic acid)	R:36/37/38 S:26-36
2,4,6-Trinitrobenzene sulfonic acid	R: 1-22-36/38-43 S: 26-36/37
Sodium chloride	R:36 S:26
Thiodiglycol	R:36 S:26
Caprylic acid	R:34 S:26-27-45-36/37/39
Brij-35	R:36/37/38 S:26-36
Sodium dihydrogen phosphate	S:22-24/25
Sodium carbonate	R:36 S:22-26
Calcium carbonate	R:41-37/38 S:26-39
Bromocresol Green	S:22-24/25
Thymolphthalein	S:22-24/25
Potassium oxalate	R:34 S:26-27-36/37/39

Risk Phrases

Indication of Particular Risks

R1: Explosive when dry

10: Flammable

22: Harmful if swallowed

31: Contact with acids liberates toxic gas

34: Causes burns

35: Causes severe burns 36: Irritating to the eyes

37: Irritating to the respiratory system

41: Risk of serious damage to eyes

43: May cause sensitization by skin contact

67: Vapors may cause drowsiness and dizziness

Combination of Particular Risks

R24/25: Toxic in contact with skin and if swallowed

36/37/38: Irritating to eyes, respiratory system and skin

36/38: Irritating to eyes and skin

37/38: Irritating to respiratory system and skin

Safety Phrases

Indication of Safety Precautions

- S13: Keep away from food, drink and animal feeding stuffs
- 22: Do not breathe dust
- 26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
- 27: Take off immediately all contaminated clothing
- 36: Wear suitable protective clothing

- 39: Wear eye/face protection
- 45: In case of accident or if you feel unwell, seek medical advice immediately (show label where
- 46: If swallowed, seek medical advice immediately and show this container or label

Combination of Safety Precautions

7/9: Keep container tightly closed and in a wellventilated place

24/25: Avoid contact with skin and eyes

36/37/39: Wear suitable protective clothing, gloves and eye/face protection

37/39: Wear suitable gloves and eye/face protection

Problem 1. Ion-exchange chromatography of amino acids

Ion-exchange chromatography is an important analytical and preparative method, which allows fractioning of charged substances. Interaction of ionic groups of the substances with counterions attached to the resin is behind the method. In this task you will have to carry out separation of a given mixture of three amino acids followed by quantitative assay of individual amino acids eluted from the column by using specific chromogenic reactions. Since queues of students are possible at spectrophotometers, we **strongly suggest you starting the exam with Problem 1**.

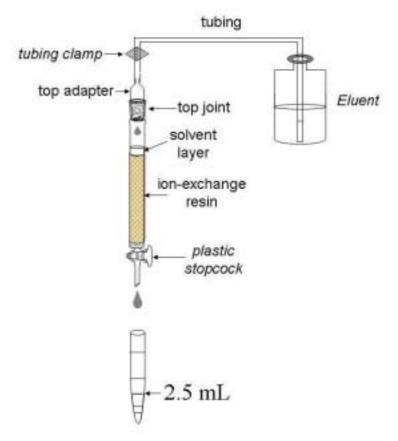
Three amino acids (see the structures above) are present in the mixture. These are histidine, cysteine, and arginine. Cross-linked sulfonated polystyrene is used as a cation-exchange resin (see the picture of the system below). At the beginning of the experiment the column is equilibrated with Eluent 1 (pH 4.9).

Procedure

Chromatography. Step 1

Apply the given solution of a mixture of amino acids to the column. First, open the stopcock to allow the solvent in the column draining into the Erlenmeyer flask labeled "Waste" so that the solvent is level with the top of packing material, still preventing the resin surface from drying off. Close the stopcock and carefully add the analyzed solution to the top of the column by using a syringe. Open the stopcock and let the sample soak inside the gel (drain the solvent into the "Waste" flask). Close the stopcock and add about 1 mL of Eluent 1 (corresponds to ~ 1 cm of liquid in the column) by carefully releasing the tubing clamp. Attach the top joint tightly, fixing the column with one hand and the adaptor with the other (be sure that the joint is fitted closely to the column). Replace the "Waste" flask at the stand with the test tubes in the rack. Release the tubing clamp and open the stopcock to let the eluent flow down through the column. Proceed with elution. (Always open the stopcock to start elution and close the stopcock to stop it).

Collect the fractions in the test tubes up to the volume of 2.5 mL (as shown in the Picture). If needed, label them with marker. After collecting each 4 to 8 fractions stop elution and carry out qualitative analysis of the collected samples.



Qualitative analysis of samples

Qualitative assay of amino acids is based on the reaction of their α -amino groups with sodium 2,4,6–trinitrobenzene sulfonate (TNBS):

The assay is carried out in the wells of a polystyrene plate, each well corresponding to a definite test tube. Before starting the assay, mix 1 mL of TNBS solution with 10 mL of carbonate buffer solution and place 0.1 mL of the resulting mixture into half of the plate wells (from A1 to H5). Then add 0.1 mL of the analyzed fraction into a well. Start with A1 well and continue with B1, C1, etc (move top to bottom and left to right). If an amino acid is present in the analyzed fraction, intense yellow coloration will develop in the corresponding well within 3 min. Use the coloration in the first well as the reference. To reliably estimate the coloration, place the plate on the white sheet of paper.

Note: all aliquots of 0.1 mL should be added by using the pipettor. We expect you to use one tip for all fractions of a single peak.

1.1a Draw the profile of coloration intensity (qualitatively) on the plate sketch in the Answer Sheet. Use the following symbols: (-) – no coloration, 1 – weak coloration, 2 – moderate coloration and 3 – intense coloration. Keep drawing the profile during the whole chromatography process.

Continue collecting fractions and analyzing them until you get at least two wells with coloration as in A1 well, which will indicate that the first amino acid has left the column completely (end of the first peak).

Chromatography. Step 2

As soon as you are finished with collecting the first peak, change to Eluent 2. To do so, close the stopcock, fix the tubing clamp (**Important!**), disconnect the tubing leading to the bottle with Eluent 1 and connect the tubing leading to the bottle with Eluent 2. Attach the top joint tightly.

1.1b *Indicate when the eluents have been changed by drawing lines between the corresponding wells on the plate sketch.*

Continue elution, collecting fractions and carrying out qualitative analysis of samples as described above.

Chromatography. Step 3

As soon as you are finished with collecting the second peak, change to Eluent 3 as described in Step 2. Continue chromatography until the third amino acid leaves the column completely.

Stop chromatography by closing the stopcock and fixing the clamp.

Based on the results of qualitative analysis, choose the fractions which contain the amino acids.

1.1c Write down in the Answer Sheet the labels of wells corresponding to the chosen fractions.

1.2 Combine the fractions from each peak and measure the volumes of combined fractions using a measuring cylinder. Report the volumes of combined fractions excluding amounts used for the qualitative analysis. Write down the obtained results in the Answer Sheet.

Pour combined fractions in the amber glass vials labeled "Peak 1", "Peak 2", "Peak 3". Prepare samples for quantitative spectrophotometric analysis as described below.

When finished with Practical exam, close the vials and leave them on the table. The combined fractions will be subsequently analyzed by lab staff.

Spectrophotometric analysis

For each probe, you should submit two cuvettes to the operator. Prepare the probes as follows. Important! When storing, always put cuvettes in the cuvette holder! All cuvettes have 2 ribbed and 2 working vertical surfaces. While operating with cuvettes, do not touch working surfaces, otherwise you may get incorrect values of absorbance.

Assay 1 (peak 1). Cysteine concentration is determined by the Ellmann reaction:

Test tube A1 (Reference). Place 0.1 mL of Eluent 1 from plastic microtube into a test tube and add 2.9 mL of Ellmann reagent (DTNB).

Test tube B1 (Sample). Place 0.1 mL of the analyzed solution into a test tube and add 2.9 mL of Ellmann reagent (DTNB).

Mix the contents of the test tubes thoroughly and transfer each mixture to the corresponding cuvettes labeled A1 (for reference) and B1 (for sample).

Assay 2 (peak 2). Determination of histidine concentration is based on the ability of imidazole moiety to react with diazonium compounds (Pauli reaction).

Test tube A2 (Reference). Place 2.8 mL of Tris-HCl buffer solution into a test tube, add 0.1 mL of Eluent 2 from plastic microtube and 0.1 mL of Pauli reagent.

Test tube B2 (Sample). Place 2.8 mL of Tris-HCl buffer solution into a test tube, add 0.1 mL of the analyzed solution and 0.1 mL of Pauli reagent.

Mix the contents of the test tubes thoroughly and transfer each mixture to the corresponding cuvettes labeled A2 (for reference) and B2 (for sample).

Assay 3 (peak 3). Determination of arginine concentration is based on the ability of guanidinium moiety to react with some phenols under alkaline and oxidative conditions (Sakaguchi reaction).

Test tube A3 (Reference). Place 0.1 mL of Eluent 3 into a test tube and add 1.5 mL of 10% NaOH solution, 1 mL of 8-hydroxyquinoline solution and 0.5 mL of sodium hypobromite solution.

Test tube B3 (Sample). Place 0.1 mL of the analyzed solution into a test tube and add 1.5 mL of 10% NaOH solution, 1 mL 8-hydroxyquinoline solution and 0.5 mL of sodium hypobromite solution.

Shake the test tubes vigorously for 2 min (**Important!**) and observe formation of orange color. Add 0.2 mL of 8 M urea solution to each test tube, mix the contents and transfer about 3 mL of each mixture to the corresponding cuvettes labeled A3 (for reference) and B3 (for sample).

All mixtures should be analyzed by spectrophotometry not earlier than 10 min and not later than 2 h after preparation. Submit the set of 6 cuvettes to the spectrophotometer operator. In case of a queue at the spectrophotometer, ask the operator to put your student code on the list at the signboard. You will be invited by the operator in due time. Meanwhile, you can answer the theoretical question and start fulfilling Problem No 2.

In case your sample(s) have not been subjected to studies within the proper time interval (which is quite improbable), prepare the sample(s) afresh.

Get the print-offs with the spectra of your samples and check it. Sign the print-offs and get the operator's signature.

1.3 Determine absorbance at the corresponding wavelengths and calculate the content (in mg) of each amino acid in the mixture you were given. The optical length is 1.0 cm. Complete the Answer Sheets taking into account that one mole of each amino acid gives one mole of the corresponding product.

Reference data:

The values of extinction coefficients:	Molar masses of the amino acids.
Product of Ellmann reaction: 13600 M ⁻¹ cm ⁻¹ at 410 nm	Cysteine 121 g/mol
Product of Pauli reaction: 6400 M ⁻¹ cm ⁻¹ at 470 nm	Histidine 155 g/mol
Product of Sakaguchi reaction: 7700 M ⁻¹ cm ⁻¹ at 500 nm	Arginine 174 g/mol

1.4. Draw three resonance structures of the species responsible for mixture coloration as a result of Ellmann reaction.

Problem 2. Determination of carbonate and hydrogen phosphate in an abrasive sample

Na₂CO₃, CaCO₃ and Na₂HPO₄ are the main constituents of abrasive powders. In this task you will have to determine carbonate and hydrogen phosphate ions in an abrasive sample by two acid-base titrations.

First, the exactly known amount of hydrochloric acid (taken in an excess) is added to the sample. As a result, hydrogen phosphates are transformed into H_3PO_4 , whereas carbonates into CO_2 which is further removed by boiling. Calcium ions initially present in the sample pass into the solution. Because of possible interference in subsequent analysis, they are precipitated as CaC_2O_4 and filtered off prior to the titration.

Next, the phosphoric acid formed is subjected to two titrations with pre-standardized NaOH solution and two different indicators: Bromocresol Green (BCG) and Thymolphthalein (TP). First, H_3PO_4 (and excess of HCl) is titrated to $H_2PO_4^-$ ion, the endpoint lying in slightly acidic medium (pH of ~4.5). It corresponds to the color change of BCG from yellow to blue. The second titration proceeds till HPO_4^{2-} is formed. The endpoint of the second titration corresponds to the color change of TP from colorless to blue (moderately alkaline medium, pH of ~10).

The content of ${\rm CO_3}^{2-}$ ions in the sample is calculated by finding the difference between:

- a) the amount of the titrant equivalent to the initial amount of HCl (taken for the sample dissolution) and
- b) the amount of the titrant corresponding to the second endpoint (TP).

The content of HPO₄²⁻ is calculated by finding the difference between the amounts of the titrant consumed to achieve two endpoints (TP and BCG).

Procedure

Step 1. Dissolution of the sample and removal of CO₂

To the sample of the abrasive powder in a beaker covered with watch glass add 10.00 mL (exactly, with a pipette! *Carefully*, not removing the glass and avoiding losses because of splashing!) of ca. 1 mol/L HCl (see the exact concentration of the acid on the label). After the most intensive stage of gas evolution is completed, heat *carefully* the solution in the beaker (covered with watch glass) on a heating plate until the gas evolution stops. Then bring the solution to boiling and boil it carefully for 2-3 min.

Step 2. Precipitation of calcium

Remove the beaker from the plate; wash the steam condensate from the watch glass down to the beaker with distilled water. Add 1-2 mL of 15% $K_2C_2O_4$ solution with measuring cylinder. Put the beaker aside until the most part of the precipitate is formed (usually takes 10 to 20 min). Spend this time for standardization of the titrant solution of NaOH (see the procedure hereunder).

Step 3. Standardization of NaOH solution

Place with a pipette 10.00 mL of HCl solution into a 100 mL volumetric flask, make up to the mark with distilled water and mix. Fill the burette with NaOH solution. Transfer with a pipette 10.00 mL of the diluted HCl solution from the volumetric flask to an Erlenmeyer flask. Add 1-2 drops of Thymolphthalein solution and titrate with NaOH solution until blue coloration stable on swirling for 5-10 s appears.

Here and after. Repeat the titrations as necessary. It is desirable that the highest and the lowest titrant volume values differ not more than by 0.10 mL. Report all the final volume values with 0.01 mL accuracy.

2.1a Complete the table in the Answer Sheet.

2.1b Calculate the concentration of NaOH solution (in mol/L).

Step 4. Filtering off calcium oxalate

After the most part of CaC₂O₄ precipitates filter the precipitate off collecting the filtrate into a 100 mL volumetric flask. Slight turbidity in the filtrate is admissible, since small amounts of calcium oxalate do not interfere in the titration. Wash the filter with distilled water; make up the solution in the flask to the mark with distilled water and mix. Put the used filter into the waste basket.

Step 5. Sample titration against Bromocresol Green

Transfer with a pipette a 10.00 mL aliquot of the sample solution coming from the step 4 from the volumetric flask to an Erlenmeyer one, and add 3 drops of BCG solution. Prepare in another Erlenmeyer flask a reference solution by adding 3 drops of 15 % NaH₂PO₄ solution and 3 drops of BCG solution to 15-20 mL of distilled water. Titrate the sample solution with NaOH solution until the color coincides with that of the reference solution.

2.2 Complete the table in the Answer Sheet.

Step 6. Sample titration against thymolphthalein

Transfer with a pipette a 10.00 mL aliquot of the sample solution coming from the step 4 from the volumetric flask to an Erlenmeyer one. Add 2 drops of TP solution and titrate with NaOH solution until blue coloration stable on mixing for 5-10 s appears.

2.3 *Complete the table in the Answer Sheet.*

Step 7. Calculations

- **2.4** Calculate the mass of CO₃²⁻ in the sample.
- **2.5** Calculate the mass of HPO₄²⁻ in the sample.

Step 8. Additional questions to the problem

Answer the additional questions in the Answer Sheets.

- **2.6a** Indicate one reaction (write down the equation) for a process interfering in the sample analysis you have carried out in the presence of Ca²⁺.
- **2.6b** A list of mistakes possible at different steps is given in the table in the answer sheet. Indicate which of the mistakes can lead to errors in CO_3^{2-} and/or HPO_4^{2-} content determination. Use the following symbols: "0" if no error is expected, "+"or "-" if the result is higher (positive error) or lower (negative error) than the true one.

1 H 1.01	Periodic Table of Elements with atomic masses														2 He 4.00		
3 Li 6.94	4 Be 9.01											5 B 10.81	6 C 12.01	7 N 14.01	8 O 16.00	9 F 19.00	10 Ne 20.18
11 Na 22.99	12 Mg 24.31											13 Al 26.98	14 Si 28.09	15 P 30.97	16 S 32.07	17 Cl 35.45	18 Ar 39.95
19 K 39.10	20 Ca 40.08	21 Sc 44.96	22 Ti 47.88	23 V 50.94	24 Cr 52.00	25 Mn 54.94	26 Fe 55.85	27 Co 58.93	28 Ni 58.69	29 Cu 63.55	30 Zn 65.39	31 Ga 69.72	32 Ge 72.61	33 As 74.92	34 Se 78.96	35 Br 79.90	36 Kr 83.80
37 Rb 85.47	38 Sr 87.62	39 Y 88.91	40 Zr 91.22	41 Nb 92.91	42 Mo 95.94	43 <u>Tc</u> 98.91	44 Ru 101.07	45 Rh 102.91	46 Pd 106.42	47 Ag 107.87	48 Cd 112.41	49 In 114.82	50 Sn 118.71	51 Sb 121.76	52 Te 127.60	53 I 126.90	54 Xe 131.29
55 Cs 132.91	56 Ba 137.3	57-71	72 Hf 178.49	73 Ta 180.95	74 W 183.84	75 Re 186.21	76 Os 190.23	77 <u>Ir</u> 192.22	78 Pt 195.08	79 Au 196.97	80 Hg 200.59	81 Tl 204.38	82 Pb 207.19	83 Bi 208.98	84 Po 208.98	85 At 209.99	86 Rn 222.02
87 Fr 223	88 Ra 226	89-103	104 Rf 261	105 Db 262	106 Sg 263	107 Bh 264	108 Hs 265	109 Mt 268									

57	58	59	60	61	62	63	64	65	66	67	68	69	70	71
La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
138.91	140.12	140.91	144.24	144.92	150.36	151.96	157.25	158.93	162.50	164.93	167.26	168.93	173.04	174.97
89	90	91	92	93	94	95	96	97	98	99	100	101	102	103
Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr
227	232	231	238	237	244	243	247	247	251	252	257	258	259	262