INSTITUTE OF CHEMICAL TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology
Department of Food Analysis and Nutrition

ANALYSIS OF FOOD AND NATURAL PRODUCTS

LABORATORY EXERCISE

Determination of mineral elements

(atomic absorption spectrometry, spectrophotometry, titrimetry)

Responsible person: Prof. Richard Koplík

CONTENT

Required knowledge

1. The preparation of solutions and the corresponding calculations: a conversion of the mass of substance to the amount of substance, a conversion of mass fraction to mass concentration, a conversion of mass concentration to molar concentration and vice versa.
2. The stoichiometry of chemical reactions, the principle of titrimetric (volumetric) analysis, the related basic terms (standard solution, titration, titration curve, equivalence point), calculations in titrimetric analysis, using volumetric glassware.
3. Precipitation reactions in aqueous solutions; solubility product.
4. The main principle of spectrophotometric measurement; Lambert-Beer's law.

Evaluative criteria

- proved theoretical knowledge
- quality of practical work execution
- compliance with laboratory work rules (safety, order)
- protocol – integrity of experimental data, results calculation and discussion

Laboratory exercise content

A. Determination of chloride ions and sodium chloride in bakery products

B. Determination of phosphoric acid in a beverage sample

Specification:

- The group of students receives a sample of bakery product for determination of sodium chloride and a sample of soft drink for determination of phosphoric acid.
- All students should participate appropriately in all activities outlined in the laboratory exercise schedule (see bellow) and should record all relevant data.
- Each student elaborates his or her protocol containing specification of task, outline of procedure, all input values (such as sample mass), the measured values and the calculated results.

Laboratory exercise schedule

General tasks	Duration	Part A NaCl determination	Duration	Part B H_3PO_4 determination	Duration
Introduction and testing	30 min				
		Sample cutting, weighing and extraction	10 min		
		Filtration, taking aliquot for Na determination	60 min	Preparation of solutions	30 min
		Deproteinization and filtration	60 min	Spectrophotometric measurement	30 min
		AAS analysis of Na: explanation, instrument setting and measurement	30 min	Calculation and evaluation of results	10 min
		Titrimetric analysis of chloride	30 min		
Glassware washing and tidying up	30 min	Calculation and evaluation of results	30 min		

DETERMINATION OF CHLORIDE IONS AND SODIUM CHLORIDE IN BAKERY PRODUCTS

1. SCOPE

This method can be used for determination of soluble chlorides in bakery products. It is a simplified method based on the extraction of chlorides from the sample. To apply the method for more general use (e.g. in protein-rich samples), a dry ashing procedure instead of the extraction procedure is recommended for sample preparation.

2. BASIC PRINCIPLE

An aqueous extract of the sample is prepared. A part of the extract (one tenth) is taken to determine sodium using flame atomic absorption spectrometry. The remaining part of the primary extract is submitted to deproteinization using CARREZ reagents. Then the mixture is filtered and chloride ions are determined using argentometric titration using potassium chromate as an indicator. The chloride containing solution is titrated by the silver nitrate standard solution of the known concentration in the presence of potassium chromate. White precipitate of silver chloride is formed until all chloride ions are converted to the insoluble compound:

$Ag^{+} + Cl^{-} \rightarrow \underline{AgCl}$

The equivalence point is indicated by the orange to brownish coloured precipitate of silver chromate that is formed, when a minor excess of silver ion is added.

3. REAGENTS

- Standard solution 0.05 mol/l $AgNO_3$ of the known exact concentration
- Standard solution of 0.05 mol/l NaCl: 1.4611 g of the dried sodium chloride (of p.a. purity grade) is accurately weighed into a beaker and dissolved in 50 ml of distilled water; then the solution is quantitatively transferred into a 500-ml volumetric flask, made up to the mark with distilled water and mixed thoroughly
- 0.5 mol/l $NaHCO_3$ solution
- 5% solution of K_2CrO_4
- CARREZ reagent I: a portion of 53 g $K_4Fe(CN)_6.3H_2O$ is dissolved in distilled water and made up to 500 ml
- CARREZOVO reagent II: a portion of 110 g $Zn(CH_3COO)_2.2H_2O$ is dissolved in distilled water together with 3 ml of concentrated acetic acid and made up 500 ml.

4. GLASSWARE AND EQUIPMENT

- Titration flasks 250 ml
- Pipettes 5 ml, 10 ml and 25 ml
- A 50-ml beaker for filling of burette with the $AgNO_3$ solution
- Beakers
- Plastic bottle with a screw cap
- Burette 10 ml

5. PROCEDURE

5.1 Preparation of the standard solution of ca 0.05 mol/l $AgNO_3$ and determination of its exact concentration

Approximately 8.5 g of $AgNO_3$ (p.a. purity) is weighed into a 250-ml beaker; then 100 ml of distilled water is added and the mixture is cautiously mixed to achieve dissolution. Then the solution is quantitatively transferred into a 1000-ml volumetric flask; the flask is made up to the mark with distilled water, closed with a stopper and mixed carefully. The solution is stored in an amber glass bottle.

The exact concentration of $AgNO_3$ in the standard solution is determined by repeated titration of the known amount of sodium chloride. Exactly 5 ml of 0.05 mol/l solution of NaCl is pipetted into a titration flask containing approx. 80 ml of distilled water; then 1 ml of 5% K_2CrO_4 solution is added and the yellow coloured solution is titrated with the silver nitrate solution. During titration, a white precipitate of silver chloride is formed. It is also accompanied by the formation of orange coloured precipitate of silver chromate that rapidly disappears as a result of mixing. Just before the end of titration the disappearing of orange colour becomes slower. The end of titration is indicated by just noticeable but stable orange to brownish colour. The titration should be done at least three times. Then the exact concentration of $AgNO_3$ standard solution is calculated:

$c_{\mathrm{Ag}} = 0.05 . 5 / V_{\mathrm{Ag}}$ [mol/l]

V_{Ag} is the average consumption of $AgNO_3$ standard solution expressed in ml.

Remarks to the use of a burette:

- Use a 10-ml burette.
- Before use, water tightness of the burette's cock should be tested; or possibly the cock is taken to pieces, cleaned and lubricate with a small amount of Ramsay's fat.
- The burette should be rinsed with the standard solution and then filled with the standard solution again; during this procedure a beaker is placed under the burette.
- A portion of the standard solution, stored in an amber glass bottle, should be poured from the storing bottle to a small and dry beaker; using this beaker and a small funnel the burette is filled; the filled burette must not contain any air bubbles.
- When the burette is filled and the level of solution is above the zero mark, the funnel is removed; just before the start of titration, the excess of standard solution is let out to adjust the level exactly on the zero mark; any drop of the solution hanging at the burette's tip must be removed.
- During titration, the left hand is usually used to operate with the burette's cock while the right hand is used to mix the titrated solution via extensive circular movement of titration flask.
- When the analysis is finished the burette is thoroughly rinsed with distilled water.

5.2 Sample preparation (extraction and deproteinization)

The portion of 5 g of the sample cut into cubes is accurately weighed into a conical flask. Then 100 ml of distilled water is added, the flask is closed with a stopper and its content is extensively shaken for three minutes. The content of the flask is transferred into a special beaker, inside which the suspension is decanted (drained) using a sifter (sieve) on a central bar via pressing the sample. The liquid is drained and filtered using paper filter (no. 389) and a funnel into a 250-ml volumetric flask. The conical flask, inside which the extraction took place, is rinsed with three portions of distilled water (each of 40 ml) and the water is added to filtered liquid. The filtration take approx. an hour. The clear filtrate in the 250-ml volumetric flask is made up to the mark with distilled water and the solution (primary extract) is mixed. Using a glass pipette, a portion of 25 ml of primary extract is taken and transferred into a clean and dry plastic bottle with a screw cap. (This portion will be used to determine sodium content by atomic absorption spectrometry.)

Into the 250-ml volumetric flask with remaining 225 ml of primary extract, two millilitres of CARREZ reagent I and two millilitres of CARREZ reagent II are added. The content of the flask is moderately mixed by circular movement of the flask after addition of each reagent. Then it is left to stand for 10 min and the mixture with precipitated zinc hexacyanoferrate is made up to the mark with distilled water again. Then the suspension is filtered using filter paper (no. 389) and funnel into another conical flask. (These steps, i.e. precipitation and filtration, provide removal of proteins from the primary extract that interfere with chloride determination.) The filtrate is taken for titrimetric determination of chloride.

5.3. Determination of chloride ions

A portion of filtrate of the deproteinizated extract (usually 75 ml) is pipetted into a 250-ml titration flask. As the solution is acidic, 1 ml of 0.5 mol/l solution of $NaHCO_3$ must be added to adjust pH to 7 to 9. (It is recommended to check the pH value using indicator paper). Then 1 ml of 5 % K_2CrO_4 solution is added and the solution is titrated dropwise by the standard solution of $AgNO_3$ of the known exact concentration until the orange-yellow colour of silver chromate becomes stable (for explanation, see paragraph 5.1). The titration should be done three times. If more than 10 ml of silver nitrate solution was consumed in the first titration, a lower aliquot volume of the sample should be taken for the second and third titration (e.g. 50 ml) and appropriate volume of distilled water should be added.

5.4. Determiation of sodium

The primary extract of the sample is diluted five times to ten times before AAS analysis. The dilution also provides an addition of hydrochloric acid (final conc. 0.1 mol/l) and deionising agent CsCl (final concentration of Cs 2 mg/ml). For details, see pp. 9–13.

5.5. Calculation and evaluation of results

Total amount of chloride ions in the sample is given by the following formula:

$$n(\mathrm{Cl}^-) = (V_1/V_2)\,.\,(V_3/V_4)\,.\,c(\mathrm{AgNO_3})\,.\,V(\mathrm{AgNO_3}) \qquad [\mathrm{mmol}]$$

V_1 is the total volume of primary extract (=250 ml)
V_2 is the volume of primary extract remaining after taking off the portion for sodium determination (=225 ml)
V_3 is the total volume of deproteinizated extract (=V_1 = 250 ml)
V_4 is the aliquot volume of deproteinizated extract pipetted for titrimetric analysis (=75 ml).
$V(\mathrm{AgNO_3})$ is the average consumption of silver nitrate standard solution [ml] and $c(\mathrm{AgNO_3})$ is its exact concentration [mol/l].

Mass of chloride ions in the sample is $m(\mathrm{Cl}^-) = n(\mathrm{Cl}^-)\,.\,M(\mathrm{Cl})$ [mg]

$M(\mathrm{Cl})$ is molar mass of chlorine (= 35.453 g/mol).

Molar content of chloride ions is $x(\mathrm{Cl}^-) = n(\mathrm{Cl}^-) / m_s$ [mmol/g]

m_s is sample mass [g]

Molar content of sodium is $x(\mathrm{Na}) = f_d \,.\, \rho(\mathrm{Na}) \,.\, V_1 / (M(\mathrm{Na}) \,.\, m_s)$ [mmol/g]

f_d is dilution ratio corresponding to dilution of primary extract before AAS measurement of sodium (usually $f_d = 10$ or 5)
$\rho(\mathrm{Na})$ is the mass concentration of sodium in diluted primary extract determined using AAS [mg/l]
V_1 is the volume of primary extract expressed in the litter unit (=0.25 l)
$M(\mathrm{Na})$ is the molar mass of sodium (=22.9898 g/mol) and
m_s is sample mass [g].

The rounded values of molar contents of chloride $x(\mathrm{Cl}^-)$ and sodium $x(\mathrm{Na})$ should be compared. The lower value of both values is equal to molar content of sodium chloride $x(\mathrm{NaCl})$. Then the percentage of sodium chloride in the sample is calculated:

$p(\mathrm{NaCl}) = x(\mathrm{NaCl}) \,.\, M(\mathrm{NaCl}) / 10$ [%]

$M(\mathrm{NaCl})$ is molar mass of sodium chloride (=58.443 g/mol).

The result should be rounded to three significant figures and evaluated in relation to EU regulation 1924/2006. According to the regulation, only the foodstuffs containing maximum 0.12 % sodium (or 0.305 % sodium chloride) may be designated as low-sodium or low-salt food. You also should decide if the sample has to have a declared content of salt at the packaging. According to national legislation of Czech Republic (Decree 113/2005 Sb.), the declaration is obligatory for the foodstuffs containing more than 2.5 % NaCl with the exception of dried food, flavouring materials, sauces and dressings.

DETERMINATION OF SODIUM AND POTASSIUM IN FOOD USING ATOMIC ABSORPTION SPECTROMETRY

1. SCOPE

The method is suitable for the determination of sodium and potassium in foodstuffs and some other biological matrices. For sample preparation, various procedures can be applied; they include microwave pressure decomposition with nitric acid, dry ashing followed by dissolution of ash in diluted hydrochloric or nitric acid or sample solubilisation in diluted hydrochloric acid under slight heating. To determine soluble sodium content (e.g. sodium chloride content) in food, a method of sample extraction in pure water could be used.

The method was adapted from literature [1] and it is consistent with the standard method of ČSN EN 1134 (56 0405) *Fruit and vegetable juices – Determination of sodium, potassium, calcium and magnesium content by atomic absorption spectrometry (AAS)*.

2. BASIC PRINCIPLE

The liquid sample is introduced into air-acetylene flame as aerosol using pneumatic nebulizer. Light absorption caused by free atoms of sodium and potassium is measured at the specific spectral lines of 589.0 nm and 766.5 nm, respectively. The samples and calibration solutions have to contain the deionising agent of CsCl at the same concentration. The concentrations of elements are determined from the corresponding values of absorbance using calibration curves.

3. INSTRUMENTS AND EQUIPMENT

- Atomic absorption spectrometer GBC Avanta P
- Cylinders of pressurized air and acetylene (flame photometry grade)
- 10-ml volumetric flasks for the dilution of samples
- A 100-ml polypropylene volumetric flask and three 50-ml polypropylene volumetric flasks for preparation of calibration solutions
- Pipettes with plastic tips 200–1000 µl and 1–5 ml

Note: all glassware and plastic ware should be washed using distilled water and decontaminated by leaching with diluted nitric acid(1:10) for at least 24 hours. The recommended time of leaching is three weeks. Then it should be rinsed thoroughly with deionised water and optionally dried in a clean room and stored in plastic bags until use.

4. CHEMICALS AND SOLUTIONS

High purity chemicals (p.a. grade or better) and distilled deionised water should be used.

- Sodium standard solution (1000 mg/l Na in 0.5 mol/l HNO_3), MERCK, (product no. 1.70238.500) or an equivalent standard

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[1] Rowe C.J.: Food Analysis by Atomic Absorption Spectroscopy, Varian Techtron, Springvale 1973

- Potassium solution (1000 mg/l Na in 0.5 mol/l HNO_3), MERCK, (product no. 1.70230.500) or an equivalent standard
- Stock solution of Na and K (500 mg/l): into a clean a dry plastic bottle with a screw cap, the equal volumes of Na standard solution (1000 mg/l) and K standard solution (1000 mg/l) are pipetted (e.g. 10 ml + 10 ml) and the content of bottle is mixed.
- 1% Cs solution (deionising agent): into a clean and dry plastic bottle with a screw cap, a portion of 1.27 g CsCl (p.a. or Suprapur purity grade) is weighed and 98.73 g of deionised water is added; then the mixture is mixed to achieve dissolution.
- 1 mol/l solution of HNO_3 or HCl: they are prepared from concentrated nitric acid (65 %, ca 15 mol/l) or concentrated hydrochloric acid (37%, ca. 12 mol/l) by dilution with deionised water.

5. PROCEDURE

The starting samples for AAS analysis are the acidic solution prepared from original samples using dry ashing and ash dissolution or using microwave pressure wet decomposition with nitric acid. The beverages can be usually analysed without decomposition. To determine sodium chloride content, a clear aqueous extract can be used.

5.1 Sample dilution

A suitable dilution ratio for the dilution of samples ranges from 5 to 20. To dilute the liquid samples (i.e. solutions of ashed samples in diluted HCl or HNO_3 or digestates obtained by microwave pressure decomposition in HNO_3 or aqueous extracts of solid samples), 10-ml volumetric flasks are used. The acid concentration (HCl or HNO_3) is adjusted to approx. 0.1 mol/l. It is also necessary to add the deionising agent (the solution of CsCl containing 1 % Cs). The final concentration of Cs in the diluted samples should be 2 mg/ml. The sample and the acid solution (1 mol/l HCl or 1mol/l HNO_3) are pipetted according to table 1; finally the solutions are made up to the mark (10 ml) with deionised water and mixed.

Tab. 1

	Dilution ratio $f_d = 5$	Dilution ratio $f_d = 10$	Dilution ratio $f_d = 20$
Sample solution	2 ml	1 ml	0.5 ml
CsCl solution (1 % Cs)	2 ml	2 ml	2 ml
1 mol/l HCl or 1 mol/l HNO_3	1 ml of 1M HCl (for an aqueous extract) or 0.6 ml of 1M HCl (for sample solution in 0.2M HCl) or 0.2 ml of 1M HNO_3 (for diluted digestate containing 3 ml HNO_3/100 ml)	1 ml of 1M HCl (for an aqueous extract) nebo 0.8 ml 1M HCl (for sample solution in 0.2M HCl) nebo 0.6 ml of 1M HNO_3 (for diluted digestate containing 3 ml HNO_3/100 ml)	1 ml of 1M HCl (pro vodný extrakt) nebo 0.9 ml 1M HCl (for sample solution in 0.2M HCl) nebo 0.8 ml of 1M HNO_3 (for diluted digestate containing 3 ml HNO_3/100 ml)
Deionised water	up to 10 ml	up to 10 ml	up to 10 ml

5.2 Preparation of calibration standards

The calibration blank, which serves to adjust the instrument to zero absorbance, is prepared in a 100-ml plastic volumetric flask. Individual standards are prepared using 50-ml plastic volumetric flasks. The solution of acid (1 mol/l HCl or 1 mol/l HNO_3), the deionising agent of CsCl and mixed solution of Na and K (500 mg/l) are pipetted to the volumetric flasks according to table 2.

Tab. 2

	Blank	Standard 1 -	Standard 2 -	Standard 3
1 mol/l solution of HCl or HNO_3	10 ml	5 ml	5 ml	5 ml
CsCl solution (1 % Cs)	20 ml	10 ml	10 ml	10 ml
Na+K stock solution (500 mg/l)	0 ml	0.50 ml	1.00 ml	2.00 ml
Deionised water	up to 100 ml	up to 50 ml	up to 50 ml	up to 50 ml
Final concentration of Na and K	0	5 μg/ml	10 μg/ml	20 μg/ml

Note: The rsulting concentration of the acid in the blank and standards is ca. 0.1 mol/l. If another way of sample preparation (e.g. wet ashing) is used, the calibration standards have to contain the same concentration of the same acid as the diluted samples.

5.3 Preliminary steps before AAS analysis

- Insert the appropriate element lamp into the lamp holder.
- Switch the instrument and the computer on, check the lamp table and load the measuring method for the corresponding element.
- Check the setting of lamp current.
- Check other parameters of the method (the recommended values are summarized in the following paragraph).
- Check the burner type (the burner for air-acetylene flame is used; it has a 10 cm long slot).
- Set the lamp position to achieve the maximum light energy and set the optimum photomultiplier voltage.
- Check the passing of light beam above the burner slot.
- Check the rotational position of the burner: for the measurement of high concentrations (up to 20 μg/ml of Na or K), the burner is rotated perpendicular to the optical axis, while for low concentration (up to 0.5 μg/ml for sodium and 1.5 μg/ml for potassium) the burner slot is parallel with optical axis
- Set the recommended vertical position of the burner (10 mm under the light beam).
- Check the level of liquid in the waste container.
- Open the main vents on gas cylinders and check the outlet pressure values (they should range from 300 to 400 kPa for air and from 55 to 96 kPa for acetylene).
- Fill the large plastic beaker in the front of the instrument with deionised water and place the aspiration capillary in it.
- Switch the fume hood on.
- Ignite flame and measure the liquid flow rate when water is aspirated from calibrated cylinder (it normally ranges from 5 to 7 ml/min).

5.4 Measurement

When the preliminary steps are finished, an operator can start the measurement. The measurement parameters are summarized in table 3. To measure the element concentration, three files have to be opened:

- the method file defining the measurement of the element (open either Na.mth or K.mth),
- the sample file defining a sequence of individual solutions to be analysed including a calibration set (i.e. blank and three standards) and individual samples (open e.g. Samples1.sam),
- the result file for data storing (create a new file *.res).

When the analysis is started, the capillary is placed into the blank solution and zero absorbance is set. Then the three standards are consecutively analysed. When the calibration is finished, the operator should check the calibration curve (linear or slightly bent curves are normal). Then the individual samples are aspirated and analysed according to the sequence defined in the sample file. Between samples, the capillary is placed into the beaker with water for few seconds. The instrument displays the results as the calculated concentration in µg/ml, the average absorbance and the corresponding relative standard deviation.

Tab. 3

	Na	**K**
Lamp current	10 mA	10 mA
Wavelength	589.0 nm	766.5 nm
Spectral bandwidth	0.5 nm	0.5 nm
Background correction	no	no
Measurement mode	integration	integration
Read time	3 s	3 s
Number of replicates	3	3
Time constant	0.2 s	0.2 s
Calibration mode	„Concentration least square“ (non-linear least square method)	
Concentrations of standards	5.0; 10.0; 20.0 µg/ml (for perpendicular burner position)	5.0; 10.0; 20.0 µg/ml (for perpendicular burner position)
Vertical burner position (observation height)	10 mm	10 mm
Air flow	10 l/min	10 l/min
Acetylene flow	1.7–1.9 l/min (for perpendicular burner position) or 1.0–1.2 l/min (for parallel burner position)	1.7–1.9 l/min (for perpendicular burner position) or 1.0–1.2 l/min (for parallel burner position)

5.5 Calculation of results

The element content expressed in mg/kg in an individual sample is calculated according to following formula:

$$w = f_d \cdot (\rho - \rho_0) \cdot V / m_s \quad \text{[mg/kg]}$$

ρ is the mass concentration of the element in the diluted sample [µg/ml]
f_d is dilution ratio for the dilution applied before AAS measurement
ρ_0 is the average mass concentration of the element in the series of corresponding blank samples [µg/ml]
V is final volume of sample solution (= total volume of the ash solution or the digestate or the extract) [ml]
m_s is sample mass [g].

From the results of repeated analyses (decompositions + measurements) of the same sample, the arithmetic mean and standard deviation are calculated. The mean result is rounded to three significant figures.

SPECTROPHOTOMETRIC DETERMINATION OF PHOSPHATES, PHOSPHORIC ACID OR TOTAL PHOSPHORUS

1 SCOPE

This method was adapted according to the original papers of Narasaraju [2] and Karvánek [3]. It can be applied for determination of phosphates, phosphoric acid or total phosphorus in water samples, biological materials or food and beverages.

2 BASIC PRINCIPLE

Solid samples of biological origin are decomposed using wet digestion with a mixture of nitric and sulphuric acid. As a result of decomposition, all compounds of phosphorus are converted to phosphoric acid H_3PO_4. By reaction with molybdate ions in an acidic solution, phosphoric acid yields molybdophosphoric acid $H_3[P(Mo_3O_{10})_4]$. The next step, which is the reduction of molybdophosphoric acid, results in the formation of a blue-coloured product called molybdenum blue. If the solution is measured spectrophotometrically, the corresponding absorbance is proportional to phosphorus concentration. The wavelength of measurement used in various procedures ranges from 650 to 840 nm.

Molybdenum blue is represented by a mixture of hetero-poly acids of pentavalent and hexavalent molybdenum. The exact composition of molybdenum blue in a solution as well as the spectrum (shape of absorption curve) depends on the chemical condition of product formation (namely the kind of reduction agent and acidity). The most commonly used reduction agents are tin (II) chloride, ascorbic acid and hydrazine. In the following procedure ferrous ions are used to reduce molybdophosphoric acid. The procedure is simple and does not take much time. Figure 1 shows the absorption curve of molybdenum blue prepared as described bellow.

3 GLASSWARE AND EQUIPPMENT

- Glass beakers, 100 ml, high form and watch glasses
- Hot plate with temperature control and aluminium block with holes for beakers
- Volumetric flasks, 50 ml, 100 ml and 500 ml
- Automatic pipette, 1-5 ml and tips
- Graduated glass cylinders, 25 ml, 100 ml and 250 ml
- Spectrophotometer and r ectangular glass spectrophotometric cells, 1 cm
- Analytical balance.

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[2] Narasaraju T.S.B., Singh R.P., Rao V.L.N.: *Fresenius Z. Anal. Chem. 251, 300, 1970*
[3] Karvánek M., Eisenberger B., Suong D.N.: *Krmivářství a služby 14, 141-3, 1978*

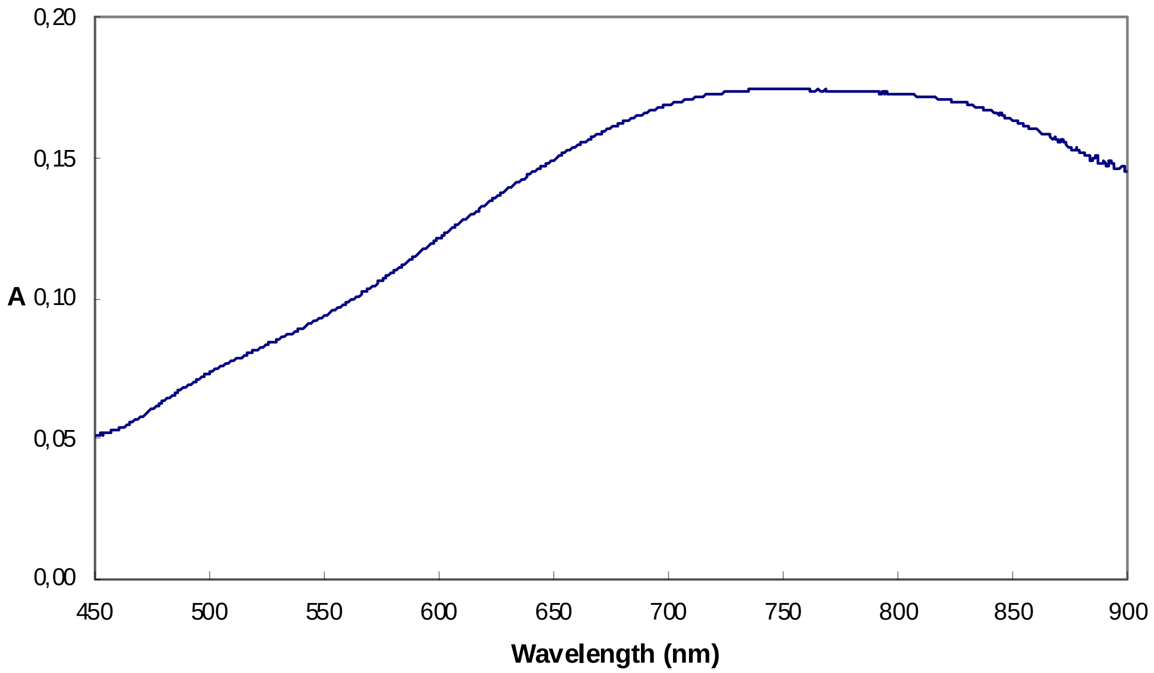

Fig. 1
Absorption spectrum of molybdenum blue solution measured in a 1-cm cell,
$c(PO_4^{3-})$ = 50 µmol/l

4 CHEMICALS, REAGENTS AND SOLUTIONS

- Distilled or deionised water
- Sulphuric acid 96 %, p.a.
- Nitric acid 65 %, p.a.
- Ammonium dihydrogenphosphate, p.a.
- Ammonium molybdate, tetrahydrate, $(NH_4)_6Mo_7O_{24}.4H_20$, p.a.
- Ammonium-ferrous sulphate, hexahydrate, $(NH_4)_2Fe(SO_4)_2.6H_20$ (Mohr's salt), p.a.
- Phosphate stock solution c = 100 mmol/l: 0.5752 g $NH_4H_2PO_4$ is dissolved in a beaker in 20 ml of water, the solution is transferred to a 50-ml volumetric flask, acidified by 1 ml of conc. H_2SO_4, made up to the mark with water and mixed thoroughly.
- Phosphate working solution c = 1 mmol/l: 1 ml of stock phosphate solution is pipetted into a 100-ml volumetric flask, made up to the mark with water and mixed thoroughly.
- 5.4 mol/l (i.e. 30 % v/v) solution of sulphuric acid: 150 ml of concentrated sulphuric acid is added in several small portions into a 600-ml beaker containing 250 ml of water; the mixture is mixed using a glass rod and cooled in a water bath; after cooling to approx. 20 °C, the solution is transferred into a 500-ml volumetric flask using additional water, cooled again, made up to the mark with water carefully and mixed thoroughly.
- 7 % solution of ammonium molybdate: 35 g of $(NH_4)_6Mo_7O_{24}.4H_2O$ is weighed into a plastic bottle with a screw cap and 465 ml of water is added; after closing of the bottle the mixture is mixed thoroughly and allowed to stand overnight to achieve complete dissolution; the solution is mixed finally.

- 14 % solution of ammonium-ferrous sulphate: 35 g of $(NH_4)_2Fe(SO_4)_2.6H_2O$ is weighed into a plastic bottle with a screw cap, 215 ml of water is added and the mixture is acidified with 1 ml of concentrated H_2SO_4, and mixed to achieve dissolution; the resulting solution should be clear and green-blue coloured; the reagent is stable for one week only.

5 PROCEDURE

5.1 Sample preparation

5.1.1 Sample preparation using decomposition

This procedure is applied for solid, semi-solid and liquid samples with an organic matrix, in which the total phosphorus is to be determined. **The procedure is carried out in a fume hood!**
A sample weight equivalent to 0.2-0.5 g of dry matter is weighed (at the precision of 0.1 mg) into a 100-ml beaker. Triplicate or at least duplicate analysis is commonly needed. After careful addition of 10 ml of conc. nitric acid and 5 ml of conc. sulphuric acid, the beakers are covered with watch glasses and inserted into cylindrical holes of the aluminium block positioned on the hot plate. Temperature is set up at 90ºC and after 2 h of heating is raised to 120 ºC and after further 3 h to 140 ºC. Then the beakers are allowed to cool down and 2 ml of nitric acid is added into each beaker. The heating continues at 140ºC for 1 h, at 160 ºC for 2 h and at 180 ºC for 2 h. Sample appearance is gradually changed: after some time the evolution of brown gas (NO_2) weakens and the solution is discoloured. If the discoloration is temporary and the solution darkens at higher temperatures again the decomposition is not completed yet. In that case the samples are allowed to cool down and some nitric acid (2–3 ml) is added again. Then the samples are heated by stepwise increase of temperature from 160 to 200 ºC until the complete and permanent discoloration of the sample is achieved.*

After the completion of the sample decomposition the condensed liquid on a watch glass is rinsed (sprinkled) using wash bottle with distilled water and collected into the beaker and approx. 5 ml of water is added. The beakers without watch glasses are heated on the hot plate at 110-150ºC to evaporate all water. During this step the residual nitric acid is removed as nitric acid interfere with spectrophotometric determination of phosphorus. After cooling the decomposed sample in the beaker is transferred into a 50-ml volumetric flask and made up to the mark with water. Blank samples are prepared by an analogous way as the samples.

* *Note*: Some samples cannot be completely decomposed using HNO_3+H_2SO_4 - mixture. In that case, a more efficient agent (perchloric acid) has to be applied to achieve complete destruction of resistant components of the sample matrix. After 2 hours of heating at 180ºC the samples are allowed to cool down and both nitric acid and perchloric acid are added (2 ml 65 % HNO_3 and 1 ml 70 % $HClO_4$). The heating in a fume hood continues at 180ºC for 1 h and at 200ºC for 30 to 60 min. The procedure using **perchloric acid is very dangerous** (risk of explosion) and therefore **any use of perchloric acid is forbidden for students**.

5.1.2 Sample preparation using dilution

Aqueous samples containing only inorganic phosphorus compounds (water samples or soft drinks like Coca-Cola) can be analyzed without decomposition. The only sample preparation consists in dilution of the sample (see paragraph 5.2). Carbon dioxide should be removed from carbonated beverages prior to analysis.

5.2 Preparation of calibration solutions and sample solutions for spectrophotometric analysis

The calibration of spectrophotometric determination of phosphate (or phosphorus) is performed in the range 0 to 80 μmol/l PO_4^{3-}. Phosphate concentrations in individual calibration solutions are 0; 20; 40 and 80 μmol/l.

To a series of four 50-ml volumetric flasks 0; 1.0; 2.0 a 4.0 ml of working standard solution of phosphate (c = 1 mmol/l) is pipetted. The volume in the flasks is adjusted to approx. 25 ml by addition of water using a graduated cylinder. Then the following three reagents are added:
5 ml of 5.4 mol/l H_2SO_4, 5 ml of ammonium molybdate solution and 5 ml of ammonium-ferrous sulphate solution. After addition of each reagent, the solutions are gently mixed by rotating and rocking the flasks. The solutions should turn blue when the last reagent is added. Then the solutions in volumetric flasks are made up to the mark with water and mixed thoroughly.

To determine phosphate content in the decomposed samples, an aliquot volume of the sample solution (2 to 15 ml according to expected phosphate concentration) is taken from the 50-ml volumetric flask and pipetted into another 50-ml volumetric flask. Then 5.4 mol/l sulphuric acid solution is added. The volume of sulphuric acid solution depends on the sample volume taken (see table 4).

Table 4

The volume of sample solution	2 ml	5 ml	10 ml	15 ml
The volume of 5.4 mol/l H_2SO_4	4.3 ml	3.3 ml	1.7 ml	0

After addition of the sulphuric acid solution water is added to dilute the solution to the volume of approx. 25 ml. Then the two remaining reagents are added: 5 ml of ammonium molybdate solution and 5 ml of ammonium-ferrous sulphate solution. The solutions are gently mixed after addition of each reagent and finally made up to the mark with water and mixed thoroughly. Blank samples are prepared by the same way as the samples.

When liquid samples (e.g. beverages like Coca-Cola) are analysed, the aliquot of the sample is weighed directly into the 50-ml volumetric flask (0.2 to 0.5 ml of the degassed sample is transferred into the previously weighed flask and the gain in mass of the flask is ascertained by precise weighing). Then the sample is diluted to approx. 25 ml with water and the three reagents are consecutively added (5 ml of 5.4 mol/l sulphuric acid, 5 ml of ammonium molybdate solution and 5 ml of ammonium-ferrous sulphate solution). Thus the colour-forming reaction of phosphate ions is completed. The volume is made up to the mark with water and the content of the flask is mixed well. Then the blank sample for the correction of beverage colour is prepared. To another 50-ml volumetric flask, the same portion of the beverage is dosed, 5 ml of 5.4 mol/l sulphuric acid is added, the volume is made up to the mark with water and the resulting solution is mixed well.

5.3 Measurement

The calibration solutions (0; 20; 40; 80 µmol/l), sample solutions and blanks can be measured immediately after preparation. Nevertheless, a time delay between preparation and measurement up to five hours has no effect on the results.

The absorbance values are measured at the wavelength of 700 nm in a 1-cm rectangular cell against the second cell with water. Setting up the spectral bandwidth at 1.5 nm or higher is convenient for this analysis. Absorbance values of calibration solutions are plotted against concentrations of phosphate expressed in µmol/l. A simple linear calibration model ($A = \mathrm{a} + \mathrm{b}\, c$) is applied. Concentrations of phosphate ions in sample solutions and blanks are ascertained from the corresponding absorbance values using calibration line or calculated from the equation of the calibration line.

When the diluted solutions of beverages are analysed the absorbance of the sample solution is measured against the blank solution.

5.4 Calculation and evaluation of results

The analyte content expressed as a mass fraction of phosphate ions (PO_4^{3-}), phosphoric acid (H_3PO_4) or phosphorus (P) is calculated for each sample according the formula

$$w = (V_t/V_a) \cdot 0.05 \cdot (c - c_b) \cdot M / m_s \qquad [\mathrm{mg/kg}],$$

where c and c_b are the concentration of phosphate ions in the sample and the average concentration in the series of blanks, respectively [µmol/l]
M is molar mass of the analyte
$M(PO_4^{3-}) = 94.971$ g/mol, $M(H_3PO_4) = 97.995$ g/mol, $M(P) = 30.974$ g/mol
m_s is sample weight [g]
V_a is the aliquot volume of diluted decomposed sample taken for the analysis [ml].
V_t is total volume of diluted decomposed sample, $V_t = 50$ ml
0.05 is the volume of the solutions measured expressed in litter (the volume of corresponding volumetric flasks is 50 ml).

Based on two or three results of analyses of a sample the average result is calculated and rounded to three significant figures.

If samples of beverages are analysed the formula is simplified to

$$w = 0.05 \,.\, c \,.\, M / m_s \quad [\mathrm{mg/kg}],$$

where c is phosphate concentration [μmol/l] ascertained from the absorbance value of the sample measured against the blank.

When the content of phosphoric acid is determined in the beverage, the result is compared with the maximum limit value of phosphoric acid (food additive compound no. E 338), which was specified by Czech legislation (i.e. 700 mg/kg expressed as P_2O_5).