Amino acid composition of peptides

Materials:		**Methods:**
dipeptide sample	standards:	N-terminal labeling
2,4-dinitrofluorobenzene (DNB)	amino acids	extraction
acetone	DNP-amino acids	TLC
sodium carbonate		
diethyl ether		
6N HCl	ninhydrin and $Cu(NO_3)_2$	
indicator paper		

eluants:
E1: n-buthyl alcohol:acetic acid:water (4:1:1)
E2: chloroform:acetic acid (10:1)

Theory:

1. Amino acids and peptides. Primary structure of peptides.
2. Methods used for peptide sequence determination.
3. Labeling of the N-terminal residues. Labeling reagents.
4. Peptide hydrolysis.
5. Theory and practice of extraction.
6. Thin layer chromatography.

Introduction:

1. How does DNB react with amino acids and peptides? Find the mechanism for this reaction. Write the reaction of DNB with Ala-Gly-Val tripeptide.
2. Find the products of acidic hydrolysis of Ala-Thr-Asn-Lys-Tyr peptide and DNP-Ala-Val-Phe-Gly labeled peptide.
3. Write schemes of all reactions occurring in the experiment.
4. Select solvents which could be used for extraction of aqueous solution: benzene, diethyl ether, acetic acid, THF, methylene chloride, chloroform.
5. The progress of substance in TLC is described by R_f value. How is this value calculated? Draw a scheme of TLC plate, mark spots for substance A of $R_f = 0.3$ and B of $R_f = 0.8$.

Experiment:

1. Determination of amino acid composition.

To a sample of peptide in glass ampoule (5 mg) is added 6N HCl, the ampoule is sealed by using micro-burner and left in the oven (100°) for 6 hours.

(next week)

After opening, the solution from the ampoule is evaporated to dryness on glass watch set on water bath. The residue is dissolved in water (1 ml) and used for TLC analysis. Use the amino acid standards and eluant E1. Visualize the chromatogram using ninhydrin solution (CAUTION) and copper nitrate solution. Compare the analyzed sample with standards, calculate the R_f values and determine the composition of peptide.

2. Determination of the N-terminal amino acid residue.

The sample of peptide (20 mg) is dissolved in water (5 ml). Sodium carbonate (200 mg) is added to the solution and the mixture is warmed to 40° on water bath. A solution of DNB (20 mg) in acetone is added to the warm mixture and the reaction is kept for 30 minutes at 40° with occasional shaking.

The unreacted BNB is removed by extraction with ether (10 ml). The water phase is acidified with concentrated HCl (CAUTION) and extracted with ether (four times by 10 ml). The combined ether extracts are evaporated on water bath, the residue is dissolved in 6N HCl and transferred into ampoule. The ampoule is sealed by using micro-burner and left in the oven (100°) for 6 hours.

(next week)

After opening, the solution from the ampoule is diluted with water (15 ml) and extracted with ether (4 times by 5 ml). The combined ether extracts are evaporated , the residue is dissolved in acetone and used for TLC analysis. Use the DNP-amino acid standards and eluant E2. The chromatogram does not require visualization. Compare the analyzed sample with standards, calculate the R_f values and determine the N-terminal residue of the peptide.

Report:

1. Briefly describe the experiment.
2. Draw the schemes of TLC plates, calculate the Rf values.
3. Draw the scheme of labeling procedure, with pH change steps and extractions.
4. Write the labeling and hydrolysis reactions of the peptide using the sequence determined in the experiment.

The proper safety measures have to be observed (personal protection, MSDS)

All experiments have to be carried out in fume hood.

All organic solvent waste has to be disposed into special container.