XI. letní škola elektrochemie

XI. Summer school of electrochemistry

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ELECTROCHEMICAL PRETREATMENT OF SOLID STATE ELECTRODES

A) Effect of different electrochemical pretreatments on detection of ascorbic acid and dopamine

It is known that electrochemical determination of drugs and biological compounds is often difficult with bare electrodes due to their poor responses and high over-potentials. To overcome these limitations, modification of electrode surfaces using different approaches has been performed. For carbon electrodes, typical procedure is the electrochemical treatment which relies on the electrochemical oxidation or reduction at certain conditions. This treatment can dramatically change the electrochemical properties and the surface area of electrodes relative to the untreated ones due to the formation of specific surface groups (e.g. oxygen containing), generation of graphitic edge sites etc. These properties of the surface of electrodes depend on the electrochemical treatment conditions such as applied potential interval, supporting electrolyte, potential scan rate and so on and also the selectivity and sensitivity of the electrochemically treated electrodes varied with the nature of both analytes and supporting electrolytes used during the electrode preparations. Therefore, the effects of all parameters should be optimized for every analytes type [1].

Dopamine (DA) is an important neurotransmitter molecule of catecholamine group which is widely distributed in the mammalian central nervous system, renal, hormonal and cardiovascular systems [2]. DA is also involved in the regulation of congnitive functions. Various electrochemical techniques have been proved to be advantages in the selective and sensitive determination of DA concentration. The fact that DA and other catecholamine's are easily oxidizable compounds makes their detection possible by electrochemical method based on anodic oxidation [3].

Ascorbic Acid (AA) is a soluble vitamins present in many biological systems and in multi vitamin preparations which are commonly used to supplement inadequate dietary intake and as anti-oxidants. AA has been used for the prevention and treatment of the common cold, mental illness, infertility and even cancer. Now-a-days AA has been more interested in medical, veterinary science, toxicology, diagnosis of certain metabolic disorder and in the determination of nutrition value of foods [3, 4].

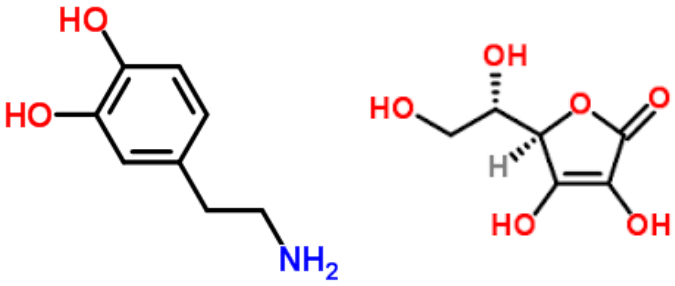

Fig 1. Structures of dopamine (Left) and ascorbic acid (Right)

APPARATUS, ACCESSORIES AND REAGENTS

- AUTOLAB PGS30 Analyzer (EcoChemie, Netherlands)
- NOVA software (Metrohm, Switzerland)
- Electrodes (working – Pencil graphite electrode, e.g. leads Tombow, 0.5 mm, reference – Ag/Ag Cl/3M KCl, counter electrode – Pt); voltammetric vessel
- Dopamine, ascorbic acid, potassium chloride, phosphoric acid, sodium hydroxide, miliQ water (18.2 MΩ·cm)

SOLUTIONS

Supporting electrolyte

1. Phosphate buffer silane pH 7.5 = 0.1 mol/L, 2. c (NaOH) = 0.1 mol/L, 3. c (H_3PO_4) = 0.1 mol/L,

Samples

Dopamine (1mM solution in 1M KCl), Ascorbic Acid (1mM solution in 1M KCl)

EXPERIMENT

Electrochemical pretreatment methods:

1. Cyclic Voltametry

   Pretreatment protocol [5]: potential cycling between −0.3V and +2.0V with a scan rate of 50mVs^{-1} for 5 scans in 0.1M Acidic (H_3PO_4), neutral (PBS, pH=7.5), and alkaline (NaOH) electrolytes
2. Chronoamperometry

   Pretreatment protocol [6]**:** The surface of PGE was pretreated by applying a potential of +1.4 V for 60 s in 0.1M Acidic (H_3PO_4), neutral (PBS, pH=7.5), and alkaline (NaOH) electrolytes

PARAMETERS (CYCLIC VOLTAMMETRY)

CV measurement

Dopamine: Start potential -0.29 V; stop potential -0.29 V; upper vertex potential 1.0 V; lower vertex potential -0.3 V; scan rate 100 mV/s, room temperature

Ascorbic acid: Start potential 0.01 V; stop potential 0.01 V; upper vertex potential 1.0 V; lower vertex potential 0.0 V; scan rate 100 mV/s, room temperature

MEASUREMENT

a) Clean the voltammetric vessel by diluted nitric acid, and miliQ water; then add 3 mL of Dopamine (1mM solution in 1M KCl) or Ascorbic Acid (1mM solution in 1M KCl)
b) Insert working electrode into a pencil holder and connect all electrodes.
c) Immerse three electrode set into the measuring vessel with solution of sample.
d) Record the voltammogram with the specified voltammetric parameters.
e) Save the voltammograms.
f) Load overlay total voltammograms from all experiments and compare them.

CURVE EVALUATION

a) The evaluation of CV peak height and peak potential for all measurements before and after pretreatments of electrodes
b) How to use Nova software for determination of CV peak height and peak potential
c) Evaluation of effect of different electrochemical pretreatment
d) The evaluation of effect of different electrolytes

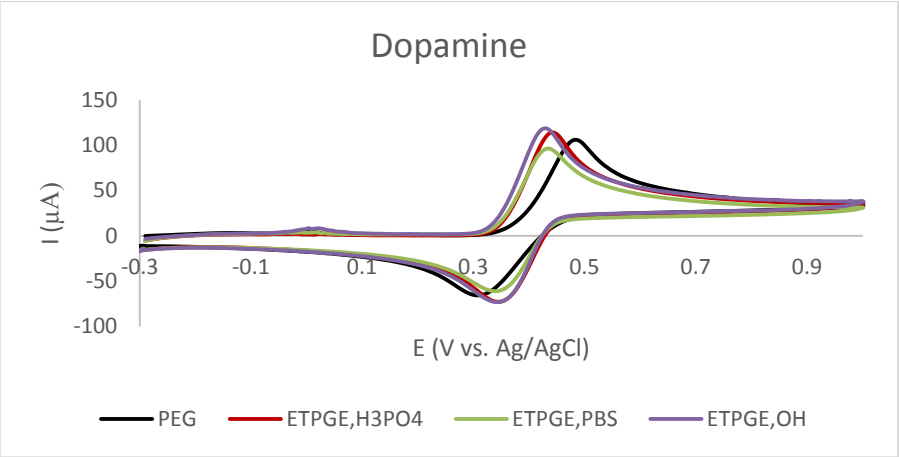

Fig 2. Cyclic voltammograms of dopamine before and after pretreatment of electrode by chronoamperometry carried out in different supporting electrolytes

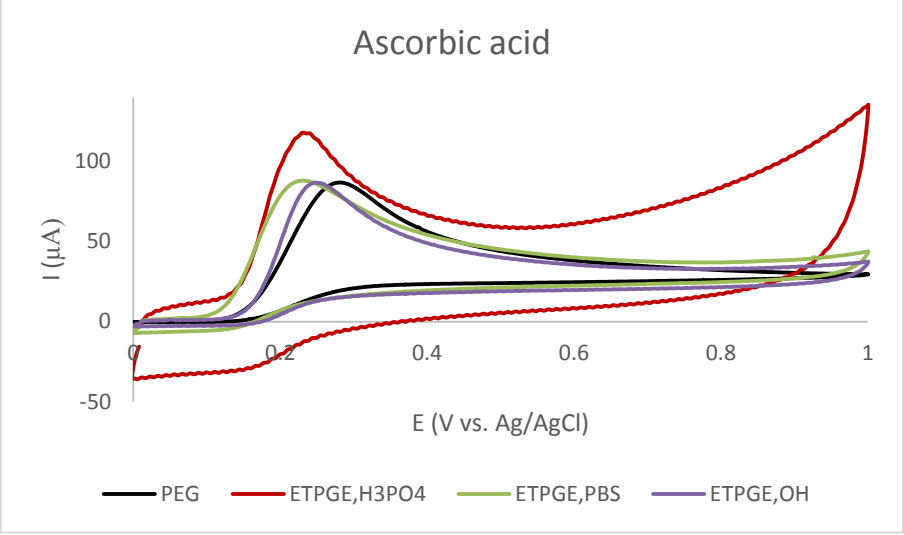

Fig 3. Cyclic voltammograms of ascorbic acid before and after pretreatment of electrode by cyclic voltammetry carried out in different supporting electrolytes

RESULTS

Pretreatment Method	Electrolyte	Dopamine		Ascorbic Acid	
			I_p	E_p	I_p
Cyclic voltammetry	H_3PO_4 (0.1M)				
	PBS,pH7.5 (0.1M)				
	NaOH (0.1M)				
Chronoamperometry	H_3PO_4 (0.1M)				
	PBS,pH7.5 (0.1M)				
	NaOH (0.1M)				
PGE	-				

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DISCUSSION AND REMARKS

DISCUSSION AND REMARKS

B) The processing an electroanalytical experiment

Peak-shaped signals are observed in various branches of analytical and physical chemistry, such as chromatography electrophoresis, atomic and molecular spectrometry, thermal and X-ray diffraction analysis and, of course, in electrochemistry, typically for voltammetric methods.

For *symmetric peak* fitting in voltammetry some arbitrary peak shape models can be used:

Gaussian model

$$G = a_1 \cdot exp\left(\frac{x - a_2}{a_3}\right)^2$$

where a_1 = const/($\sqrt{\pi}$ * a_3), a_2 is position of the peak maximum, and a_3 is peak dispersion parameter. This model is most commonly used [1, 7, 10, 13, 23].

Lorentz or ***Cauchy model***

$$L = \frac{a_1}{1 + \left(\frac{x - a_2}{a_3}\right)^2}$$

where a_1 = const/(π * a_3), a_2 is position of the peak maximum, and a_3 is peak dispersion parameter. This model is more suitable model for peaks with heavy long tails; it is often used for fitting of voltammetric peaks [1, 7, 10, 13].

pseudo-Voigt model is a sum of fractional contributions of components Gaussian (α) and Lorentzian (1 - α) peak shapes.

$$pV = \alpha \cdot G + (1 - \alpha) \cdot L$$

It is typical for atomic and molecular spectrometry [4, 14, 20], in electrochemistry is used sporadically[7]. It was applied as a simple approximation of Voigt spectral line profile, which is complicated to compute [3]. The main disadvantage of this model is a big number of optimized parameters. The model has no physical foundations, but it is often used as a pragmatic solution [4, 14].

Generalized Gaussian (exponential power) model

$$GG = a_1 * \exp\left(\frac{x - a_2}{a_3}\right)^n$$

where a_1 = (const * n)/(2 * a_3 *$\Gamma(1/n)$), a_2 is position of the peak maximum, and a_3 is peak dispersion parameter. Generalized Gaussian is a parametric family of symmetric distributions. It includes all uniform, Gaussian and Laplace distributions. It was also used for voltammetric peaks fitting [1].

Pearson VII model

$$P_{VII} = \frac{a_1}{\left[1 + \left(\frac{x - a_2}{\sqrt{m} * a_3}\right)^2\right]^m}$$

where a_1 = const/($\sqrt{\pi}$ * m * a_3) * $\Gamma(m)$/ $\Gamma(m$ - 1/2), a_2 is position of the peak maximum, and a_3 is peak dispersion parameter. This model is a general distribution based on Pearson system of frequency curves. Lorentz (m = 1), modified Lorentz (m = 2) and Gaussian ($m = \infty$) distributions are included to this system [11]. Modification of this distribution is symmetrical Fraser-Suzuki distribution [9].

Sigmoidal model (theoretical voltammetric peak)

$$S = \frac{a_1 * \exp\left[\frac{(x - a_2)}{a_3}\right]}{\left\{1 + \exp\left[\frac{(x - a_2)}{a_3}\right]\right\}^2}$$

where a_1 = -const/a_3, a_2 is position of the peak maximum, and a_3 is peak dispersion parameter. It is derived directly from current-potential curves [2, 7, 5, 12].

Derivative of logistic function

$$LD = \frac{a_1}{\left\{\exp\left[\frac{(x - a_2)}{a_3}\right] + \exp\left[\frac{(x - a_2)}{-a_3}\right]\right\}^2}$$

where a_1 = const, a_2 is position of the peak maximum, and a_3 is peak dispersion parameter. It is also model used for approximation of voltammetric peaks [18, 21].

For two non-separated peaks equation contains two terms, e.g. for gaussian peaks

$$G = a_1 * \exp\left(\frac{x - a_2}{a_3}\right)^2 + a_4 * \exp\left(\frac{x - a_5}{a_6}\right)^2$$

and analogically for the other models. Fitting of model to the data is usually made using non-linear regression. To be reliable, appropriate model selection and curve fitting should be accompanied by some *a priori* knowledge of the studied system.

More complicated is the situation in case of *asymmetric peaks*, where special mathematical models (see e.g. [6, 8, 9, 16, 17, 20]; etc.) should be applied.

Background correction is necessary to use in some cases, however, it can dramatically alter the results of fitting and also subsequent physical interpretation. Long tails of a Lorentzian function can be erroneously masked if the background is overestimated [4] and application of Gauss or pseudo-

Voigt function in this case may cause serious errors [4, 14]. Thus it is preferred to fit both baseline and envelope simultaneously if possible [14].

Practical application

For illustration voltammetrical data, a mixture of two symmetrical peaks, were employed (Fig. 1 - Gaussian model). The peaks correspond to reoxidation signals of guanine moieties in deoxyoligonucleotide dG3 on a mercury electrode. The calculations were made with freely available statistical software **R** (*www.r-project.org*). Baseline was corrected using robust local regression approach [19], included to R library *IDPmisc*. Minimization of the residual sum of squares in non-linear regression was made here via the Nash variant of the Marquardt algorithm [15] using R library *nlmrt*. The best fits yield sigmoidal, logistic derivative, Gaussian, Generalized Gaussian and Pearson VII models. Pseudo-Voigt and Lorentzian (Cauchy) models were found as unsuitable for presented data.

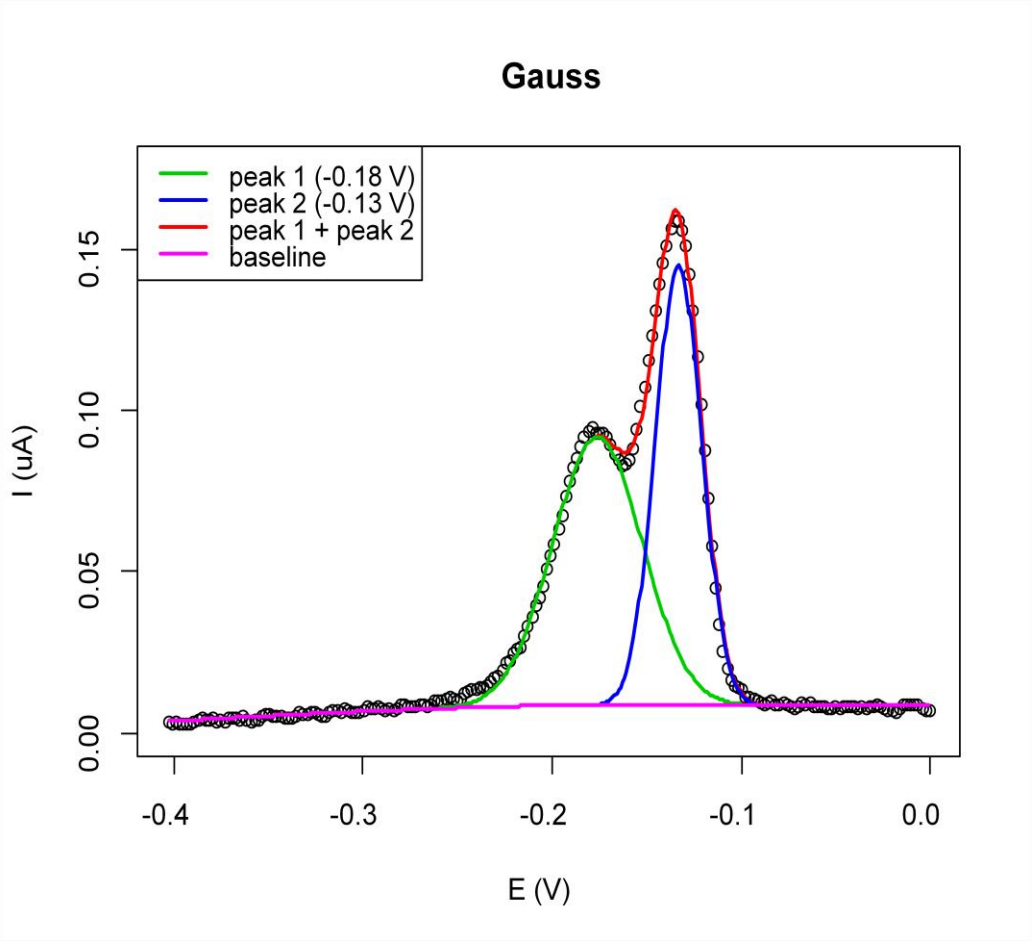

Fig.1 The Gauss model for the guanine reoxidation double peak in dG3

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DISCUSSION AND REMARKS

DISCUSSION AND REMARKS

ANALYSIS OF EQUILIBRIUM USING POTENTIOMETRIC TITRATIONS

Determination of protonation constants of purine derivatives

Potentiometry, widespread electroanalytical technique, is primarily used not only for pH measurements in different samples and for the determination of ionic constituents in biological fluids (e. g. blood, urine), but also as a transduction mode in monitoring selective interactions in molecular sensor devices or in the course of chemical reactions [1, 2]. One of the major types of potentiometry based on the monitoring of the indicating electrode potential change as a function of the reagent addition to the sample, potentioemtric titration, is a suitable method for the investigation of protonation – deprotonation equilibrium of biologicaly important compounds (purine derivatives, cytokinins, etc.) and it enables very precise determination of protonation constants pK_a.

Adenine (6-aminopurine) is one of the two most commonly occurring purines in nucleic acids, and it is involved in the process of protein synthesis and transmission of genetic information. 2-aminopurine (2-AP), isomer of adenine, is a fluorescent molecular marker used in nucleic acid research[3]. It most commonly pairs with thymine as an adenine-analogue, but can also pair with cytosine as a guanine-analogue[4]. For this reason it is sometimes used in the laboratory for mutagenesis. 2,6-diaminopurine(2,6-DAP) is a compound used to treat leukemia[5].

The knowledge of their protonation equilibrium is very important not only in biochemical processes and in electroanalysis, but also for the study of metal complexes in which aminopurines take the role of the ligand. Adenine exhibits two dissociation constants (pK_{a1} = 4.12 and pK_{a2} = 9.83)[6].

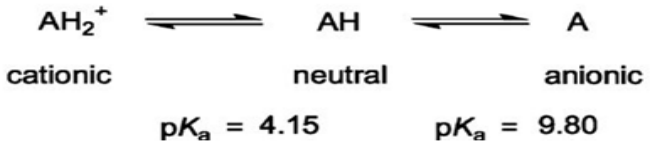

Fig.1: Protonation-deprotonation of adenine

The protontion constants pK_a of 2-AP is 3.8 and protonaion constant pK_a of 2,6-DAP is 5.3.

APPARATUS, ACCESSORIES AND REAGENTS

- titrator Titrando 835 (Metrohm, Switzerland), thermostat Julabo F25 – EH
- Tiamo 02/03 software (Metrohm, Switzerland)
- ion – selective electrode LL Ecotrode plus
- potentiometric cell

- argon (99.999%)
- NaOH; HCl; NaCl; milli Q water (18.2 MΩ·cm, 25 °C)
- adenine, 2- aminopurine (2-AP) and 2,6 – diaminopurine (2,6-DAP)

SOLUTIONS

Stock solutions

c (NaOH) = 0.1 mol/L; c (HCl) = 0.1 mol/L; c (NaCl) = 1 mol/L; c (aminopurine) = $1 \cdot 10^{-3}$ mol/L

The electrode calibration

5 mL of HCl + 4.5 mL of NaCl + 40.5 mL of milli Q water

Sample measurement

0.5 mL of HCl (0,1mol/L) + 5 mL of adenine (mmol/L) + 4.95 mL of NaCl (1 mol/L) + 39.55 mL of milli Q water

EXPERIMENT

The electrode calibration

5 mL of HCl (0.1mol/L); 4.5 mL of NaCl (1mol/L) + 40.5 mL of milli Q water

Sample measurement

0.5 mL of HCl (0.1mol/L) + 5 mL of aminopurine (mmol/L) + 4.95 mL of NaCl (1 mol/L) +39.55 mL of milli Q water

PARAMETERS

1) *Electrode calibration*: the addition of standard titrant solution (0.1M NaOH) = 0.01 mL; the total volume of 0.1M NaOH = 10 mL; the dosing rate is "maximal" and the titration mode is set as "optimal". Other parameters are "0" or "off"

2) *Sample measurement:* the addition of 0.1M NaOH = 0.001 mL; the total volume of 0.1M NaOH = 1 mL; the dosing rate is "maximal" and the titration mode is set as "optimal". Other parameters are "0" or "off"

Inert atmosphere is ensured by bubbling (argon; 99.999%) and stirring (magnetic stirrer, speed 3); temperature 15 °C, 25 °C and 37 °C.

MEASUREMENT

a) Start PC, open Tiamo 2.3 software (see manual below) and clean the automatic burette system with standard titrant solution (10 mL of 0.1 mol/L NaOH).

b) Put measured solution (for electrode calibration or sample measurement) in the potentioemtric cell, equipped with LL Ecotrode and dosing capillary. Ensure inert atmosphere (bubbling and stirring).

c) Set titration parameters in the selected method.

d) Start the measurement.

e) Record the titration curve and export data.

Note: LL Electrode plus is filled with 3M KCl, the filing hole should be opened during the measurement. After measurement don´t let the electrode in basic solution for long time, clean it and put it in stock solution (3M KCl).

POTENTIOMETRIC CURVE EVALUATION

The acidic part of calibration curve is used for the determination of the slope (q) and equilibrium potential (E^0) by linear regression. The equivalence point allows to calculate concentration of HCl. The basic part of calibration curve helps to calculate pK_w (should be for temperature 25^o C about 13.78). All these values are important for calculation of pH and degree of titration *z* and subsequently of p*K*a value based on these equations:

$$pKa_1 = -\log\left[H^+\right] - \log\left(\frac{z}{1-z}\right) \qquad pKa_2 = -\log\left[H^+\right] - \log\left(\frac{1-z}{z}\right)$$

$$z = \frac{\left(\frac{\left(c_{NaOH}\cdot V_{ekv} - c_{NaOH}\cdot\Delta V\right)}{\left(V_{celk}+\Delta V\right)} - \left[H^+\right] + \left[OH^-\right]\right)}{\left(\frac{c_L}{\left(V_{celk}+\Delta V\right)}\right)}$$

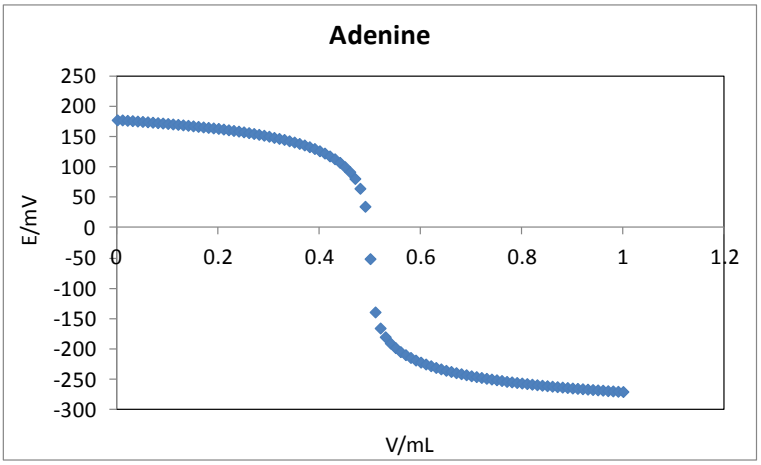

Fig.1: An example of measured data - titration curve of adenine in water

RESULTS AND REPORT

Calibration	
slope q	**E_0**

Measurement		
	pK_{a1}	**pK_{a2}**
Ade		
2-AP		
2,6-DAP		

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DISCUSSION AND REMARKS

DISCUSSION AND REMARKS

ELECTROANALYSIS AT MERCURY AND SOLID ELECTRODES

A) Electrochemical detection of nucleic acid fragments

Linear sweep voltammetry (LSV) is one of the most used electrochemical methods. It requires a potentiostat and a three-electrode set, consisting of a working electrode, an auxiliary electrode, and a reference electrode [1]. The current is measured between the working and an auxiliary electrode while the potential between the working electrode and a reference electrode is swept linearly in time. The slope of the potential vs. time is called the scan rate v and the polarization process can be described by the equation: $E = E_{initial} + vt$. The oxidation or reduction processes of electro-active species are registered as current maxima (peaks I_p) providing the determination of their concentration in solutions.

$$I_p = 2.99 \cdot 10^5 n(\alpha n_a)^{1/2} A D^{1/2} c v^{1/2} \quad \text{(Delahay equation)}$$

Where: I_p stands for the current peak, n stands for the number of electrons participating in reaction and n_a for the number of electrons participating in a rate determining step, α is the electron transfer coefficient, A is the effective area of electrode, D is the diffusion coefficient, c is the concentration, v is the scan rate.

DNA fragments provide on mercury electrodes reduction signals of adenine and cytosine moieties, and oxidation signals of guanine moieties; on graphite electrodes (e.g., a pencil graphite electrode - PeGE) well readable oxidation signals of adenine and guanine [2-3].

APPARATUS, ACCESSORIES AND REAGENTS

- µAUTOLAB TYPEIII Analyzer (Metrohm, Switzerland)
- NOVA software (Metrohm, Switzerland)
- Electrodes (working – Hg electrode or PeGE with leads Tombow, 0.5 mm, reference – Ag/Ag Cl/3M KCl, counter electrode – Pt); voltammetric vessel
- argon
- phosphate-acetate buffer pH 5.5; c (CH_3COOH) = 0.4 mol/L, c (H_3PO_4) = 0.4 mol/L, c(NaOH) = 2 mol/L
- short DNA fragments (oligonucleotides; ONs); miliQ water (18.2 MΩ·cm)

SOLUTIONS

Supporting electrolyte

phosphate-acetate buffer pH 5.5; c (CH_3COOH) = 0.4 mol/L, c (H_3PO_4) = 0.4 mol/L, c(NaOH) = 2 mol/L

Samples

DNA fragments (c = $2 \cdot 10^{-6}$ mol/L for Hg electrode and c = $1 \cdot 10^{-5}$ mol/L for PeGE)

EXPERIMENT on HMDE (Hanging Mercury Drop Electrode)

- Voltammetric experiment of ONs on HMDE

  1 mL of supporting electrolyte + 4 mL of miliQ H_2O + 200 µL of ONs (stock solution $5 \cdot 10^{-5}$ mol/L)

EXPERIMENT on PeGE

- Voltammetric experiment of ONs on PeGE

  0.6 mL of supporting electrolyte + 2.4 mL miliQ H_2O + 15 µL of ONs

PARAMETERS (CV OR LSV - CYCLIC OR LINEAR SWEEP VOLTAMMETRY)

LSV measurement of reduction signals

Start potential 0 V; upper vertex potential -1.7 V; scan rate 200 (400, 800) mV/s; time of accumulation 60 s, room temperature

LSV measurement of oxidation signals

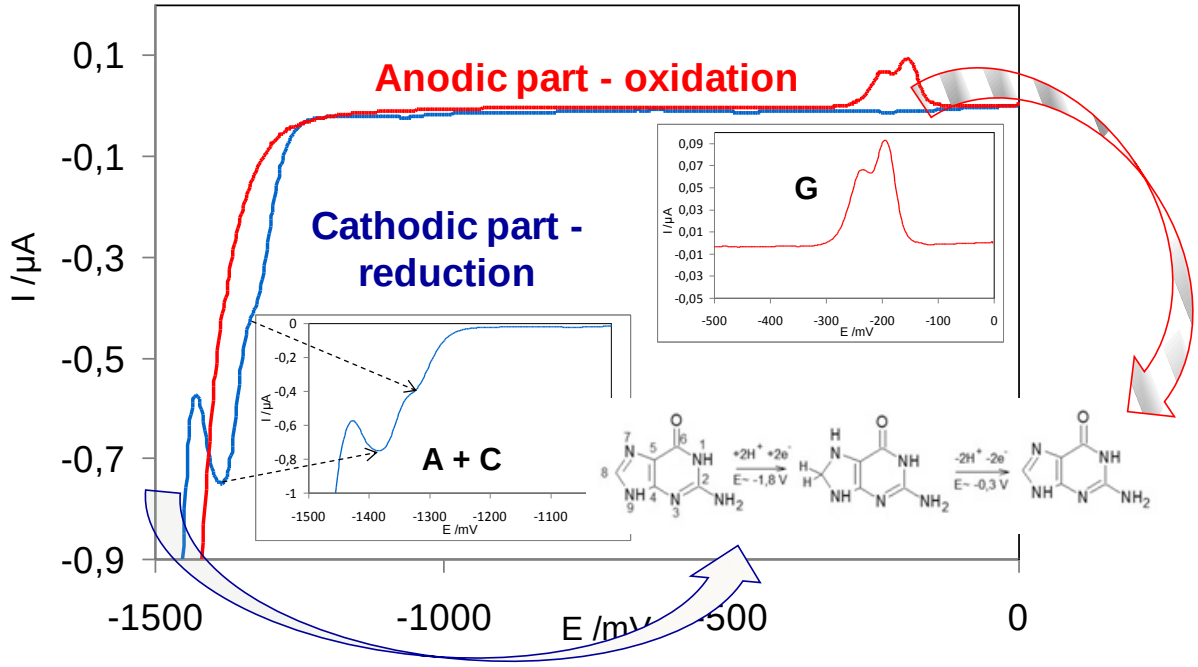
Start potential -0.1 V; stop potential -0.2 V; upper vertex potential 1.4 V; lower vertex potential -0.1 V; scan rate 200 (400, 800) mV/s; time of accumulation 120 s, room temperature

MEASUREMENT

a) Clean voltammetric vessel (diluted nitric acid, then miliQ water) and add the sample into vessel
b) Insert working electrode into pencil and connect all electrodes.
c) Immerse three electrode set into the measuring vessel with solution of sample.
d) Record the voltammogram with the specified voltammetric parameters.
e) Smooth voltammetric curves by Savitzky-Golay filter (level 2).
f) Save the smoothed curves.
g) Load overlay total voltammograms from all experiments and compare them.

CURVE EVALUATION

Measurements of the fragments DNA: cathodic and anodic processes on mercury and graphite electrode



Voltammogram of DNA fragment.
Red line: oxidation processes
Blue line: reduction processes

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DISCUSSION AND REMARKS

DISCUSSION AND REMARKS

B) Techniques and procedures increasing sensitivity of voltammetric methods

The elimination voltammetric procedure (EVP) is a mathematical processing of voltammetric data enabling achievement of better sensitivity and detection limits of studied compounds compared to voltammetric methods on which it relies. It works with elimination voltammetric functions (EVF), eliminating or conserving some chosen partial currents. EVP is based on different dependences of these partial currents on scan rate. In other words, the variable parameter is the scan rate and EVF are presented as linear combinations of total currents measured at different scan rates. In EVP must be satisfied two necessary conditions:

1) An eliminated current can be expressed as a product of two independent functions - the scan rate function and electrode potential function.

$$I_j = Y_j(E) \cdot W_j(\nu)$$

where $Y_j(E)$ is potential function and $W_j(\nu)$ is scan rate function.

2) A total voltammetric current is expressed as a sum of partial currents.

$$I = \sum_{j=1}^{n} I_j = I_d + I_c + I_k + \ldots$$

where I_d is the diffusion current, I_c the charging current and I_k the kinetic current.

Advantages of the approach consist in simplicity and availability. EVP is not time consuming procedure and it is able: (i) to provide better sensitivity, (ii) uncover of minor processes hidden in major processes, (iii) to extend potential range and (iv) to yield new information about electrode processes. The interaction of partial currents can be considered as a partial drawback. This disadvantage is sometimes reflected in an elimination signal distortion, as for example in the case of strongly adsorbed electroactive substances. Then the disadvantage is changed in the advantage because the elimination signal is much more higher and has the form (peak-counterpeak) which does not need a baseline correction [1].

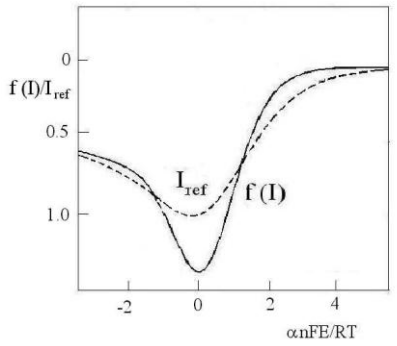

Fig.1: A substance transported to an electrode only by diffusion

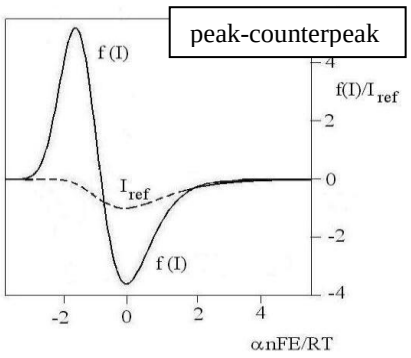

Fig.2: A totally adsorbed electroactive substances

I_{ref} – reference current, $f\ (I)$ – the aplication of the elimination function E4 (I_k +I_c elimination and I_d conservation), the values on the axe x are dimensionless.

elimination function E4: $f\ (I) = a_1 I_{(v1/2)} + a_2 I_{(v)} + a_3 I_{(v2)}$

$$f(I) = -11.657 I_{1/2} + 17.485 I - 5.8284 I_2$$

- Export data from NOVA 1.10 (or GPES) to Excel.
- Use elimination voltammetric procedure EPV E4 for measured data

PARAMETERS

- Scan rates with integer 2: e.g., 100, 200, 400 and 800 mV/s
- Elimination voltammetric functions with calculated coefficients

f(I)	Characteristics	EVLS equation
E1	**$I_d \neq 0$; $I_k = 0$ (I_c dist. By 1.707)**	**$f(I) = -3.4142 \cdot I_{1/2} + 3.4142 \cdot I$**
E2	**$I_d \neq 0$; $I_c = 0$ (I_k dist. By 2.414)**	**$f(I) = 4.8284 \cdot I_{1/2} - 2.4142 \cdot I$**
E3	**$I_k \neq 0$; $I_d = 0$ (I_c dist. By -0.707)**	**$f(I) = 3.4142 \cdot I_{1/2} - 2.4142 \cdot I$**
E4	**$I_d \neq 0$; $I_k = 0$; $I_c = 0$**	**$f(I) = -11.657 \cdot I_{1/2} + 17.485 \cdot I - 5.8284 \cdot I_2$**
E5	**$I_k \neq 0$; $I_d = 0$; $I_c = 0$**	**$f(I) = 6.8284 \cdot I_{1/2} - 8.2426 \cdot I + 2.4142 \cdot I_2$**
E6	**$I_c \neq 0$; $I_d = 0$; $I_k = 0$**	**$f(I) = 4.8284 \cdot I_{1/2} - 8.2426 \cdot I + 3.4142 \cdot I_2$**

CURVE EVALUATION

- Evaluate the EVP curves of the ONs

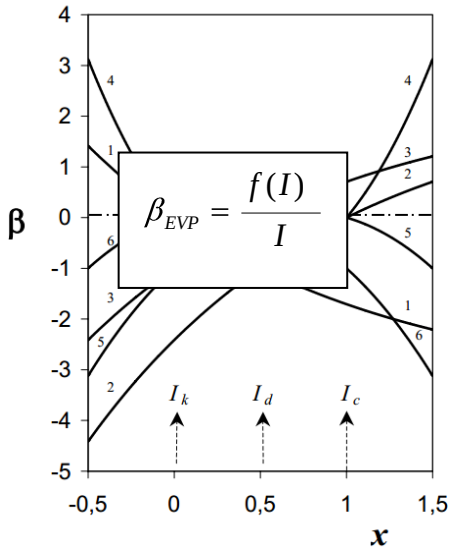

Fig. 3: Dependence of elimination current coefficient β_{EVP} on scan rate coefficient x for EVFs

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DISCUSSION AND REMARKS

DISCUSSION AND REMARKS

ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY (EIS)

EIS of guanine on modified and unmodified graphite electrodes

Electrochemical Impedance Spectroscopy (EIS) is a powerful technique for the characterization of electrochemical systems. The promise of EIS is that, with a single experimental procedure encompassing a sufficiently broad range of frequencies, the influence of the governing physical and chemical phenomena may be isolated and distinguished at a given applied potential.

In recent years, EIS has found widespread applications in the field of characterization of materials. It is routinely used in the characterization of coatings, batteries, fuel cells, and corrosion phenomena. It has also been used extensively as a tool for investigating mechanisms in electro–deposition, electro–dissolution, passivity, and corrosion studies. It is gaining popularity in the investigation of diffusion of ions across membranes and in the study of semiconductor interfaces.

Principles of EIS measurements

The fundamental approach of all impedance methods is to apply a small amplitude sinusoidal excitation signal to the system under investigation and measure the response (current or voltage or another signal of interest1). In the following figure, a non-linear i-E curve for a theoretical electrochemical system is shown in Figure 1.

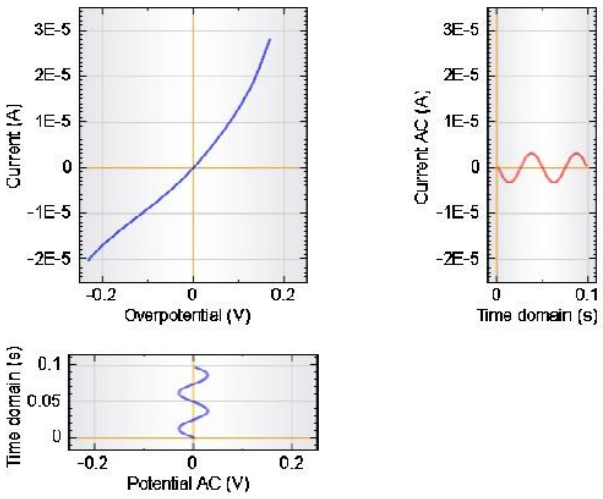


Fig. 1: Potential and current modulation recorded during an impedance measurement

A low amplitude sine-wave $\Delta E \cdot \sin(\omega t)$, of a particular frequency ω, is superimposed on the DC polarization voltage E_o. This results in a current response of a sine wave superimposed on the DC

current $\Delta i \cdot \sin(\omega t+\varphi)$. The current response is shifted with respect to the applied potential (see Figure 2).

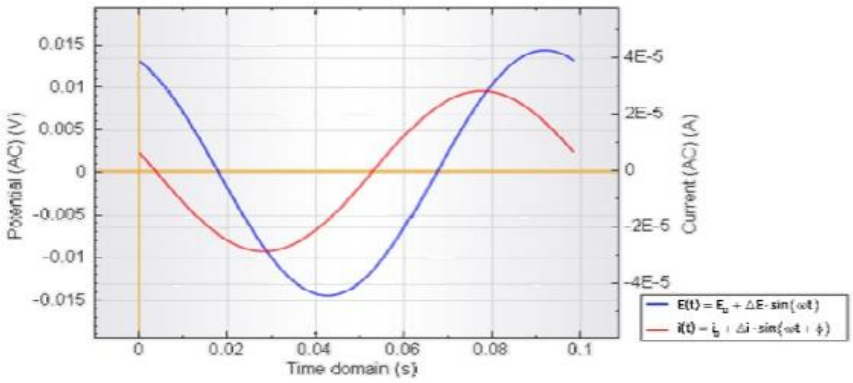


Fig. 2: Time domain plots of the low amplitude AC modulation and response

The Taylor series expansion for the current is given by:

$$\Delta i = \left(\frac{di}{dE}\right)_{E_o,i_o} \Delta E + \left(\frac{d^2 i}{dE^2}\right)_{E_o,i_o} \Delta E^2 + ...$$

If the magnitude of the perturbing signal ΔE is small, then the response can be considered linear in first approximation. The higher order terms in the Taylor series can be assumed to be negligible. The impedance of the system can then be calculated using Ohms law as:

$$Z(\omega) = \frac{E(\omega)}{i(\omega)}$$

This ratio is called impedance, $Z(\omega)$, of the system and is a complex quantity with a magnitude and a phase shift which depends on the frequency of the signal. Therefore by varying the frequency of the applied signal one can get the impedance of the system as a function of frequency. Typically in electrochemistry, a frequency range of 100 kHz – 0.1 Hz is used. The impedance $Z(\omega)$, as mentioned above is a complex quantity and can be represented in Cartesian as well as polar coordinates. In polar coordinates the impedance of the data is represented by:

$$Z(\omega) = |Z(\omega)| e^{\varphi\omega}$$

Where $|Z(\omega)|$ is the magnitude of the impedance and is the phase shift. In Cartesian coordinates the impedance is given by:

$$Z(\omega) = Z'(\omega) - j\,Z''(\omega)$$

Where Z'(ω) is the real part of the impedance and Z"(ω) is the imaginary part and .

Data presentation

The plot of the real part of impedance against the imaginary part gives a Nyquist plot, as shown in Figure 3. The advantage of Nyquist representation is that it gives a quick overview of the data and one can make some qualitative interpretations. While plotting data in the Nyquist format the real axis must be equal to the imaginary axis so as not to distort the shape of the curve. The shape of the curve is important in making qualitative interpretations of the data. The disadvantage of the Nyquist representation is that one loses the frequency dimension of the data. One way of overcoming this problem is by labeling the frequencies on the curve.

The absolute value of impedance and the phase shifts are plotted as a function of frequency in two different plots giving a Bode plot in Figure 4. This is the more complete way of presenting the data.

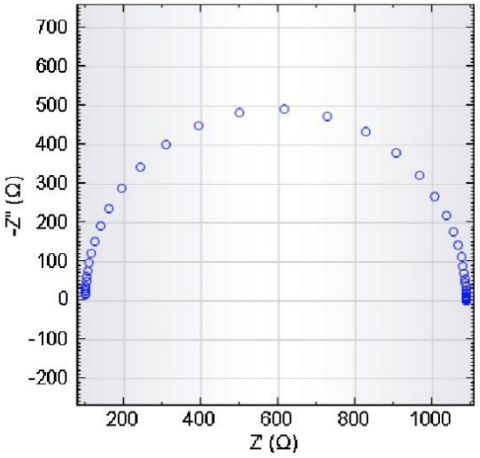


Fig. 3: A typical Nyquist plot

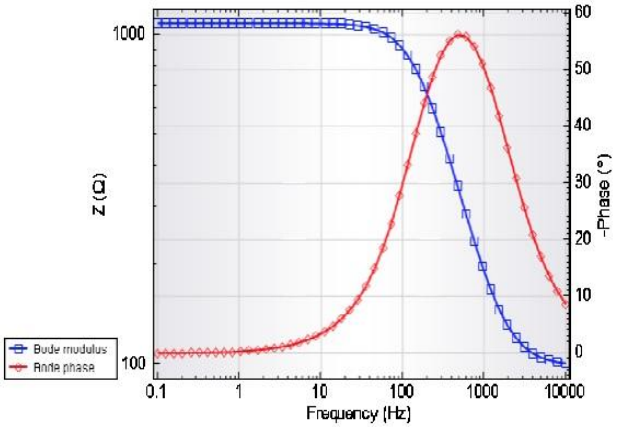


Fig.4: A typical Bode plot

A third data presentation mode involving a 3D plot, is available. In this presentation mode the real and imaginary components are plotted on the X and Y axis, respectively and the logarithm of the frequency is plotted on the Z axis (see Figure 5).

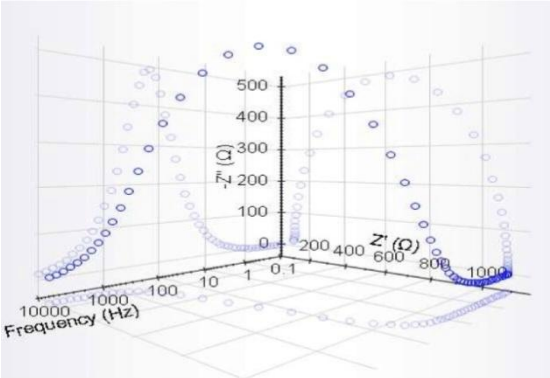


Fig.5: 3D plot

The relationship between the two ways of representing the data is given by:

$$|Z|^2 = (Z')^2 + (-Z'')^2 \qquad tg(\varphi) = \frac{-Z''}{Z'}$$

Alternatively, the real and imaginary components can be obtained from:

$$Z' = |Z|\cos\varphi \quad \text{and} \quad -Z'' = -|Z|\sin\varphi$$

APPARATUS, ACCESSORIES AND REAGENTS

- AUTOLAB PGSTAT20 Analyzer (Metrohm AUTOLAB, Netherlands)
- NOVA software 1.10
- Electrodes (working - PeGE with leads Tombow, 0.5 mm, reference – Ag/Ag Cl/3M KCl, counter electrode – Pt); voltammetric vessel
- argon
- phosphate-acetate buffer pH 5.5; c (CH_3COOH) =0.4 mol/L, c (H_3PO_4)=0.4 mol/L, c(NaOH)=2 mol/L
- adenine; copper sulphate; miliQ water (18.2 MΩ·cm)

SOLUTIONS

Supporting electrolyte

phosphate-acetate buffer pH 5.5; c (CH_3COOH) =0.4 mol/L, c (H_3PO_4)=0.4 mol/L, c(NaOH)=2 mol/L, c($CuSO_4$)=$1 \cdot 10^{-3}$ mol/L

Samples

adenine = $1 \cdot 10^{-3}$ mol/L; (or guanine)

EXPERIMENT

- EIS experiment for adenine in the absence of copper ions – 2 mL supporting electrolyte + 8 mL miliQ H_2O + 100 µL adenine (guanine)

PARAMETERS

- Mesurement method potentiometric electrochemical impedance spectroscopy
- Start 0.1 V, End potential 1.2V, logarithmic step 0.2V
- Frequency 10 000- 0.1 Hz, 50 steps (logarithmic)
- RPM 0.1V

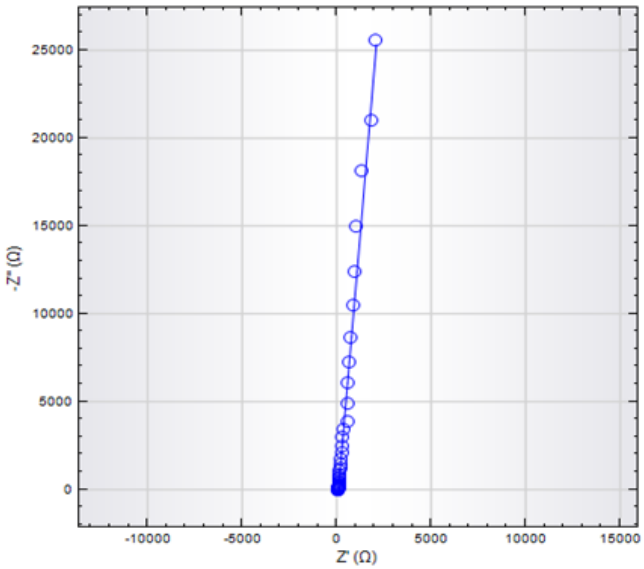
MEASUREMENT

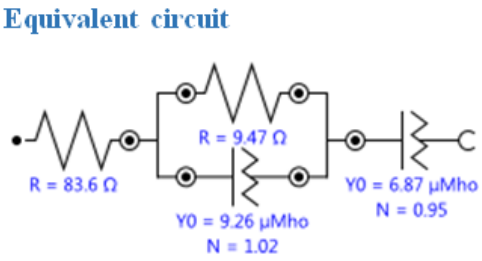
a) Clean voltammetric vessel (diluted nitric acid, than miliQ water). Than we added in the vessel 2 mL of supporting electrolyte, 8 mL miliQ H_2O and 100 µL of adenine (guanine)
b) Insert working electrode into pencil and connect all electrodes.

c) Start potentiometric EIS
d) Save data
e) Data analysis, Fit and Simulation
f) Insert equivalent circuit and Fit data
g) Export results

Measured data

Equivalent circuit





Results

Element	Parameter	Value
R1	R	83.613
R2	R	9.4724
Q1	Y0	9.2585E-06
	N	1.0198
Q2	Y0	6.8726E-06
	N	0.94971
	χ^2	0.033201

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DISCUSSION AND REMARKS

DISCUSSION AND REMARKS

ELECTROCHEMICAL QUARTZ CRYSTAL MICROBALANCE (EQCM)

The quartz crystal microbalance (QCM) is capable to sensitively measure the mass of thin films adhering to the surface of quartz crystal. The technique is based on the monitoring the changes in the frequency of quartz crystal resonant oscillations. The term quartz crystal used in QCM represents a thin (fraction of millimeter) plate cut from the quartz crystal in certain crystallographic orientation (AT-cut quartz wafer). The plate, usually a circle approx. 1 cm in diameter, is equipped with two metal contacts which are used to establish an electric field across the crystal. Driven by appropriate electronics, the crystal is made to oscillate at its resonant frequency, typically used are crystals resonating in the range from 5 to 30 MHz. The measured frequency is dependent on the thickness of the quartz wafer, metal electrodes, and material deposited on the quartz crystal microbalance surface. Because the resonance is very sharp, high precision frequency measurements allow the detection of minute amounts of deposited material, as small as 100 picograms per square centimeter, corresponding to submonolayer coverage. In the combination of QCM with electrochemistry i.e., EQCM, one of the metal contacts on the crystal serves as a working electrode and is a part of the common thre-electrode electrochemical cell. In EQCM, the typical technique is cyclic voltammetry with simultaneous recording of frequency change of QCM oscillations. Depositing material onto working electrode results in decrease in the frequency of oscillations while stripping the deposit from the electrode is accompanied with an increase in the frequency. The frequency change occuring in the event of a mass change on the QCM surface is governed by Sauerbrey equation:

$$\Delta f = \frac{2 f_0^2}{A\sqrt{\rho_q \mu_q}} \Delta m,$$

where f_0 is the resonant frequency (Hz), Δf is frequency change (Hz), Δm is mass change (g), A piezoelectrically active crystal area (Area between electrodes in cm^2), ρ_q is density of quartz (2.643 g/cm^3) and μ_q is the shear modulus of quartz for AT-cut crystal (2.947×10^{11} $g \cdot cm^{-1} \cdot s^{-2}$). Sauerbrey's equation only applies to systems in which the following three conditions are met: the deposited mass must be rigid, the deposited mass must be distributed evenly and the frequency change $\Delta f <$ 0.02.

A) Deposition and stripping of copper on gold

APPARATUS, ACCESSORIES AND REAGENTS

- AUTOLAB PGSTAT100 (Metrohm, Switzerland)
- NOVA software
- EQCM cell and crystals
- Reference nad auxiliary electrodes
- 1 mM copper sulphate solution in 0.1 M H_2SO_4
- 0.1 M H_2SO_4
- 1 mM lead nitrate solution in 0.1 M $HClO_4$
- 0.1 M $HClO_4$

EXPERIMENT

The purpose of the experiment is to find a calibration factor for the crystal using the copper electrodeposition method. By the use of the known calibration factor, molar mass of the „unknown" electrodeposited species (lead) will be estimated. Finally, the total mass of the deposited lead determined from EQCM frequency change is compared with the total mass of the deposited lead evaluated from Faraday Law.

MEASUREMENTS

The crystal electroactive area is determined using the ferro-ferricyanide electrochemistry. For this purpose, the cyclic voltammogram of ferricyanide is measured from +500 mV vs. Ag/AgCl (initial potential) to -100 mV vs. Ag/AgCl (vertex potential) using lower value of scan rate (20-50 mVs^{-1}).

EQCM experiment is performed for copper deposition (scan limits are: +200 mV vs. Ag/AgCl (initial potential) to -700 mV vs. Ag/AgCl (vertex potential), scan rate (20 mVs^{-1}) and lead deposition (scan limits are: +200 mV vs. Ag/AgCl (initial potential) to -800 mV vs. Ag/AgCl (vertex potential), scan rate (20 mVs^{-1}) for overpotential deposition, if the vertex potential is set to -400 mVs^{-1}, underpotential deposition of lead can be observed.

EVALUATION

The electroactive area of the crystal is calculated from peak current using Randles-Ševčík equation:

$$I_{pa} = I_{pc} = 2.69 \cdot 10^5 An^{3/2} D^{1/2} cv^{1/2},$$

where $I_{pa} = I_{pc}$ is anodic resp. cathodic peak current, A is electrode area in cm^2, n is a number of exchanged electrons, D diffusion coefficient in cm^2s^{-1}, c is concentration in $mol \cdot cm^{-3}$ (note unusual unit!), v is scan rate in Vs^{-1}.

The slope of Δf vs. charge curve is obtained by fitting the data using linear regression. The calibration factor for the crystal is then calculated according to the equation:

$$C_f = \frac{slope \cdot FAn}{M_{Cu} \cdot 10^6},$$

where F is the Faraday Constant, A is the electroactive area, M_{Cu} is the molar mass of Cu, and n is the number of electrons. The 10^6 is used to convert from grams to micrograms.

To determine molar mass of lead, the measured Δf data are expressed as change in mass (Δm). Then, Δm versus charge plot is constructed. The molar mass is calculated from the slope of the aforementioned plot:

$$M = \frac{|slope|Fn}{10^6}.$$

Finally, compare the total mass of the deposited lead determined from EQCM frequency change with mass evaluated from Faraday Law.

RESULTS

Theoretical value of calibration factor for copper is 226 Hz $cm^2/\mu g$, the determined value should be close to this value.

B) Monitoring of long alkyl chain thiols self-assembly on gold

APPARATUS, ACCESSORIES AND REAGENTS

- AUTOLAB PGSTAT100 (Metrohm, Switzerland)
- NOVA software
- EQCM cell and crystals
- Reference nad auxiliary electrodes
- 1-Dodecanethiol (resp. alkylthiols of different alkyl chain length)
- Ethanolic solution of 0.1M $NaClO_4$ or similar supporting electrolyte
- 0.1M tetrabutylammonium perchlorate solution in dimethylformamide

EXPERIMENT

Adsorption of thiols such as 1-dodecanethiol on gold is enhanced by application of positive potentials to the electrode surface and that adsorption proceeds faster than in the case of open circuit deposition. Monolayers can be removed by cycling in alkaline solution or in dilutesulfuric acid. EQCM is able to monitor these processes.

MEASUREMENTS

Monitoring of 1-dodecanethiol self assembled monolayer formation can be followed by EQCM under constant potential. At different applied potentials, the addition of 1-dodecanethiol into ethanolic solution of 0.1M $NaClO_4$, so that the final 1-dodecanethiol concentration is 1mM causes a decrease in the frequency of QCM crystal. While at open circuit potential the reaction exhibits slow kinetics (halftime of ca 1800 sec), at 0.5 V vs. Ag/AgCl the assembly proceeds considerably faster (halftime of ca 300 sec). The desorption process can be performed electrochemically, it can be monitored by cyclic voltammetry with EQCM around -1.4 V vs. Ag/AgCl. For this purpose, 0.1M tetrabutylammonium perchlorate solution in dimethylformamide is recommended as supporting electrolyte.

EVALUATION

The formation of self assembled monolayer of 1-dodecanethiol prodeeds according to equation $RSH\ (Au) \rightarrow RS\text{-}Au + H^+ + e^-$. In the absence of potential, formation of hydrogen takes place.

RESULTS

Using calibration factor obtained from the experiment with copper, the mass of 1-dodecanethiol can be estimated from the decrease in QCM frequency. Calculate the density of molecules per unit area of the QCM electrode.

DISCUSSION AND REMARKS

DISCUSSION AND REMARKS

SPECTROELECTROCHEMISTRY

Spectroelectrochemical investigation of the ferro-ferri redox system

Spectroelectrochemistry is a hybrid technique that enables to measure UV-vis spectra of the analyte that is exposed to a given potential. The technique therefore provides the volt-ampere characteristics together with the information about the absorption spectra of generated intermediates. In the classical mode, cyclic voltammetry is coupled with the UV-vis spectrometry, but other methods like chronoamperometry or linear sweep voltammetry can be used as well. The technique provides much deeper insight into redox processes in comparison to classical electrochemical methods and therefore becomes very popular in recent years. [1] The main drawback of this technique is a need of special equipment, a diode-array UV-vis spectrometer with a suitable cuvette space and the spectroelectrochemical cell. The construction and performance of different types of spectroelectrochemical cells can significantly differ and the commercially available types are usually very costly.

The OTTLE cell (optically transparent thin layer electrochemical cell) depicted in Figure 1 is one of the most advanced commercially available cells. The set of three electrodes is placed in a thin measuring window. The working electrode (WE, Fig. 1, right part) is usually fully transparent (ITO electrode) or is a semitransparent grid from a thin wire. The pseudoreference electrode is a thin wire pointing towards WE. The auxiliary electrode (AUX) is located at the opposite side of the measurement window. The electrode set is fixed into the plastic spacer which determines the thickness of the measurement window and therefore the length of the optical pathway. The whole setup is covered with the optically transparent crystal (SiO_2, CaF_2, KCl, Fig. 1, left part) and fitted into a gas-tight housing.

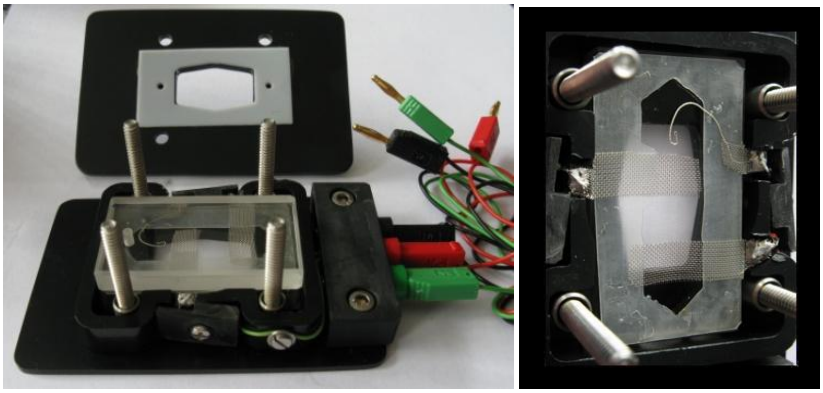

Fig 1. OTTLE spectroelectrochemical cell
(https://www.reading.ac.uk/web/FILES/chemistry/SEC_Products.pdf)

Since the electrochemical process in the cell must occur fast and quantitatively, the volume of the measurement window is usually very small (~100 μL). The optical pathway is also very short (0.1 mm) which results in a need of usage of higher concentrations of the analyte (~ 1 mM) in comparison to the UV-vis spectrometry (~ 10 μM).

The ferro-ferri system is a standard reversible water-based redox system commonly used in electrochemistry as a benchmark. It is based on the redox interconversion of potassium ferrocyanide ($K_4[Fe(CN)_6]$) to potassium ferricyanide ($K_3[Fe(CN)_6]$) and vice versa. The ferrocyanide ion in aqueous solution is not stable and gets oxidized by oxygen dissolved in the solution. Therefore the more stable ferricyanide is used as a stock solution and the Fe(II) species are generated electrochemically *in situ* by a pretratment. [2]

APPARATUS, ACCESSORIES AND REAGENTS

- AUTOLAB PGSTAT101 Analyzer (EcoChemie, Netherlands)
- NOVA software (Metrohm, Switzerland)
- OTTLE spectroelectrochemical cell (prof. Hartl, University of Reading, UK), electrodes (working – platinum grid, pseudoreference – Ag wire, counter electrode – Pt grid); optical pathway 0.1 mm
- Agilent 8453 (Agilent, CA, USA)
- potassium ferricyanide stock solution: $c(K_3[Fe(CN)_6])$ = 0.02 mol/L; c(KCl) = 0.1 mol/L
- deionized water
- argon
- degassing sealed vial with septum

- balloon with a needle
- acetone

SOLUTIONS

Supporting electrolyte

potassium chloride solution: c = 0.1 mol/L

Samples

potassium ferricyanide stock solution: $c(K_3[Fe(CN)_6]) = 0.02$ mol/L; c(KCl) = 0.1 mol/L

EXPERIMENT

- Blank measurement of degassed KCl solution
- Electrochemical pretreatment
- Estimation of the maximal scan rate
- Measurement of the sample of $K_3[Fe(CN)_6]$
- Data analysis, comparison of electrochemical and spectral result

PARAMETERS (CV OR LSV - CYCLIC OR LINEAR SWEEP VOLTAMMETRY, SPECTROSCOPY)

Blank measurement

Start potential -0.1 V, stop potential -0.1 V, upper vertex potential 1.0 V, lower vertex potential -1.0 V, scan rate 100 mV/s, number of cycles 3

Electrochemical pretreatment

Start potential -0.1 V, waiting time 60 s, spectroscopic measurement – interval time 1 s, wavelengths followed 230 nm, 303 nm, 410 nm

Estimation of the maximal scan rate

Start potential -0.1 V, scan rate 200 mV/s (100, 20, 10 mV/s), stop potential 0.6 V, spectroscopic measurement – interval time 1 s, wavelengths followed 230 nm, 303 nm, 410 nm

Sample measurement

Start potential -0.1 V, determined pretreatment wait time, determined scan rate, stop potential 0.6 V, spectroscopic measurement – interval time 1 s, wavelengths followed 230 nm, 303 nm, 410 nm

MEASUREMENT

g) Clean the OTTLE cell (acetone, distilled water).
h) Degass the blank solution in a vial sealed by a septum by bubbling with nitrogen for 10 minutes
i) Fill the blank solution into the OTTLE cell
j) Measure the CV scan of the blank solution
k) Degass the sample solution

l) Fill the OTTLE cell with the sample solution
m) Perform the electrochemical pretreatment, determine the time needed for quantitative reduction of the sample
n) Refill the OTTLE cell with the fresh degassed solution of the sample
o) Measure the sample at different scan rates, estimate the maximum suitable scan rate
p) Refill the OTTLE cell with the fresh degassed solution of the sample
q) Measure the sample with the appropriate pretreatment and the determined scan rate
r) Determine the redox potential of the ferro-ferri system based on the UV-vis spectra and voltammogram analysis

RESULTS AND DISCUSSION

The typical voltammogram of the ferro-ferri system is shown in Figure 2. The oxidation from ferrocyanide to ferricyanide occurs at approximately +0.24 V vs SCE.

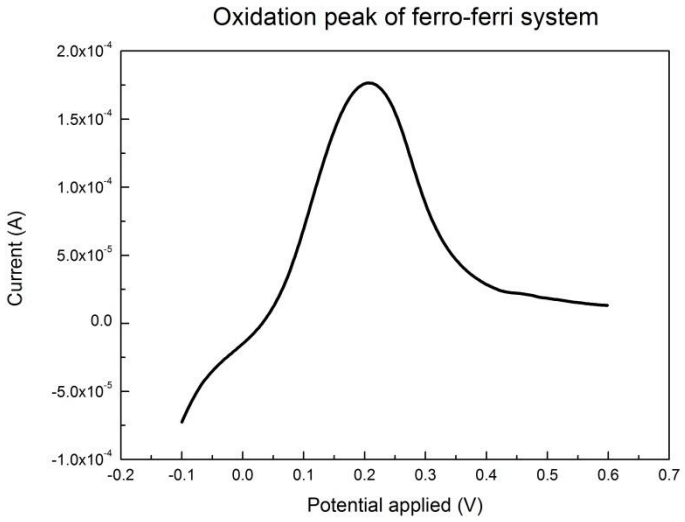

Fig 2. Voltammogram of the ferro-ferri system

The absorption spectrum of $K_3[Fe(CN)_6]$ is shown in the Figure 3.

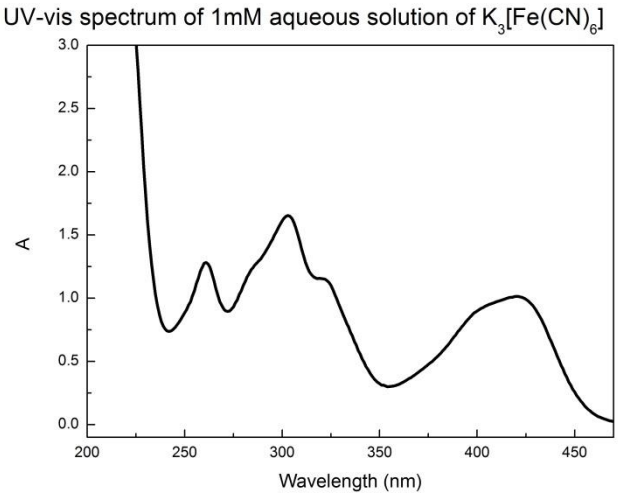

Fig 3. Absorption spectrum of $K_3[Fe(CN)_6]$

Stacked spectra of ferro-ferri system at varying potential are depicted in Figure 4. The blue curve corresponds to the ferricyanide ion, the red spectrum is the initial ferrocyanide.

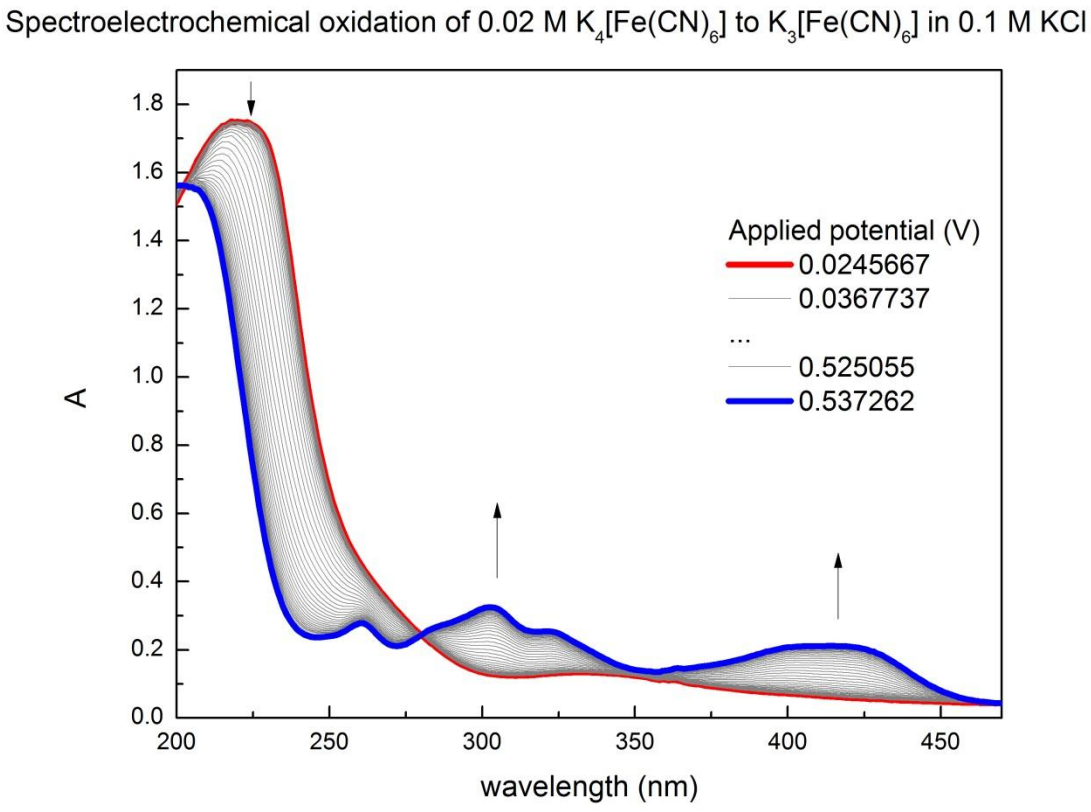

Fig 4. Stacked absorption spectra of ferro-ferri system at different potentials

The distribution digram of ferrocyanide and ferricyanide determined from different wavelengths (ferrocyanide: 230 nm, ferricyanide: 303 nm, 410 nm; normalized from 0 to 1) at varying potential is shown in Figure 5.

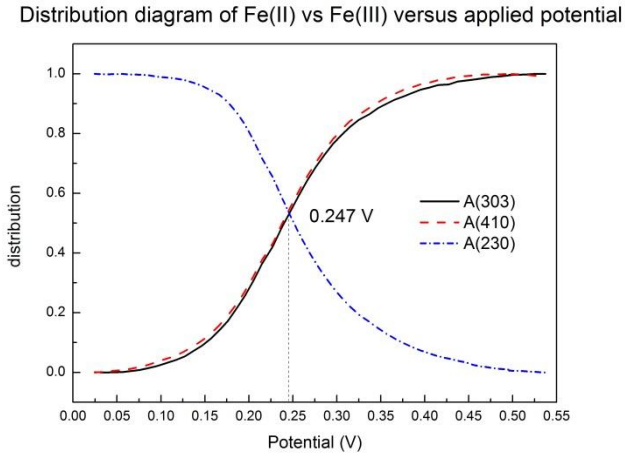

Fig 5. Dependence of the distribution of forms of ferrocyanide and ferricyanide at different potential

RESULTS

Pretreatment time [s]	

Scan rate determination (absorbance at 0.6 V)			
Scan rate (mV/s)	**A^{230}**	**A^{303}**	**A^{410}**
10			
20			
100			
200			

Determined potential	A^{230}	A^{303}	A^{410}
spectroscopical			
electrochemical			

REFERENCES

[1] Kaim W., Fiedler J. *Chem. Soc. Rev.*, **2009**, *38*, 3373-3382.

[2] Huang T. H., Salter G., Kahn S. L., Gindt Y. M. *J. Chem. Educ.*, **2007**, *84*, 1461-1463.

DISCUSSION AND REMARKS

DISCUSSION AND REMARKS

ION CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

Anions in drinking water

Ion chromatography belongs to the separation methods. It is a high-performance version of ion-exchange chromatography. The aim is to separate ions from the sample between two immiscible phases – mobile and stationary. The ion chromatograph consists of a reservoir of mobile phase, pump, injector, switching valve, anion/cation exchange column, thermostat, suppressor and detector.

The stationary phase, which consists of an ion exchanger, is located in the column. For separation of cations, catex is used, it contains acidic functional groups (e.g. carboxyl) with negative charge. The separation of anions is done using annex, which contains basic functional group (e.g. amino) with positive charge. The mobile phase consists of buffer (e.g. carbonate).

The pump allows to adjust flow rate of the mobile phase through the column, where the ion exchange takes place according to differences in charges of the ions. Injector is used for the injection of the sample into switching valve. Then the sample is mixed with the mobile phase and goes into the column. The column is heated by thermostat on adjustable temperature. Some chromatographs can be equipped by suppressor, which decreases the background conductivity of the eluent, minimizes baseline noise, enhances the signal-to-noise ratio and increases the sensitivity of the measuring system. The suppressor is located behind the column.

The most common type of detectors for ion chromatography is based on conductivity measurement. However, also other detectors can be used, e.g. UV-VIS, fluorescence, electrochemical or the mass spectrometry. Ion chromatography allows also combination of more detectors in series.

The evaluation and control device represents computer with appropriate software. The record of the analysis is called chromatogram. The detector response for given analyte has the form of a peak generated at a certain time (retention time). The retention time is utilized for qualitative analysis, the area (or the height) of the peak is related to the quantity of the observed analyte. The peak area is directly proportional to the concentration of the analyte, the height evince this property only for constant peak shape.

Thermodynamic aspects of the ion exchange process

Ion exchangers normally consist of solid phases on whose surface ionic groups are fixed. Because ofthe condition of electroneutrality there is always an oppositely-charged counter-ion in the vicinity of the functional group. The counter-ion usually originates from the mobile phase and is therefore also known as the eluent ion (**Fig. 1**).

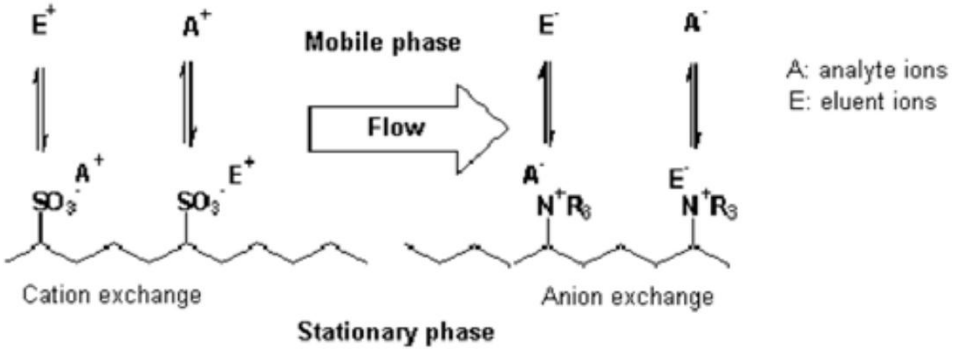

Fig. 1 Principle of anion and cation exchange

Ion chromatography has wide range of use. This technique is suitable for inorganic and organic cations and anions in either simple or complicated matrices. Its main advantages are short analysis time, high sensitivity, high selectivity, simple sample preparation and small sample volume.

Drinking water is our most important foodstuff. It is obtained mainly from ground water and surface water. Surface waters include water from lakes and reservoirs, bank filtrate, groundwater enriched with surface water and river water. According to DIN 2000, drinking water must satisfy the following requirements: It should be colorless, clear, cool and free from foreign odor and taste. If possible it should be naturally free from pathogens and substances that are hazardous to health. It should not contain too many salts, particularly hardness components, iron, manganese, as well as organic substances (peat and humic substances). It should not cause corrosion. The amount available should be sufficient to supply all the requirements of the population to be supplied with it.

Depending on the degree of pollution, various methods are used for the treatment of drinking water.
Screening, removes coarse soil and larger particles.
Sand filters, for filtration; biodegradation processes also occur in the sand and help with purification.
Active charcoal filters adsorb dissolved organic substances, e.g. pesticides.

Removal of iron and manganese by the oxidation of Fe(II) and Mn(II); this process would otherwise take place in the drinking water pipeline and result in brown turbidity or flocs in the drinking water.

Disinfection is always necessary when the water is not free of pathogens. Chlorine, ozone, chlorine dioxide and UV irradiation are all used.

Preventive chlorination before release into the drinking water pipeline in order to prevent growth of microorganisms on the way to the consumer.

The concentration of inorganic anions and cations is one of the most important parameter which is to be analyzed in drinking water. These concentration limits and analytical procedures are specified in official standards and regulations according to the US EPA 300.1 method.

TASK

- Investigate the quality of your tap water
- Investigate the quailty of mineral water and compare the data given on the bottle

Limiting values for drinking water (*EU standards*):

Chloride 100 mg/L, Nitrate 50 mg/L, sulfate 250 mg/L

EXPERIMENTAL CONDITIONS OF THE IC METHOD

column	Metrosep A Supp 5 150/4.0 mm
eluent	3.2mM Na_2CO_3 + 1mM $NaHCO_3$
sample	Tap water and mineral water
flow	0,7 mL/min
pressure	9 MPa
analysis time	Up to 15 minutes
loop	20 µL
supresor	Catex sequential supresor, regenerant 50 mM H_2SO_4, autostep with fill
polarity	+

EXPERIMENTAL CONDITIONS OF THE IC METHOD

1) Prepare the eluent listed in **Table 1** with concentrations given
2) Switch on the IC instrument and select the method „Sumer school anions“ and start equlibration of the system with your eluent.
3) Prepare the mixtures of the calibration standards according to the concentrations mentioned in the **Table 2**

Table 2 – concentrations of the calibration standards

standards	Std 1 [mg/l]	Std 2 [mg/l]	Std 3 [mg/l]
chloride	2	50	100
nitrate	1	25	50
sulfate	5	125	250

4) Measure the standards in order to obtain the calibration curve of chloride, nitrate, sulfate
5) Determine the concentration of the tap water and mineral water and compare the data with the ones on the bottle label.

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