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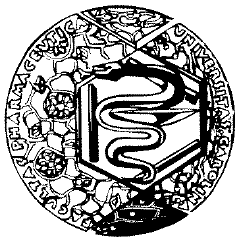

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FACULDADE DE FARMÁCIA
UNIVERSIDADE DO PORTO

Vývoj nové pyruvát-selektivní elektrody pro kontrolu kvality vína

Diplomová práce

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Hradec Králové, 2013

CHARLES UNIVERSITY IN PRAGUE

FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

DEPARTMENT OF ANALYTICAL CHEMISTRY

UNIVERSITY OF PORTO

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Development of a new pyruvate-selective electrode for wine control

Diploma thesis

Thesis author: Lucie Zelená

Thesis supervisors: Prof. RNDr. Petr Solich, CSc.

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Hradec Králové, 2013

Prohlášení

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V Hradci Králové, dne 15. května 2013 ……..

Lucie Zelená

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"I declare that this thesis is my original author work. All literature and bibliographic sources that I used for this work are listed in the references and cited properly. This work was not used to obtain the same or another title."

Hradec Králové, 15th May 2013 ……..

Lucie Zelená

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Abstract

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Title of master's thesis: Development of a new pyruvate-selective electrode for wine control

Pyruvate and other organic acids can influence the properties of wines as for example flavor, color, aroma or stability. New fast, low-cost, simple and sensitive methods have been developed for wine quality control.

A development of a new selective electrode for potentiometric determination of pyruvate in wine samples was the aim of this work. Sensors based on PVC membrane using 5,10,15,20-Tetraphenyl-21*H*,23*H*-porphine manganese (III) chloride as ionophore were under study. Several solvent mediators and lipophilic additives were tested in optimization of response characteristics of sensors.

The most interesting response showed membrane with 2-Nitrophenyl phenyl ether as a solvent mediator and Potassium tetrakis(2-chlorophenyl)borate as a lipophilic additive. Sensors with the best characteristics are still under the study.

Abstrakt

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Název diplomové práce: Vývoj nové pyruvát-selektivní elektrody pro kontrolu kvality vína

Pyruvát a další organické kyseliny mohou ovlivňovat vlastnosti vína jako je například chuť, barva, vůně nebo stabilita. Nové rychlé, levné, jednoduché a citlivé metody pro kontrolu kvality vína jsou neustále vyvíjeny.

Cílem této práce byl vývoj nové selektivní elektrody pro potenciometrické stanovení pyruvátu ve vzorcích vína. Studovány byly elektrody s PVC membránami, ve kterých byl jako ionofor použit chlorid 5,10,15,20-tetrafenyl-21*H*,23*H*-porfin manganitý. Během optimalizace složení membrán bylo testováno několik rozpouštědel a lipofilních aditiv.

Nejzajímavější výsledky ukázala membrána obsahující 2-nitrodifenylether jako rozpouštědlo a tetrakis(2-chlorofenyl)borát draselný jako aditivum. Elektrody s nejlepšími vlastnostmi jsou stále předmětem studia.

List of abbreviations

2-FNDPE	2-Fluorophenyl 2-nitrophenyl ether
2-NPPE	2-Nitrophenyl phenyl ether
a_A	Activity of the ion A
DBP	Dibutyl phthalate
DBS	Dibutyl sebacate
E	Potential of electrode
EMF	Electromotive force
F	Faraday constant (96485 $C.mol^{-1}$)
HPLC	High performance liquid chromatography
IC	Ion chromatography
ISE	Ion-selective electrode
$K^{pot}_{A,B}$	Potentiometric selectivity coefficient for ion A against ion B
KTpClPB	Potassium tetrakis(2-chlorophenyl)borate
LLLR	Lower limit of linear range
LOD	Limit of detection
MnTPPBr	5,10,15,20-Tetraphenyl-21*H*,23*H*-porphine manganese (III) chloride
NAD	Nicotinamide adenine dinucleotide
PVC	Poly(vinyl chloride)
R	Universal gas constant (8.314 $J.K^{-1}.mol^{-1}$)
RE	Reference electrode
T	Absolute temperature in Kelvins
TOABr	Tetraoctylammonium bromide
z_A	Charge number of the ion A

Content

1. Introduction

Electrochemical methods can be divided into two major groups:
A) methods based on electrochemical reaction (e.g. potentiometry, polarography, voltammetry or coulometry),
B) methods based on electrical properties of solution (e.g. conductometry) (1).

Direct potentiometry is an analytical method that provides non-destructive, simple, low-cost and no time consuming analysis with minimal consumption of testing sample. Low concentrations of analytes can be detected and the response is not affected by colored or high viscous samples. Thanks to short response time these methods can be connected as semi-automatic or fully automatic systems.

Ion selective electrodes (ISE´s) are commonly used as indicator electrodes in potentiometry. ISE´s provides determination of analyzed ion thanks to selective membrane through a process of transduction of chemical potential into electrical potential.

Potentiometry method can be applied in environmental, clinical, pharmaceutical or food analysis or in monitoring processes.

The aim of this work was a development of a new pyruvate-selective electrode for fast, simple and specific potentiometric determination of pyruvate in wine samples. The method can be used as a new alternative for quality control of wines in the future against the officially recommended method.

Pyruvate is an anion of pyruvic acid. It is important substance in several metabolic pathways, for example glycolysis, gluconeogenesis and alcoholic or malolactic fermentation. Pyruvate and other organic acids can influence properties of wines as color, flavor, taste or microbiological stability and so it is important for analytical control.

2. The aim of the work

The aim of this work was a development of a new pyruvate-selective electrode for fast, simple and specific potentiometric determination of pyruvate in wine samples. The method can be used as a new alternative for quality control of wines in the future against the officially recommended method.

Several potentiometric membranes with different composition will be prepared and tested. The developed solid contact ion-selective electrode will based on a PVC membrane incorporating a metalloporphyrin as an ionophore, a mediator solvent and a lipophilic additive. Different membranes with different proportions of ionophore, different mediator solvents and lipophilic additives will be prepared and evaluated in order to optimize the response characteristics of the electrode.

The potentiometric sensor with the best response characteristics will be chosen for practical applications on wine samples.

3. Theoretical part

3.1. Potentiometry

Potentiometry is an electroanalytical method that determines analytes by measuring the potential of an electrochemical cell under the zero current flow condition. The electrochemical cell is formed by two electrodes, an indicator electrode and a reference electrode, immersed in an analyzed solution. The measured potential is related to the activity of ions in the analyzed solution.

Potentiometric measurements can be divided in three types: direct potentiometry, standard addition method and potentiometric titration.

On direct potentiometry the potential difference of the electrochemical cell immersed in the sample is compared with the calibration curve previously made using standard. The standard addition method includes measuring of a potential of an electrochemical cell before and after additions of exact volume of standard solution of an analyte. In potentiometric titration the measured potential is plotted as a function of titrant volume, where a rapid change of potential indicates the end of titration (equivalence point).

3.1.1. Direct potentiometry

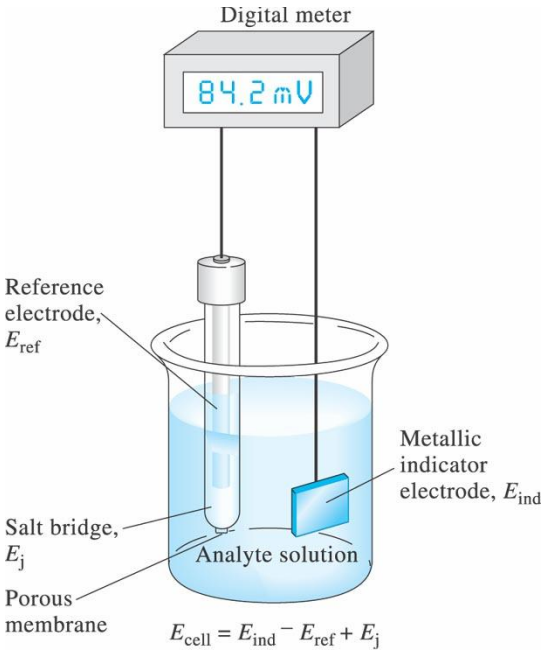
Direct potentiometry based on measuring of a potential of an electrochemical cell and is used for determination of the activity or concentration of analyzed ions.

Device for potentiometric measurement includes a reference electrode, an indicator electrode and a potential measuring device (e.g. voltmeter, pH meter).

A reference electrode is a half-cell with potential E_{ref} that is independent on the activity of ions in solution. Silver/silver chloride reference electrode (Ag/AgCl) and saturated calomel reference electrode (SCE) are the most commonly used.

Indicator electrode or working electrode is a half cell with potential E_{ind} that depends on activity of ions in analyzed solution and can be selective in response for different ions. As indicator electrodes can be used electrodes of the first kind (e.g. mercury electrode for determination of mercury ions), electrodes of the second kind (e.g. silver/silver chloride electrode for determination of Cl^- or Ag^+ ions), redox electrodes (e.g. platinum electrode for redox titrations) or ion-selective electrodes for determination of analyzed ions (e.g. pH glass electrode).

Figure 1: The scheme of electrochemical cell for potentiometric analysis (2).



The scheme of electrochemical cell can by written:

reference electrode (E_{ref}) | salt bridge (E_j) | analyte solution | indicator electrode (E_{ind})

The potential of electrochemical cell corresponds to the potential difference between the indicator electrode and the reference electrode plus the liquid junction potential formed in the salt bridge connecting the two electrodes (3):

$$E_{cell} = \left(E_{ind} - E_{ref}\right) + E_j \qquad (1)$$

The E_j can be combined with other constants into single constant, assuming that the liquid-junction potential doesn´t differ significantly from solution to the next. E_{ref}, E_{ind} and E_j are lumped together into constant *k* (3):

$$k = E_{ind} - E_{ref} + E_j \tag{2}$$

Then (for a 1:1 reaction),

$$\mathrm{E_{cell}} = \mathrm{k} - \frac{2.303\mathrm{RT}}{\mathrm{zF}} \log \frac{\mathrm{a_{red}}}{\mathrm{a_{ox}}} \tag{3}$$

The constant *k* is determined by measuring the potential of a standard solution in which the activities are known.

If the ionic strength is constant, activity coefficients are constant and can be included in *k*. After that concentrations can be determined from measured cell potentials (3):

$$E_{cell} = k - \frac{2.303RT}{zF} log \frac{C_{red}}{C_{ox}} \tag{4}$$

Potential of indicator electrode depends on ions presented in sample solution. This fact can be used for determination of ions activities (concentrations). Relation between potential of electrode and activity of ion is expressed by Nernst equation:

$$E_{ind} = E^0 \pm \frac{RT}{zF} ln(a_i) \; = \; E^0 \pm \frac{0.059}{z} ln(a_i) \tag{5}$$

where E_{ind} is potential of the indicator electrode, E^0 is standard potential of the reference electrode, *R* is an universal gas constant (8.314 $J.K^{-1}.mol^{-1}$), *T* is absolute temperature in Kelvins, *z* is charge of the analyzed ion i, *F* is a Faraday constant (96485 $C.mol^{-1}$) and a_i is the activity of the ion i. Sign in equation depends on analyzed ion – positive for cations and negative for anions.

3.2. Ion-selective electrodes

Ion-selective electrode (ISE) is an electrochemical sensor, based on thin films or selective membranes as recognition elements, and is an electrochemical half-cell equivalent to other half-cells of the zero (inert metal in a redox electrolyte), 1st, 2nd or 3rd kinds. The ISE must be used in conjunction with a reference electrode to form a complete electrochemical cell (4).

Classification of ISE´s according to IUPAC recommendations (5):

A) Primary ion-selective electrodes
    1) Crystalline electrodes
        a) Homogenous membrane electrodes
        b) Heterogenous membrane electrodes
    2) Non-crystalline electrodes
        a) Rigid, self-supporting, matrix electrodes
        b) Electrodes with mobile charged sites:
            (1) Positively charged hydrophobic cations
            (2) Negatively charged hydrophobic anions
            (3) Uncharged “carrier“ electrodes based on solutions of molecular complexing agents of cations and anions
            (4) Hydrophobic ion-pair electrodes of plasticized polymer containing a dissolved hydrophobic ion pair
B) Compound or multiple membrane (multilayer) ion-selective electrodes
    1) Gas sensing electrode
    2) Enzyme substrate electrode
C) Metal contact or all-solid-state ion-selective electrodes

3.2.1. Ion-selective electrodes with polymeric membranes

This type of electrode is very versatile and relatively easy to prepare. Membrane contains dissolved lipophilic ionophore that selectively reacts with the ion of interest and is not leached from membrane in aqueous solution (3).

Membrane is continuous layer, usually consisting of semi-permeable material, with controlled permeability covering a structure, such as carbon or an inert metal, or separating two electrolyte solutions. The membrane separates internal components of ISE from the test solution (4).

Polymeric membranes are usually composed of polymeric matrix (about 33 wt%), solvent mediator (about 66 wt%), ionophore (about 1 wt%) and lipophilic additive (usually in amount from 5 to 20 mol% relative to the ionophore).

Polymeric matrix is part of the membrane that provides mechanical stability. The most common used polymer is poly(vinyl chloride), usually plasticized in tetrahydrofuran. Some other used polymers are for example silicon rubber (6–8), some methacrylates (9–11), some polyurethanes (12–14).

Solvent mediator (or plasticizer) is part that provides homogeneity of all compounds of membrane. Solvent mediator affects membrane´s elasticity. Solvents with different polarity (dielectric constants) are used and can affect the behavior of the selective membrane, especially in terms of selectivity.

Apolar plasticizers are for example Dibutyl sebacate (DBS), Dioctyl sebacate (DOS) or Dibutyl phthalate (DBP). Polar plasticizers are for example 2-Nitrophenyl octyl ether (2-NPOE), 2-Nitrophenyl phenyl ether (2-NPPE) or 2-Nitrophenyl 2-fluorophenyl ether (2-FNDPE).

Ionophore (or ion exchanger or charged or neutral carrier) is part that mainly provides selectivity of the membrane. Ionophore is compound that can carry ions through the membrane. It forms strong but reversible complex with ions, ideally selective complexes with analyzed ion with minimal interference. It has also form complex with polymer matrix to don´t be washed out from membrane into the sample.

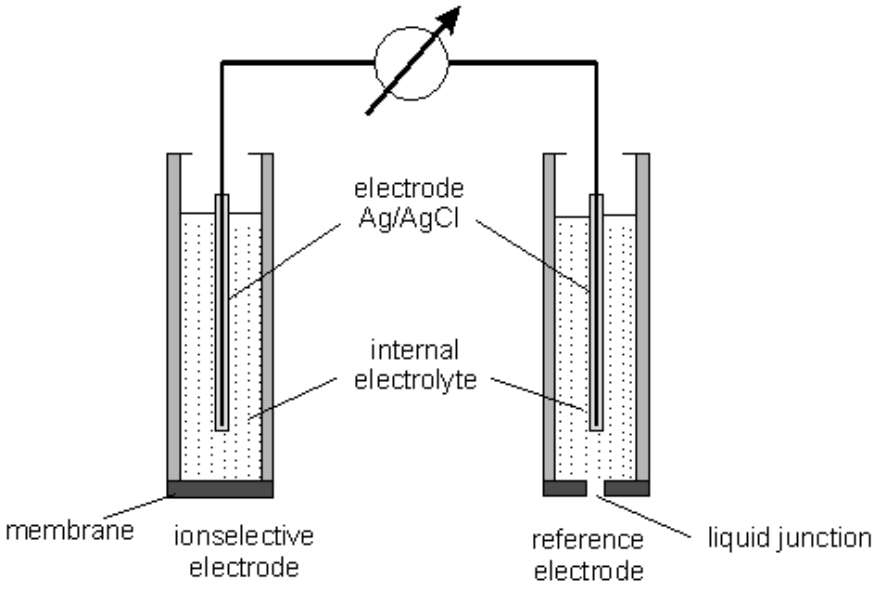
As the ionophore can be used for example metalloporphyrines (15–18), cyclodextrins (19–21), crown ethers (for metal ions, especially alkali and alkaline ions) or valinomycin (for potassium ISE (22,23)).

Lipophilic additives (salts) can be added to improve properties of membrane, mainly selectivity. These salts have no ion-exchanger properties, but can minimize interference from co-extracted ions (ions with the same charge as analyzed ion extracted from analyzed solution) by keeping total concentration of measuring ions in the membrane higher that co-extracted ions (3). Additionally make membrane more selective for divalent over monovalent ions by increasing the ionic-strength in the membrane (24). Tetraphenylborate derivates are used as anionic sites, tetraalkylammonium salts as cationic sites.

3.2.2. Response mechanism of ion-selective electrodes

Electrochemical cell consists of two half cells, the ion selective electrode (as an indicator electrode) and the reference electrode, immersed in analyzed solution.

Figure 2: Scheme of electrochemical cell with ISE (25).



The total potential difference (electromotive force, *EMF*) measured under zero current condition between two electrodes is the sum of local potential differences arising at each electrochemical interface (24):

$$EMF = E_{const} + E_M + E_{D,ref} \quad (6)$$

where E_M (membrane potential) and $E_{D,\mathrm{ref}}$ (liquid-junction potential) are sample dependent and E_{const} (refers to potential that can be expressed as constant contribution) is sample independent.

The liquid-junction potential, $E_{D,ref}$, arises between two dissimilar solutions of electrolytes, the analyzed solution and the bridge electrolyte of the reference electrode. It is caused by different mobilities of electrolytes and can be kept constant by using concentrated bridge electrolyte where mobilities of cations and anions are similar, e.g. 1 M KCl, NH_4NO_3, NH_4Cl (26).

The membrane potential, E_M, contain three separate parts: the *inner phase boundary potential* at the membrane and inner filling solution interface of RE (usually sample independent), the potential on water-membrane interface of RE between RE and sample solution and the *diffusion potential* within the membrane of ISE (sample dependent, interface can be kept constant) (21, 1).

The Nernst equation describing membrane potential difference, if the activities of the ions inside the membrane are sample independent (24):

$$E_I = EMF - E_{D,ref} = E_I^0 + s_I \ln a_{I,S} \quad (7)$$

where E_I is membrane-depending potential difference that responds to changes in $a_{I,S}$, E_I^O refers to all constant potential differences in the measuring cell, s_I refers to slope of ISE (theoretically 59.16 mV at 25°C for monovalent ions), $s_I = 2.303RT/(z_I F)$.

Ionophore is part that is responsible for selective response of ISE. Membrane selectivity is determined by complexation (or precipitation) of ions of interests with the ionophore if the membrane contents sufficient amount of the ionophore.

If the membrane contains no ionophore, but only lipophilic additive, extraction of ions from the sample to the membrane is the only potential determining process (26).

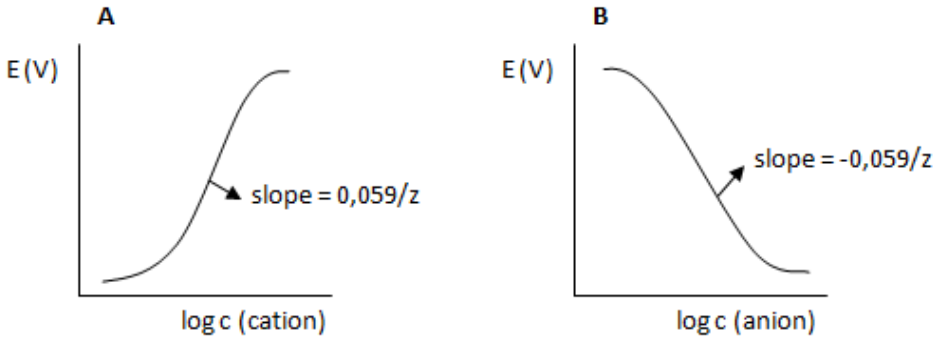
3.2.3. Characterization of ion-selective electrodes

Characterization of ISE is usually expressed by parameters that are important not only for usage of ISE, but are important also for comparison between different sensors. Some of these parameters are listed below.

Selectivity as one of the most important parameter for ISE´s is described in chapter 3.2.4.

Slope is a gradient of calibration curve that shows the extent of thermodynamic reversibility of the reaction that occurs at the interface membrane and characterizes the behavior of electrode. Calibration curve is a plot, where the measured potential of the cell, *E* (mV), is plotted versus the logarithm of ionic activity (concentration), *log* a_i (*log* c_i) of analyzed ion (5). The plot is linear if the correlation coefficient is close to 1. The theoretical value of Nernstian slope is (according to the Nernst equation) $2.303RT/z_iF$, it means 59.16 mV/dec at 25°C for monovalent ions and 29.58 mV/dec at 25°C for divalent ions.

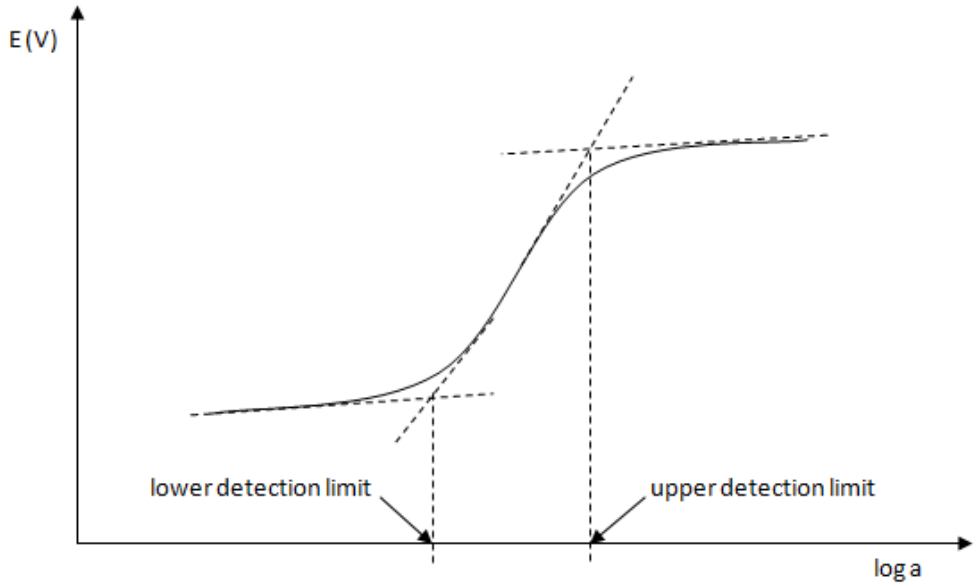
Figure 3: Scheme of calibration plots of cations (A) and anions (B).



Detection limits (upper and lower) are values that limit the linear range where ISE shows Nernstian response. According to IUPAC recommendations (5) upper and lower detection limit can be taken as ion activity (concentration) at the point of intersection of two extrapolated linear segments of the calibration curve, as shown in Fig. 4.

Range of linear response is the range of activity (concentration) over which the measured potential difference does not deviate from that predicted by the slope of the electrode by more than ± 2 mV (27). It is determined by the upper and lower detection limits.

Figure 4: Scheme of determination of detection limits by intersection of two extrapolated linear segments of calibration curve by IUPAC recommendations (5).



Reproducibility is the standard deviation of emf data collected in a series of emf measurements in solutions of different concentrations, after removal and washing and/or wiping of the electrodes (5).

pH range of electrode is that range over which a change in pH will not cause a significant change in the measured potential. It is the plateau on a graph of pH against potential (mV) at constant concentration of the detected ion. Outside this range, a change in pH may cause a significant change in the measured potential (mV) (27). pH adjustment can be used for keeping of the operational pH.

Response time (t_R) is time necessary to obtain a stable electrode potential when the electrode is removed from one solution and placed in another of different concentration. Response time is affected for example by the electrode type, temperature, the presence of interfering ions and if the samples are stirred or measured in static conditions (27).

Lifetime of electrode is period of time after which electrode change the behavior and loose it´s parameters. It can be caused for example by pH or ionic strange adjustment, by concentration of analyzed solutions, by washing out of membrane components or by effect of environmental conditions (26).

3.2.4. Selectivity of ion-selective electrodes

As mentioned above, the selectivity of the ISE is one of the most important properties. It is ability of the ISE to distinguish between the different ions in the same solution. Ion selective electrodes are not 100% ion-specific, most are sensitive to some other ions (27). In some cases it is necessary to use complexing agents or other components to minimize the effect of interfering ions existing in solution (5).

In presence of single analyzed ion the potential of ISE can be expressed by Nernst equation:

$$\mathrm{E} = \mathrm{E}^0 + \frac{\mathrm{RT}}{(\mathrm{z_A F})} \ln \mathrm{a_A} \qquad (8)$$

If analyzed solution contains a mixture of ions, different from the main ion, the ISE can also respond to these interfering ions. Potential of the ISE is expressed by modified Nicolsky-Eisenman equation (5):

$$\mathrm{E} = \mathrm{E}^0 + \frac{\mathrm{RT}}{(\mathrm{z_A F})} \ln\left[\mathrm{a_A} + \mathrm{K_{AB}^{pot}} (\mathrm{a_B})^{\mathrm{z_A}/\mathrm{z_B}}\right] \qquad (9)$$

where E is measured potential; E^0 is constant that includes the standard potential of the electrode, the reference electrode potential and the junction potential; z_A and z_B are charges of the primary ion A and the interfering ion B; a_A and a_B are the activities of the primary ion A and the interfering ion B; K^{pot}_{AB} is a potentiometric selectivity coefficient for the primary ion A against the interfering ion B; and R, T and F have the meanings mentioned above (see equation 5).

Several methods (25, 26) used for determination of K_{AB}^{pot} are described below.

Mixed solution methods

Fixed interference method (FIM)

The emf of the ISE cell is measured for solutions of constant activity of the interfering ion B, a_B, and varying activity of the primary ion A, a_A. The emf values are plotted vs. logarithm of activity of primary ion. The point of intersection of two extrapolated linear segments of the calibration curve indicates the value of a_A. K_{AB}^{pot} is calculated from the following equation:

$$\mathrm{K_{AB}^{pot}} = \mathrm{a_A}/(\mathrm{a_B})^{\mathrm{z_A}/\mathrm{z_B}} \qquad (10)$$

where both z_A and z_B have the same signs, positive or negative.

Fixed primary ion method (FPM)

The emf of the ISE cell is measured for solutions of constant activity of the primary ion A, a_A, and varying activity of the interfering ion B, a_B. The emf values are plotted vs. logarithm of activity of interfering ion. The point of intersection of two extrapolated linear segments of the calibration curve indicates the value of a_B. K_{AB}^{pot} is calculated from the following equation:

$$\mathrm{K_{AB}^{pot}} = \mathrm{a_A}/(\mathrm{a_B})^{\mathrm{z_A}/\mathrm{z_B}} \qquad (11)$$

Two solution method (TSM)

This method used measured potential of a pure solution of the primary ion, E_A, and a mixed solution containing the primary and interfering ions, E_{A+B}. K_{AB}^{pot} is calculated by inserting of the potential difference, $\Delta E = E_{A+B} - E_A$, into the following equation:

$$\mathrm{K_{AB}^{pot}} = \mathrm{a_A}\left(\mathrm{e}^{\Delta \mathrm{E}\, \mathrm{z_A}\, \mathrm{F}/\,(\mathrm{R}\,\mathrm{T})} - 1\right)/\,(\mathrm{a_B})^{\mathrm{z_A}/\mathrm{z_B}} \qquad (12)$$

Matched potential method (MPM)

This method is independent of Nicolsky-Eisenman equation. K_{AB}^{pot} is defined as the activity ratio of primary and interfering ions that give the same potential change under identical conditions.

At first, a known activity (a_A') of the primary ion solution is added into a reference solution that contains a fixed activity (a_A) of primary ions, and the corresponding potential change (ΔE) is recorded.

Next, a solution of interfering ion is added to the reference solution until the same potential change (ΔE) is recorded. The change in potential produced at the constant background of the primary ion must be the same in both cases.

$$\mathrm{K_{AB}^{pot}} = \left(\mathrm{a_A^{'} - a_A}\right)/\mathrm{a_B} \qquad (13)$$

Separate solution methods

Separate solution method ($a_A = a_B$) (SSM)

The potential of ISE cell is measured with two separate solutions. One solution contain the ion A in the activity a_A (but not B) and the other one contain the ion B in the same activity $a_A = a_B$ (but no A). If the measured values are E_A and E_B, respectively, K_{AB}^{pot} is calculated from the following equation:

$$\mathrm{K_{AB}^{pot}} = \mathrm{a_A}^{(1-z_A/z_B)}\mathrm{e}^{(E_A-E_B)\ z_A\ F/(R\ T)} \qquad (14)$$

Separate solution method ($E_A = E_B$) [SSM ($E_A = E_B$)]

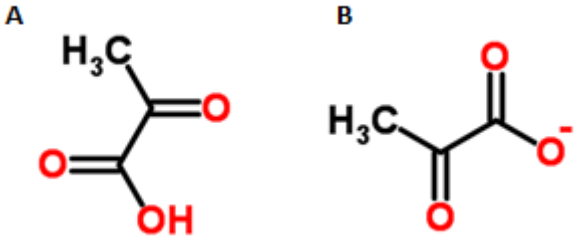
The calibration plots (where is *log a* vs. *emf* of ISE cell) for primary and interfering ions are obtained independently. Activities that correspond to the same electrode potential value are used to determine K_{AB}^{pot} value following this equation:

$$\mathrm{K_{AB}^{pot}} = \mathrm{a_A}/(\mathrm{a_B})^{z_A/z_B} \qquad (15)$$

3.3. Pyruvate

Pyruvic acid (also known as 2-oxopropionic acid, α-ketopropionic acid or acetylformic acid; Mr = 88.06 g.mol^{-1} (30)) is carboxylic acid belongs to alpha-keto acids. Anion and conjugate base of the pyruvic acid is known as a *pyruvate* and it is important substance in several metabolic pathways.

Figure 5: Structure of (A) pyruvic acid (31) and (B) pyruvate (32).



Some of chemical properties of pyruvic acid are listed in Table 1.

Table 1: Chemical properties of pyruvic acid (30,33).

IUPAC name:	2-oxopropionic acid
Formula:	$CH_3COCOOH$
Molecular formula:	$C_3H_4O_3$
Molecular weight:	88.06 g.mol^{-1}
Density:	1.272 g.mL^{-1} at 20°C
Melting point:	11 - 12°C
Boiling point:	165°C
Acidity (pK_a):	2.5

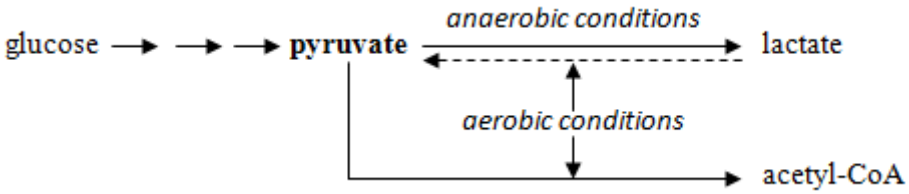
3.3.1. Biochemistry of pyruvate

Thanks to acidity (pK_a = 2.5), pyruvic acid usually exist in form of anion in the organism. Pyruvate takes part in glycolysis, gluconeogenesis, alcoholic and malolactic fermentation, Krebs cycle and metabolism of amino acids (synthesis of alanine and degradation of cysteine, serine, glycine, threonine and hydroxyproline).

Pyruvate is created as metabolite of glycolysis. Glycolysis is a process responsible for a degradation of glucose in the organisms - two molecules of pyruvate are created from one molecule of glucose. Process of glucose conversion into pyruvate is the same under the aerobic and anaerobic conditions (34).

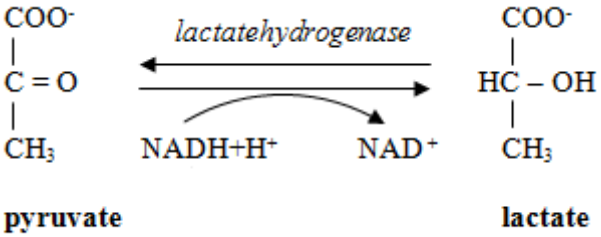
After that pyruvate can be changed in different pathways:
1) under anaerobic conditions can be converted into lactate (in mammals) or ethanol (in plants and microorganisms) in process called fermentation,
2) under aerobic conditions can be converted into acetyl-CoA, that is main substance for Krebs cycle, synthesis of fatty acids and ketogenesis,
3) pyruvate is also converted into oxaloacetate in anaplerotic reaction, that supplies amount of oxaloacetate for Krebs cycle.

Figure 6: Scheme of glycolysis (34).



Process of conversion of pyruvate under anaerobic conditions is called fermentation. In mammals organism pyruvate is converted into lactate. Enzyme responsible for this reaction is called *lactate dehydrogenase* and coenzyme is $NADH+H^+$. When conditions are changed into anaerobic, lactate can be converted back into pyruvate and can be used as source of energy (34).

Figure 7: Scheme of conversion of pyruvate into lactate (34).

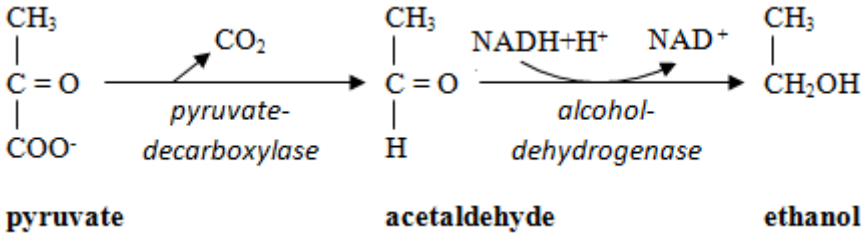


Probably the most known type is alcoholic fermentation. This process is typical for microorganisms, mainly yeasts. The conversion has two steps:

1) pyruvate from glycolysis is converted into acetaldehyde by enzyme *pyruvate decarboxylase* and coenzyme thiamine diphosphate,

2) acetaldehyde is converted into ethanol by enzyme *alcohol dehydrogenase*.

Figure 8: Scheme of conversion of pyruvate into ethanol (34).



3.3.2. Methods used for determination of pyruvate

The determination of organic acids (as acetic, citric, formic, fumaric, lactic, malic, propionic, pyruvic etc. (35)) in grape juices and wines is important because they have influence on the organoleptic properties (flavor, color and aroma) and on the stability and microbiological control of the products (36).

Some of these organic acids come directly from grapes and some are formed during winemaking process. In the case of wines, the analysis of organic acids allows to control the evolution of the acidity during the different steps of the winemaking process (alcoholic fermentation, malolactic fermentation or aging process) (37).

Several methods have been developed for identifying and quantifying these organic acids in grapes and wines, so much individually (non-enzymatic spectrophotometric and enzymatic methods) or as a group of them simultaneously (chromatographic and electrophoretic methods) (37).

Spectrophotometric methods are based on forming colored compound or complex (from acid and another analyte) and measuring of absorbance at several wavelengths. Pre-treatment of sample and isolation of acids is necessary before analysis. These methods, in addition to being not very specific, are tedious and time consuming (37).

Enzymatic methods are based on the measurement of the increase or decrease in absorbance of the coenzymes NADH or NADPH. Although these methods are highly specific, the use of enzymes is often limited, especially since there is a loss of enzymatic activity when the matrices are alcoholic. However they can be used as reference methods for validation of new methods (37).

Chromatographic methods are probably the most widely used in that area.

Gas chromatography has high sensitivity and selectivity, but for carboxylic acid analysis requires derivatization of the sample, once they are not volatile, and sample pre-treatment before derivatization is also need. The use of gas chromatography

is often limited by the sample preparation time, cost and also environmental issues. Alternatives as liquid chromatography or electrophoresis are more suitable (37).

Liquid chromatography is the technique most thoroughly used for the determination of organic acids in grape juices and wines. Separation and quantification of organic acids have been usually carried out by HPLC (normal phase or reversed phase, ion exchange or ion exclusion high performance liquid chromatography) (37).

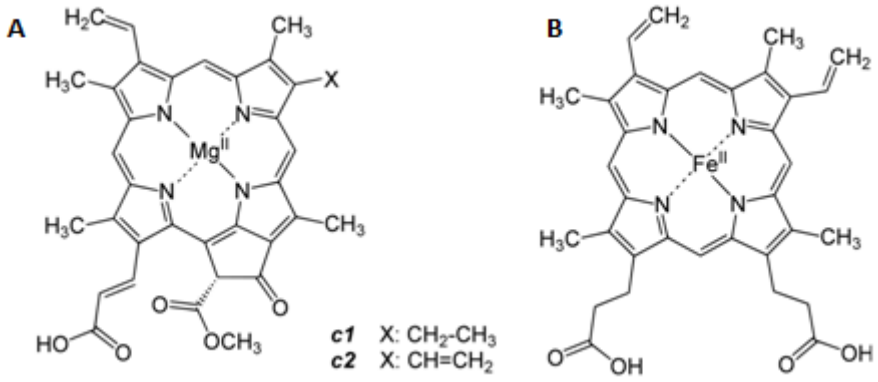
Ion chromatography with suppressed conductivity detection is the best option for the determination of pyruvate or pyruvic acid in samples with very low quantity of organic acids. The advantages are specificity, sensibility and minimization of sugar interferences. Sample pre-treatments are not necessary (37).

Electrophoretic methods are represented by capillary electrophoresis (CE). This technique has lot of advantages as a high resolution, short analysis time, low consumption of sample and reagents and also simple sample pre-treatment. Few methods for determination by capillary zone electrophoresis (CZE) appeared. Main disadvantage is its lower reproducibility, if compared with enzymatic and chromatographic methods, so some authors use internal standards or reference compounds to improve migration time reproducibility (37).

3.4. Porphyrins

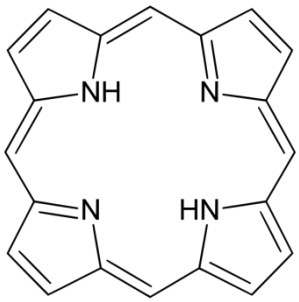
Porphyrins are naturally occurring macrocyclic organic compounds that have important role in the metabolism. The basic porphyrin is *porphine* and substituted porphines are called *porphyrins*. The most known *metalloporphyrines* are for example iron complexes in the hemoproteins or magnesium complexes in the chlorophylls and cobalt complex in vitamin B_{12} (38).

Figure 9: Structure of (A) chlorophyll 1 and 2 (39) and (B) heme (40).



The porphyrin molecule contains four pyrrole rings connected by methine bridges (=C-). The nucleus is tetradentate ligand with space for coordinated metal ions. The porphyrin ring system is very stable and exhibits aromatic character (38).

Figure 10: Structure of porphine – basic porphyrin macrocyclic system (41).



Porphyrins and their metal chelates exhibit characteristic sharp and intensive absorption bands in the visible region from 400 to 500 nm, called Soret band, that can be used for spectrophotometric detection (38).

In analytical chemistry metalloporphyrines can be used as electroactive agents in ion selective membranes in electroanalysis, as reagents in spectrophotometry and as stationary phases offering unusual resolution in HPLC (38).

Potentiometry uses porphyrins as electroactive components in membranes of ISE´s for more selective determination of analytes (15, 16, 42–44). Porphyrins are usually immobilized in PVC membrane or can be electropolymerized directly on the top of the electrode (e.g. Ag or Pt electrode) (38).

Voltammetry uses electrodes coated with electroactive film of polymer with porphyrin for detection of anions and pH measurements (38).

Spectrophotometry uses some porphyrins as reagents for determination of metal ions, complexation can be enhanced by using complexing agents (38).

HPLC with spectrophotometric detection uses porphyrins as complexing agents for the determination of transition metals. Porphyrins can be also used as stationary phases in immobilized metal ion chromatography (IMAC) (38).

4. Experimental part

4.1. Reagents and laboratory equipment

4.1.1. Reagents

Analytical chemical substances were used without further purification.

- Sodium pyruvate, from Sigma – Aldrich Chemie
- Deionized water (conductivity < 0.1µS cm^{-1})
- Poly(vinyl chloride) high molecular weight, from Fluka
- Tetrahydrofuran anhydrous, from Sigma – Aldrich Chemie
- 5,10,15,20-Tetraphenyl-21*H*,23*H*-porphine manganese (III) chloride, from Sigma – Aldrich Chemie
- 2-Nitrophenyl phenyl ether, from Fluka
- Dibutyl phthalate, from Sigma – Aldrich Chemie
- 2-Fluorophenyl 2-nitrophenyl ether, from Fluka
- Dibutyl sebacate, from Fluka
- Potassium tetrakis(4-chlorophenyl)borate, from Fluka
- Tetraoctylammonium bromide, from Fluka
- Sulfuric acid (96%), from JOSÉ MANUEL GOMES DOS SANTOS, LDA.
- Sodium hydroxide pellets, from AnalaR NORMAPUR
- Sodium chloride, from Riedel-de Haën
- Sodium sulfite anhydrous, from Sigma – Aldrich Chemie
- Sodium sulfate anhydrous, from E. Merck
- Sodium carbonate anhydrous, from Riedel-de Haën
- Sodium phosphate dibasic dihydrate, from Riedel-de Haën
- Sodium acetate anhydrous, from Riedel-de Haën
- di-Sodium tartrate dihydrate, from Sigma – Aldrich Chemie
- tri-Sodium citrate dihydrate, from Riedel-de Haën

4.1.2. Solutions

The standard pyruvate solution ($1.00x10^{-2}$ mol.dm^{-3}) was prepared every day by weighing about 0.11004 g of sodium pyruvate (Mr = 110.04 g.mol^{-1}), transferred into 100 mL volumetric flask and the volume completed with deionized water. Pyruvate solutions with lower concentration were prepared by dilution of the standard pyruvate solution with deionized water.

The solutions of interfering ions (in concentration $1.00x10^{-3}$ mol.dm^{-3}) were prepared by weighing proper amount of sodium salt of interfering anion, transferred into 100 mL volumetric flask and the volume completed with deionized water.

Concentrated acid and hydroxide were used for pH adjustment during Reilley diagrams. Concentrated sulphuric acid (96%) was used without further treatment. Concentrated sodium hydroxide was prepared by solvation of hydroxide pellet in a few drops of deionized water.

4.1.3. Laboratory equipment

A Crison 2002 pH potentiometer coupled to an electrode switcher ORION 605 was used for measuring the potential differences between reference electrode and pyruvate-selective electrodes. Double junction Ag/AgCl (KCl 3 mol.dm^{-3}) METROHM (model 6.0727.000) reference electrode was used with a solution of sodium sulfate (0.033 mol.dm^{-3}) in the external compartment.

The pH values of solutions were determined with PHILLIPS GAH 110 glass pH electrode.

4.1.4. Laboratory glass

Beakers, laboratory spoon, class A volumetric flasks and stoppers and volumetric pipettes were used during experimental work.

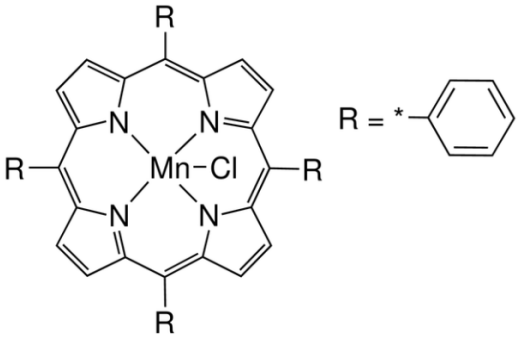
Automatic micropipettes, Gilson P100, P200 and P500, were used for adding standard solutions during calibrations.

4.2. Preparation of membranes and electrodes

PVC-based anion-selective membranes were prepared mixing 1 wt% of ionophore, 33 wt% of PVC, 66 wt% of solvent mediator and different amounts of lipophilic additives. Different membranes with different composition were prepared and evaluated (composition listed in Table 2).

The 5,10,15,20-Tetraphenyl-21*H*,23*H*-porphine manganese (III) chloride (see Figure 11) was chosen as the ionophore.

Figure 11: Structure of 5,10,15,20-Tetraphenyl-21*H*,23*H*-porphine manganese (III) chloride used as ionophore (45).



2-Nitrophenyl phenyl ether, 2-Fluorophenyl 2-nitrophenyl ether, Dibutyl phthalate and Dibutyl sebacate were evaluated as solvent mediators.

The lipophilic additives (see Fig 12) were used in amount from 5 to 20 mol % relative to the ionophore. Potassium tetrakis(4-chlorophenyl)borate was used as an anionic additive. Tetraoctylammonium bromide was used as a cationic additive.

Figure 12: Structure of (A) Potassium tetrakis(4-chlorophenyl)borate (46) and (B) Tetraoctylammonium bromide (47).

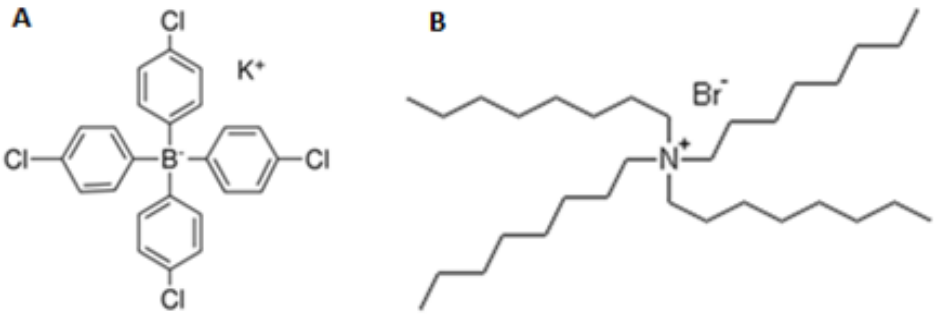
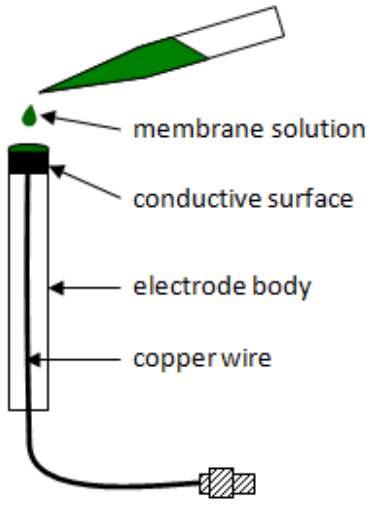


Table 2: Membrane composition (%) of pyruvate sensors under study.

Type	MnTPPBr	KTpClPB	TOABr	2-NPPE	DBP	2-FNDPE	DBS	PVC
	wt %	mol %	mol %	wt %	wt %	wt %	wt %	wt %
1	1.00	–	–	66.73	–	–	–	32.27
2	0.99	10.22	–	66.64	–	–	–	32.37
2.1	0.94	7.02	–	67.87	–	–	–	31.19
2.2	0.98	21.19	–	66.38	–	–	–	32.64
3	1.00	–	10.30	66.73	–	–	–	32.27
3.1	0.95	–	4.99	66.18	–	–	–	30.87
3.2	0.97	–	19.46	66.96	–	–	–	32.07
4.1	0.99	–	–	–	67.02	–	–	31.99
4.2	0.97	9.93	–	–	68.16	–	–	30.87
4.3	0.99	–	20.37	–	66.21	–	–	32.80
5.1	0.97	–	–	–	–	67.00	–	32.03
5.3	0.94	–	20.10	–	–	68.56	–	30.50
6.1	1.01	–	–	–	–	–	65.9	33.09

PVC was dissolved in 3.5 mL of tetrahydrofuran and after that added into the mixture of ionophore, solvent mediator and additive. For electrodes construction (see Figure 13) the membrane solution was dropped directly on the conductive surface of the electrode made from graphite powder and epoxy resin Araldite. The membrane solution was dropped in six layers. Sensors were ready for use after 12 hours of drying at room temperature.

Figure 13: Scheme of preparation of electrode.



4.3. Procedures and methods

4.3.1. Potentiometric sensors characterization

The evaluation of electrodes was carried out by obtaining the data from calibrations with standard pyruvate solution.

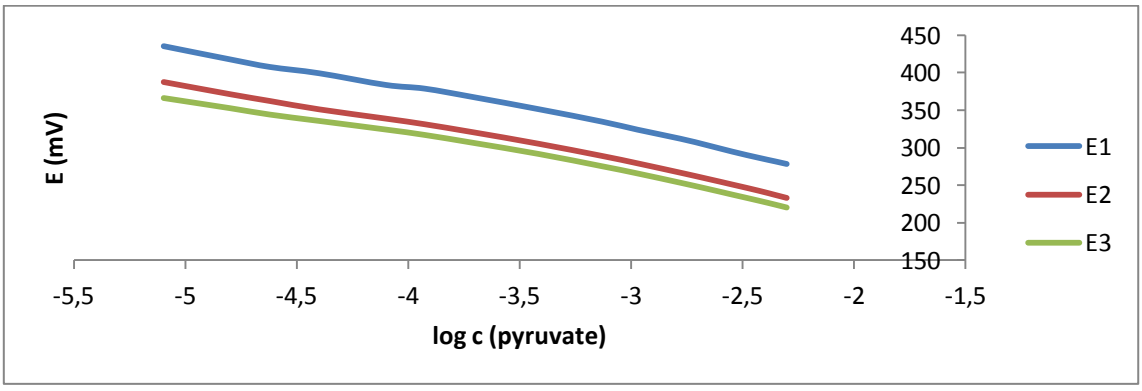
Every day before using the electrodes the hydration of membranes was done. Sensors were immersed in deionized water for 20 minutes.

After that, 25 mL of deionized water was measured into the beaker and put on the magnetic stirrer. Prepared electrodes and reference electrode were immersed in the deionized water. Several additions of standard pyruvate solution were done using micropipettes. Concentration of pyruvate solution varied from 4.00×10^{-8} mol.dm^{-3} to 4.20×10^{-3} mol.dm^{-3}. When the signal became stable (±0.1 mV), the potential was recorded.

Between calibrations of the electrodes and after the last calibration of the day the cleaning of electrodes was done. Sensors were immersed in deionized water for 30 minutes, after the first 15 minutes the water was changed.

Calibration plots were made from obtained data. The potential response of the electrodes was plotted vs. the logarithm of the pyruvate solution concentration.

Figure 14: Calibration plot of sensor with membrane 1.



The sensor characteristics were calculated as the average ± standard deviation of values obtained from 4 calibrations.

4.3.2. pH influence evaluation

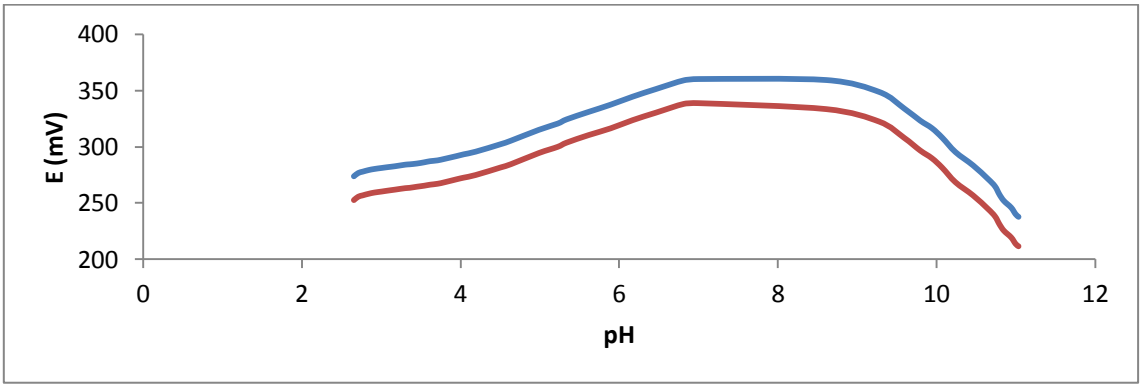
Reilley diagrams were used for evaluation of pH influence on electrode response. The aim was to find pH range, in which the change of pH does not influence the measured potential significantly.

Prior to the study of pH influence on the ion selective electrode, the pH of the glass electrode was calibrated every day with standard solutions of pH 4, pH 7 and pH 9. The potential of pH electrode was plotted as a function of pH.

Pyruvate solution in concentration 1.00×10^{-4} mol.dm^{-3} was prepared. 200 mL of this solution was measured into a beaker and pH electrode, reference electrode and working electrodes were immersed in this solution. The small amount of concentrated sulfuric acid (96%) was added to reduce the pH value to 2. Then the small amount of concentrated sodium hydroxide was added drop by drop to change the potential of pH electrode about 10 mV. The potential of all electrodes was recorded for each variation of pH. Sodium hydroxide was added until the value of pH reached 11.

The potential of studied electrodes was plotted as a function of pH.

Figure 15: Reilley diagram for sensors with membrane 1.



4.3.3. Interferences – sensor selectivity determination

Selectivity of membranes 1, 2 and 3 was studied. Fixed interference method (FIM) from the IUPAC recommendations was used.

The potential of the electrodes (ISE cell) is measured for solutions of constant activity of the interfering ion, a_B, and varying activity of the primary ion, a_A. The emf values obtained are plotted vs. the logarithm of the activity of the primary ion. The intersection of the extrapolated linear portions of this plot indicates the value of a_A that is to be used to calculate $K_{A,B}^{pot}$ from the following equation (28):

$$\mathrm{K_{AB}^{pot}} = \mathrm{a_A}/(\mathrm{a_B})^{\mathrm{z_A}/\mathrm{z_B}} \qquad (10)$$

where both z_A and z_B are charge numbers of ions A and B and have the same signs, positive or negative.

Standard pyruvate solution in concentration 1.00×10^{-2} mol.dm^{-3} and standard solutions of interfering ions in concentration 1.00×10^{-3} mol.dm^{-3} were prepared. 25 mL of interfering ion solution was measured into the beaker and put on the magnetic stirrer. Working electrodes and reference electrode were immersed in the interfering ion solution. Several additions of standard pyruvate solution were done using micropipettes. Concentration of pyruvate solution varied from 8.00×10^{-6} mol.dm^{-3} to 5.00×10^{-3} mol.dm^{-3}. When the signal became stable (±0.1mV), the potential was recorded.

Table 3 indicates a list of possible interfering compounds studied as well as their concentration values.

The potentiometric selectivity coefficients $K_{A,B}^{pot}$ were calculated according to the equation 9 listed above.

Table 3: List of potential interfering ions.

INTERFERENT		c (mol/dm^{-3})
Sodium Chloride	NaCl	0.000939
Sodium Sulfite Anhydrous	Na_2SO_3	0.000931
Sodium Sulfate Anhydrous	Na_2SO_4	0.001007
Sodium Carbonate Anhydrous	Na_2CO_3	0.001000
Sodium Phosphate Dibasic Dihydrate	$Na_2HPO_4.2H_2O$	0.000970
Sodium Acetate Anhydrous	CH_3COONa	0.001009
di-Sodium Tartrate Dihydrate	$C_4H_4Na_2O_6.2H2O$	0.001001
tri-Sodium Citrate Dihydrate	$C_6H_5Na_3O_7.2H2O$	0.001005

5. Results

To evaluate the characteristics of the different prepared membranes a set of calibrations curves were done in solutions without the ionic strength adjustment. In table 4 (Attachment 1, p. 57) are summarized the working characteristics values obtained for the prepared electrodes.

In order to evaluate the influence of the composition of the outer filling solution on the electrodes response some calibrations were made choosing as electrolyte a solution of sodium sulfate 0.033 mol.dm^{-3} instead of a commercial filling solution (NaCl 0.1 mol.dm^{-3}).

For comparison purposes the same reference electrode body was used but with sodium sulfate solution (0.033 mol.dm^{-3}) as outer filling (see Table 5) for calibrations of sensors with membrane 2 and 3.

Table 5: Characteristics of membrane 2 and 3 with different reference electrodes.

Reference	**Membrane 2**		
electrode	**Slope** (mV/dec)	**R^2**	**LLLR** (mol/l)
A	$-62.3 \pm 0.3_{n=2}$	$0.9948 \pm 0.0003_{n=2}$	$(2.39 \pm 0.00) \times 10^{-5}{}_{n=2}$
B	$-48.6 \pm 1.0_{n=2}$	$0.9954 \pm 0.0007_{n=2}$	$(7.40 \pm 0.00) \times 10^{-4}{}_{n=2}$
Reference	**Membrane 3**		
electrode	**Slope** (mV/dec)	**R^2**	**LLLR** (mol/l)
A	$-41.7 \pm 2.5_{n=6}$	$0.9993 \pm 0.0001_{n=2}$	$(7.99 \pm 0.00) \times 10^{-5}{}_{n=2}$
B	$-25.1 \pm 0.7_{n=4}$	$0.9949 \pm 0.0002_{n=4}$	$(7.99 \pm 0.00) \times 10^{-5}{}_{n=2}$

A - RE with commercial inner filling and outer solution of sodium sulfate (0.033 M).
B - RE with commercial inner and outer filling.

It was noticed that the sodium sulfate as outer filling solution allows a better response concerning to slope and LLLR. The composition of the commercial outer solution looks to behave as an interfering for the electrodes with membrane 2 and 3. Reference electrode with outer filling solution of sodium sulfate (0.033 mol.dm^{-3}) was chosen for next calibrations.

5.1. Evaluation of the potentiometric sensors

After choosing the outer filling solution for reference electrodes, all membranes were evaluated in aqueous solution of sodium pyruvate without ionic strength and pH adjustment.

Three different membranes with 2-NPPE as solvent mediator were prepared - membrane without additive, membrane with anionic and membrane with cationic additive - to see if there are any differences in response (Table 6). In the same table are represented the values obtained for the calibration parameters studied.

Table 6: Composition and characteristics of pyruvate sensors under study.
LOD – limit of detection, LLLR – lower limit of linear range.

Sensor:	1	2	3
Membrane composition:			
MnTPPBr [a)] (wt. %)	1.00	0.99	1.00
KTpClPB [b)] (mol %)	–	10.22	–
TOABr [c)] (mol %)	–	–	10.30
2-NPPE [d)] (wt. %)	66.73	66.64	66.73
DBP [e)] (wt. %)	–	–	–
2-FNDPE [f)] (wt. %)	–	–	–
DBS [g)] (wt. %)	–	–	–
PVC [h)] (wt. %)	32.27	32.37	32.27
Performance:			
Slope (mV/dec)	$-56.8 \pm 5.7_{n=9}$	$-76.4 \pm 1.2_{n=9}$	$-47.2 \pm 2.0_{n=9}$
Reproducibility:			
– between calibrations	$-54.6 \pm 6.4_{n=3}$	$-77.4 \pm 1.6_{n=3}$	$-47.5 \pm 1.9_{n=3}$
– between sensors	$-52.5 \pm 4.3_{n=3}$	$-77.4 \pm 1.5_{n=3}$	$-46.3 \pm 0.5_{n=3}$
R^2	$0.9940 \pm 0.0015_{n=9}$	$0.9945 \pm 0.0015_{n=9}$	$0.9937 \pm 0.0032_{n=9}$
LOD (mol/l)	$(1.49 \pm 0.25) \times 10^{-4}{}_{n=3}$	$(7.29 \pm 0.12) \times 10^{-5}{}_{n=3}$	$(2.19 \pm 0.70) \times 10^{-5}{}_{n=3}$
LLLR (mol/l)	$(1.65 \pm 0.34) \times 10^{-4}{}_{n=3}$	$(8.10 \pm 0.00) \times 10^{-5}{}_{n=3}$	$(5.50 \pm 0.90) \times 10^{-5}{}_{n=3}$

Membranes showed different behavior as was expected. M1 without additive showed slope (-56.8 ± 5.7 mV/dec) closest to the theoretical value (-59 mV), but it was varying quite a lot during calibrations. M2 with anionic additive showed super-

Nernstian slope (-76.4 ± 1.2 mV/dec), that means higher sensitivity for pyruvate. M3 with cationic additive showed sub-Nernstian slope (-47.2 ± 2.0 mV/dec).

Membrane 2 showed the best behavior – higher sensitivity than membrane 1 and 3, good reproducibility and small difference between LOD and LLLR values that means less interferences. Membrane 2 can be interesting for next experiments.

Figure 16: Calibration curves of sensors with membrane 1.

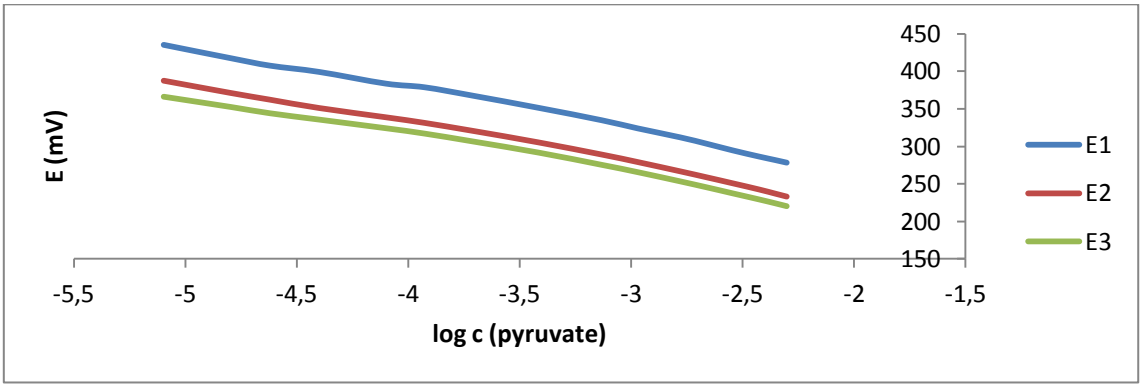


Figure 17: Calibration curves of sensors with membrane 2.

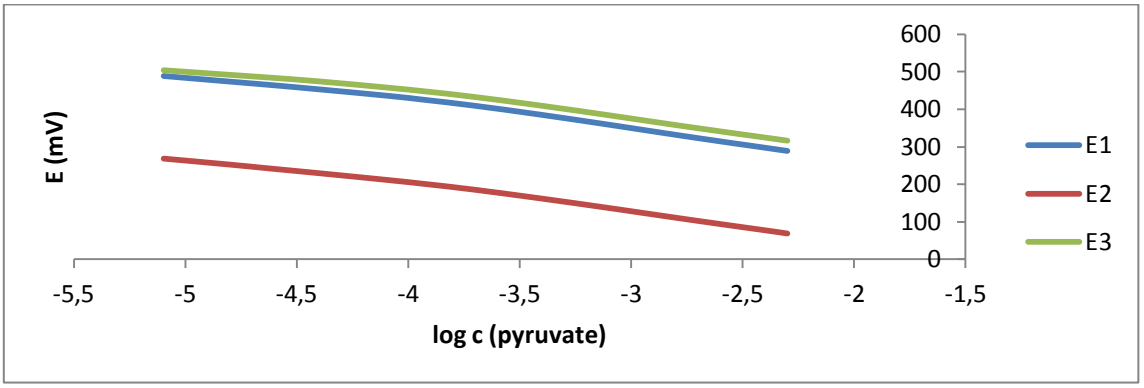
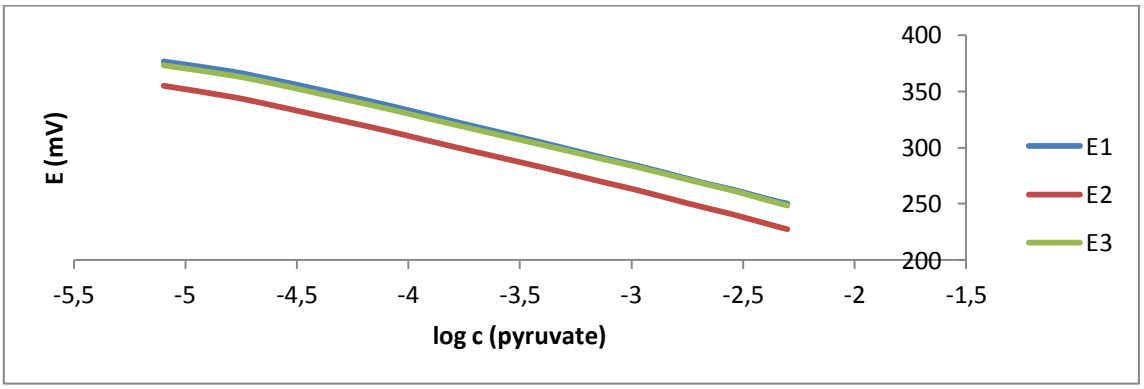


Figure 18: Calibration curves of sensors with membrane 3.



Membranes with the same solvent mediator (2-NPPE) but with different proportion of additive and ionophore were prepared to see if there are any changes in response characteristics (Table 7 and Table 8).

Table 7: Composition and characteristics of pyruvate sensors under study.
LOD – limit of detection, LLLR – lower limit of linear range.

Sensor:	2.1	2	2.2
Membrane composition:			
MnTPPBr [a)] (wt. %)	0.94	0.99	0.98
KTpClPB [b)] (mol %)	7.02	10.22	21.19
TOABr [c)] (mol %)	–	–	–
2-NPPE [d)] (wt. %)	67.87	66.64	66.38
DBP [e)] (wt. %)	–	–	–
2-FNDPE [f)] (wt. %)	–	–	–
DBS [g)] (wt. %)	–	–	–
PVC [h)] (wt. %)	31.19	32.37	32.64
Performance:			
Slope (mV/dec)	$-86.2 \pm 2.7_{n=9}$	$-76.4 \pm 1.2_{n=9}$	$-85.7 \pm 5.1_{n=9}$
Reproducibility:			
– between calibrations	$-85.5 \pm 3.4_{n=3}$	$-77.4 \pm 1.6_{n=3}$	$-87.7 \pm 6.2_{n=3}$
– between sensors	$-82.9 \pm 1.8_{n=3}$	$-77.4 \pm 1.5_{n=3}$	$-92.1 \pm 3.3_{n=3}$
R^2	$0.9948 \pm 0.0009_{n=9}$	$0.9945 \pm 0.0015_{n=9}$	$0.9945 \pm 0.0015_{n=9}$
LOD (mol/l)	$(3.42 \pm 0.30) \times 10^{-5}{}_{n=3}$	$(7.29 \pm 0.12) \times 10^{-5}{}_{n=3}$	$(2.22 \pm 0.13) \times 10^{-5}{}_{n=3}$
LLLR (mol/l)	$(5.66 \pm 1.73) \times 10^{-5}{}_{n=3}$	$(8.10 \pm 0.00) \times 10^{-5}{}_{n=3}$	$(4.44 \pm 0.00) \times 10^{-5}{}_{n=3}$

All membranes with anionic additive showed super-Nernstian response, that means higher sensitivity for pyruvate. Surprisingly both membranes with lower and higher proportion of additive/ionophore in the membrane showed higher slopes than membrane 2.

Figure 19: Calibration curves of sensors with membrane 2.1.

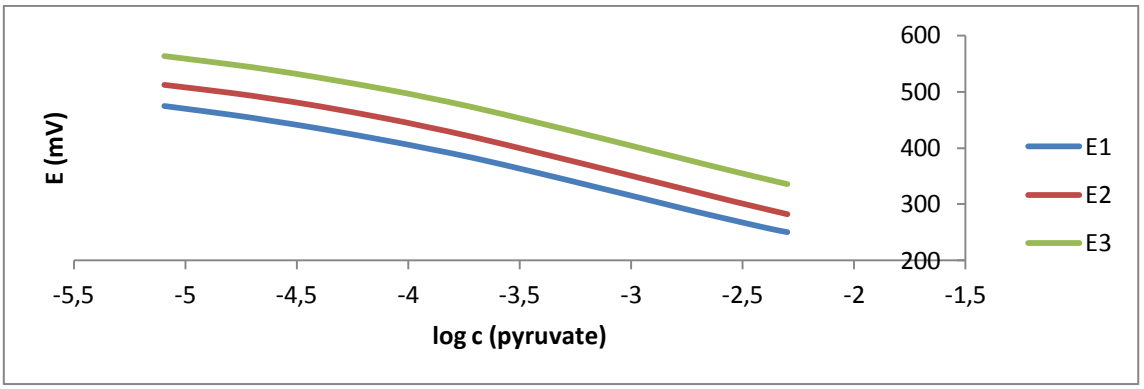


Figure 20: Calibration curves of sensors with membrane 2.2.

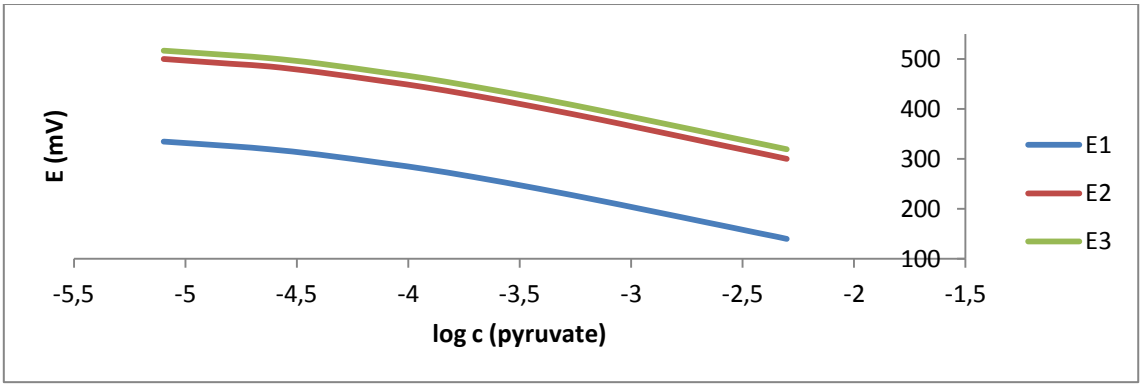


Table 8: Composition and characteristics of pyruvate sensors under study.
LOD – limit of detection, LLLR – lower limit of linear range.

Sensor:	3.1	3	3.2
Membrane composition:			
MnTPPBr [a)] (wt. %)	0.95	1.00	0.97
KTpClPB [b)] (mol %)	–	–	–
TOABr [c)] (mol %)	4.99	10.30	19.46
2-NPPE [d)] (wt. %)	68.18	66.73	66.96
DBP [e)] (wt. %)	–	–	–
2-FNDPE [f)] (wt. %)	–	–	–
DBS [g)] (wt. %)	–	–	–
PVC [h)] (wt. %)	30.78	32.27	32.07
Performance:			
Slope (mV/dec)	$-59.7 \pm 7.7_{n=9}$	$-47.2 \pm 2.0_{n=9}$	$-64.5 \pm 3.0_{n=6}$
Reproducibility:			
– between calibrations	$-59.5 \pm 8.2_{n=3}$	$-47.5 \pm 1.9_{n=3}$	$-65.0 \pm 3.2_{n=3}$
– between sensors	$-61.6 \pm 0.54_{n=3}$	$-46.3 \pm 0.5_{n=3}$	$-63.0 \pm 0.8_{n=2}$
R^2	$0.9957 \pm 0.0006_{n=9}$	$0.9937 \pm 0.0032_{n=9}$	$0.9966 \pm 0.0018_{n=6}$
LOD (mol/l)	$(4.05 \pm 0.66) \times 10^{-5}{}_{n=3}$	$(2.19 \pm 0.70) \times 10^{-5}{}_{n=3}$	$(5.26 \pm 0.48) \times 10^{-5}{}_{n=3}$
LLLR (mol/l)	$(9.31 \pm 1.72) \times 10^{-5}{}_{n=3}$	$(5.50 \pm 0.90) \times 10^{-5}{}_{n=3}$	$(9.50 \pm 1.33) \times 10^{-5}{}_{n=3}$

New membranes with cationic additive showed very similar behavior. Slopes of membrane with lower and membrane with higher proportion of additive/ionophore were surprisingly higher than slope of membrane 3. LOD and LLLR values have more or less the same interval, but bigger difference between LOD and LLLR values means more interferences.

Figure 21: Calibration curves of sensors with membrane 3.1.

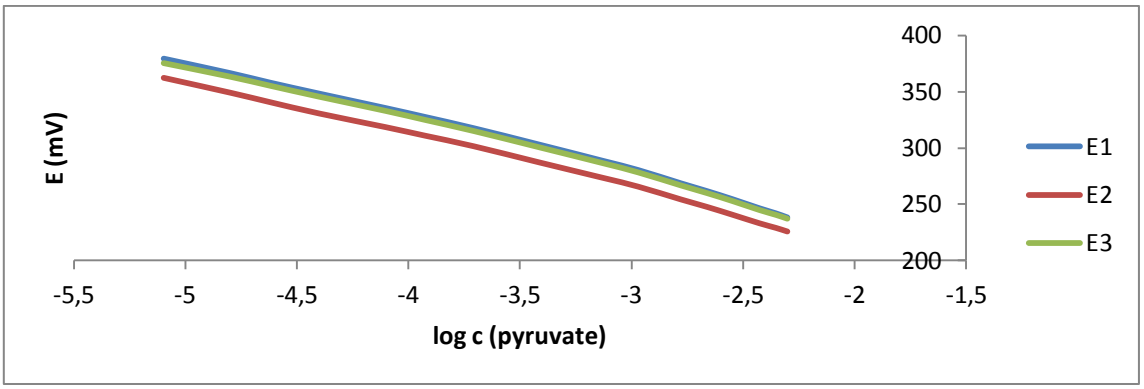
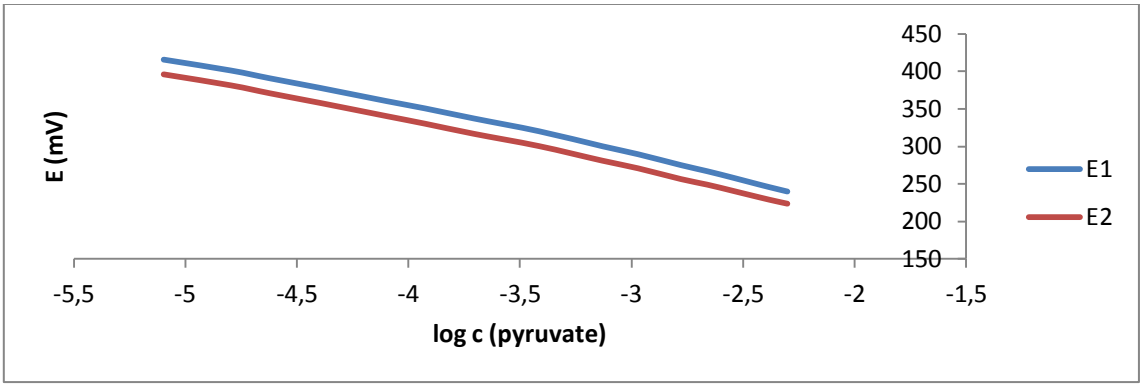
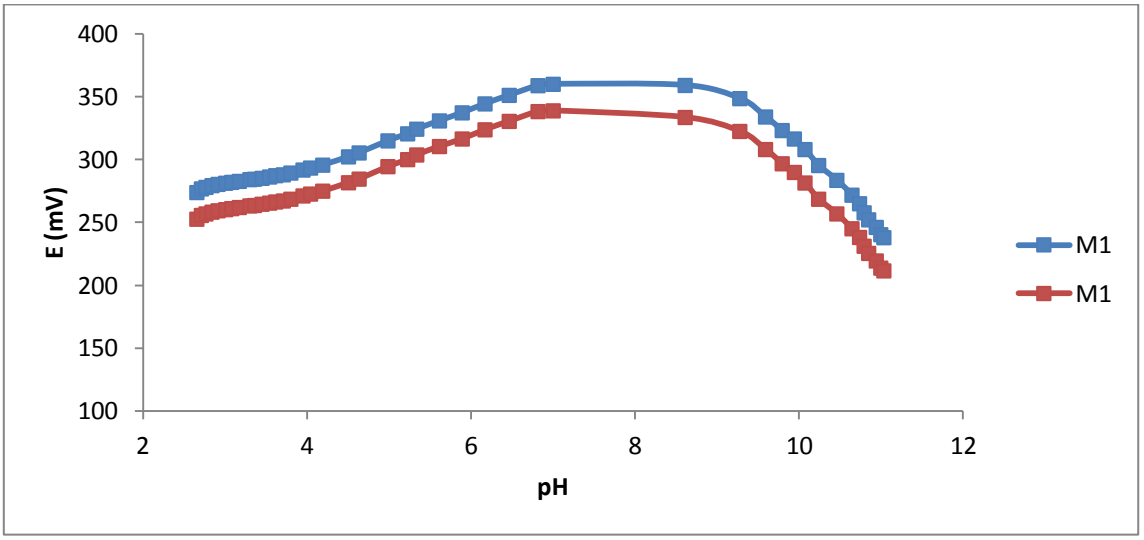


Figure 22: Calibration curves of sensors with membrane 3.2.



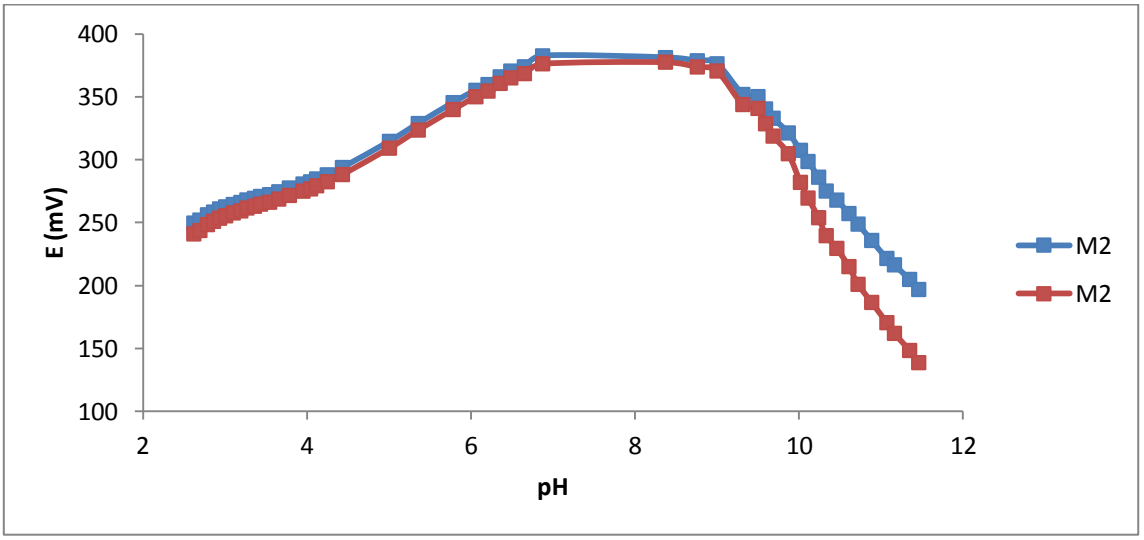
The pH influence evaluation (Reilley diagrams) described in chapter 4.3.2. was done with membrane 1, 2 and 3 in pH range from 2 to 11. pH value of aqueous solution of sodium pyruvate ($1.00x10^{-4}$ $mol.dm^{-3}$) was 5.61.

Figure 23: Evaluation of pH influence for sensors with membrane 1.



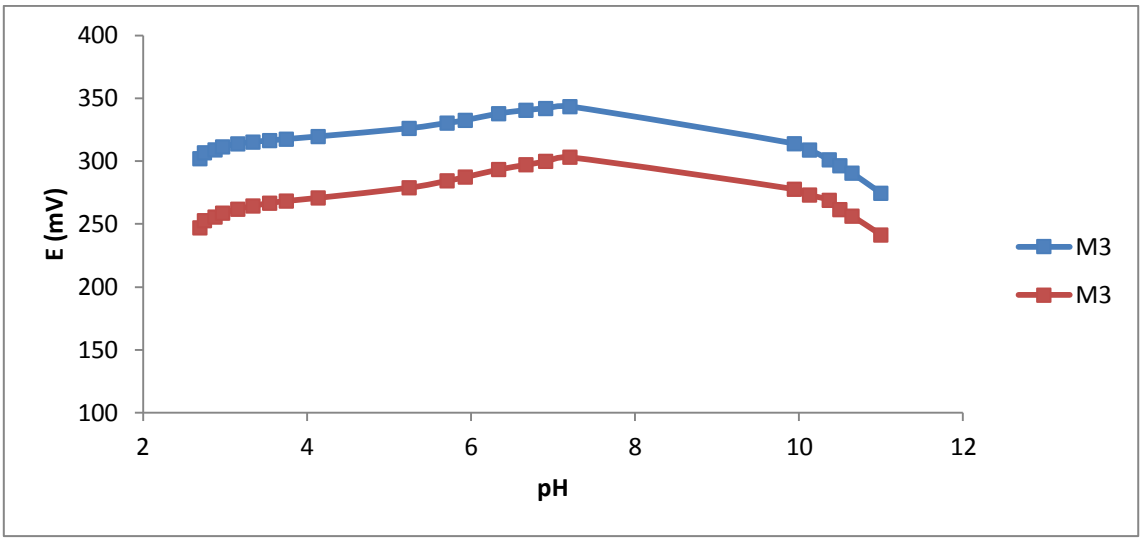
The pH range when the response was not significantly affected was 7.0-8.6 for membrane without additive.

Figure 24: Evaluation of pH influence for sensors with membrane 2.



The pH range when the response was not significantly affected was 6.9-8.4 for membrane with anionic additive.

Figure 25: Evaluation of pH influence for sensors with membrane 3.



For membrane with cationic additive was not possible to determinate exactly the pH range when the response was not significantly affected.

In one of next steps will be necessary to evaluate buffer solutions to find the best one for pyruvate determination. The improvement of response of sensors is expected.

Next step was testing of different solvent mediators. New membranes without additive were prepared. Dibutyl phthalate (DBP, ε_r = 6.4 (48)) was used as an apolar solvent, 2-Fluorophenyl 2-nitrophenyl ether (2-FNDPE, ε_r = 50 (49)) was used as a polar solvent. See Table 9 with results.

Table 9: Composition and characteristics of pyruvate sensors under study.
LOD – limit of detection, LLLR – lower limit of linear range.

Sensor:	4.1	1	5.1
Membrane composition:			
MnTPPBr [a] (wt. %)	0.99	1.00	0.97
KTpClPB [b] (mol %)	–	–	–
TOABr [c] (mol %)	–	–	–
2-NPPE [d] (wt. %)	–	66.73	–
DBP [e] (wt. %)	67.02	–	–
2-FNDPE [f] (wt. %)	–	–	67.00
DBS [g] (wt. %)	–	–	–
PVC [h] (wt. %)	31.99	32.27	32.03
Performance:			
Slope (mV/dec)	$-78.3 \pm 4.0_{n=9}$	$-56.8 \pm 5.7_{n=9}$	$-72.2 \pm 2.2_{n=9}$
Reproducibility:			
– between calibrations	$-78.1 \pm 4.3_{n=3}$	$-54.6 \pm 6.4_{n=3}$	$-71.7 \pm 2.3_{n=3}$
– between sensors	$-75.5 \pm 0.9_{n=3}$	$-52.5 \pm 4.3_{n=3}$	$-70.7 \pm 2.3_{n=3}$
R^2	$0.9958 \pm 0.0004_{n=9}$	$0.9940 \pm 0.0015_{n=9}$	$0.9957 \pm 0.0022_{n=9}$
LOD (mol/l)	$(6.64 \pm 0.32) \times 10^{-5}{}_{n=3}$	$(1.49 \pm 0.25) \times 10^{-4}{}_{n=3}$	$(1.87 \pm 0.31) \times 10^{-4}{}_{n=3}$
LLLR (mol/l)	$(8.10 \pm 0.00) \times 10^{-5}{}_{n=3}$	$(1.65 \pm 0.34) \times 10^{-4}{}_{n=3}$	$(2.31 \pm 0.94) \times 10^{-4}{}_{n=3}$

Membrane without additive with new solvent mediators showed better slope than membrane 1. Increase of slope means increase of sensitivity to the pyruvate. Membrane 4.1 with an apolar solvent showed also decrease of LOD and LLLR values that means improvement of detection limits.

Figure 26: Calibration curves of sensors with membrane 4.1.

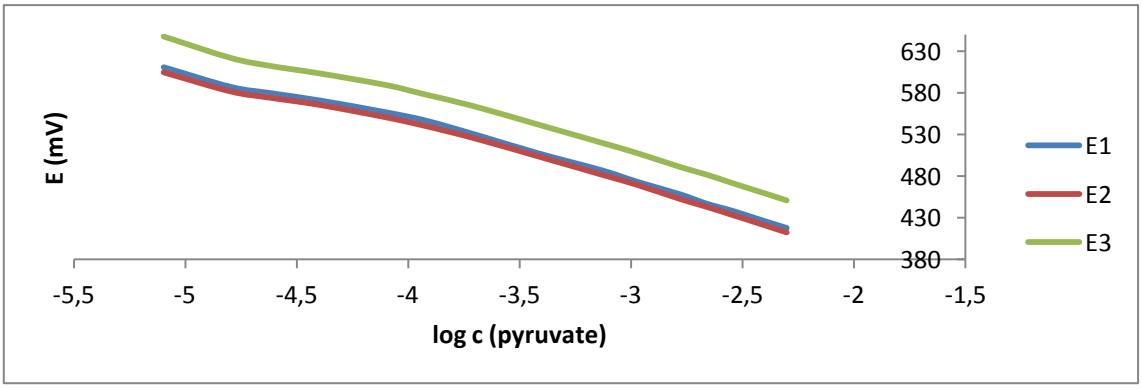
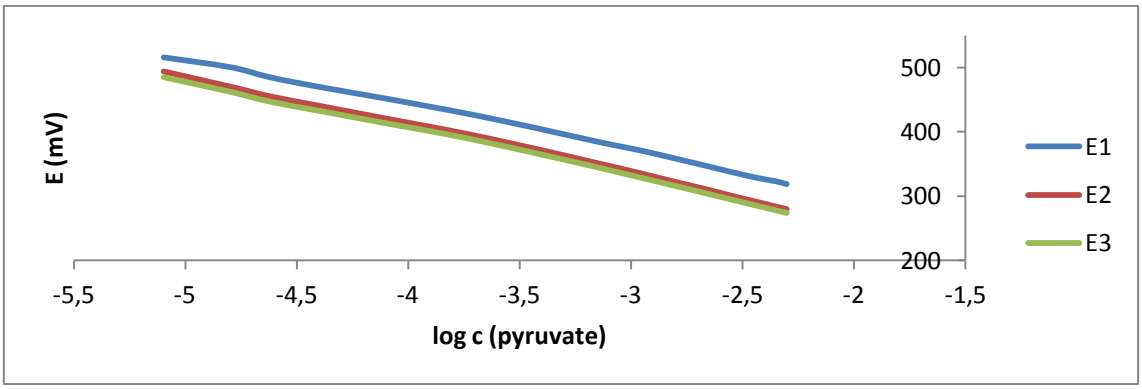


Figure 27: Calibration curves of sensors with membrane 5.1.



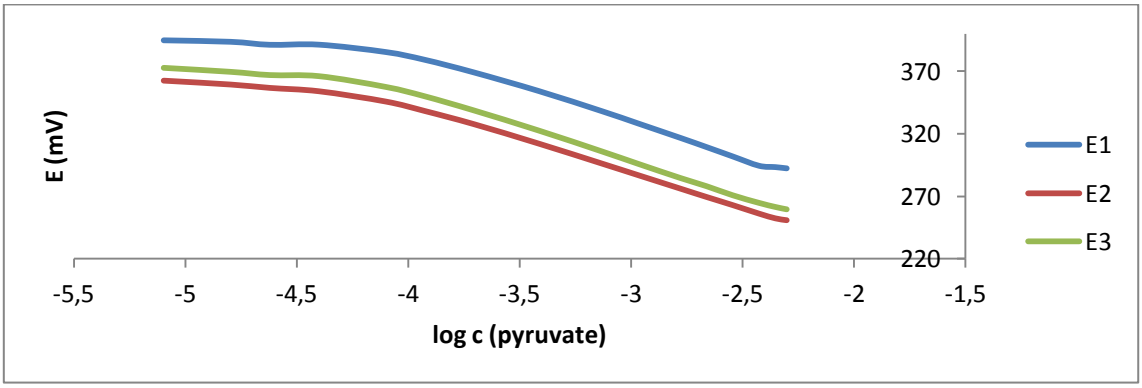
For verification of these results membrane without additive with another apolar solvent mediator was prepared. Dibutyl sebacate (DBS, ε_r = 4.5 (48)) was used as more apolar solvent mediator. Results are summarized in Table 10.

Table 10: Composition and characteristics of pyruvate sensors under study.
LOD – limit of detection, LLLR – lower limit of linear range.

Sensor:	6.1	4.1	1
Membrane composition:			
MnTPPBr [a] (wt. %)	1.01	0.99	1.00
KTpClPB [b] (mol %)	–	–	–
TOABr [c] (mol %)	–	–	–
2-NPPE [d] (wt. %)	–	–	66.73
DBP [e] (wt. %)	–	67.02	–
2-FNDPE [f] (wt. %)	–	–	–
DBS [g] (wt. %)	65.90	–	–
PVC [h] (wt. %)	33.09	31.99	32.27
Performance:			
Slope (mV/dec)	– 53.6 ± 3.0 $_{n=9}$	– 78.3 ± 4.0 $_{n=9}$	– 56.8 ± 5.7 $_{n=9}$
Reproducibility:			
– between calibrations	– 54.9 ± 2.9 $_{n=3}$	– 78.1 ± 4.3 $_{n=3}$	– 54.6 ± 6.4 $_{n=3}$
– between sensors	– 53.9 ± 3.4 $_{n=3}$	– 75.5 ± 0.9 $_{n=3}$	– 52.5 ± 4.3 $_{n=3}$
R^2	0.9950 ± 0.0025 $_{n=9}$	0.9958 ± 0.0004 $_{n=9}$	0.9940 ± 0.0015 $_{n=9}$
LOD (mol/l)	(5.92 ± 0.46) x 10^{-5} $_{n=3}$	(6.64 ± 0.32) x 10^{-5} $_{n=3}$	(1.49 ± 0.25) x 10^{-4} $_{n=3}$
LLLR (mol/l)	(8.08 ± 0.00) x 10^{-5} $_{n=3}$	(8.10 ± 0.00) x 10^{-5} $_{n=3}$	(1.65 ± 0.34) x 10^{-4} $_{n=3}$

Using of DBS didn´t verified the idea of improving the properties of membrane, but showed importance of using apolar solvent mediator for improvement of detection limits (LOD, LLLR).

Figure 28: Calibration curves of sensors with membrane 6.1.



Idea of improving of detection limits was verified with membrane 2 (anionic additive) that showed better behavior than other membranes and also with membrane 3 (cationic additive). See results in Table 11 and Table 12.

Table 11: Composition and characteristics of pyruvate sensors under study.
LOD – limit of detection, LLLR – lower limit of linear range.

Sensor:	4.2	2
Membrane composition:		
MnTPPBr [a] (wt. %)	0.97	0.99
KTpClPB [b] (mol %)	9.93	10.22
TOABr [c] (mol %)	–	–
2-NPPE [d] (wt. %)	–	66.64
DBP [e] (wt. %)	68.16	–
2-FNDPE [f] (wt. %)	–	–
DBS [g] (wt. %)	–	–
PVC [h] (wt. %)	30.87	32.37
Performance:		
Slope (mV/dec)	$-60.1 \pm 4.0_{n=9}$	$-76.4 \pm 1.2_{n=9}$
Reproducibility:		
– between calibrations	$-60.5 \pm 4.7_{n=3}$	$-77.4 \pm 1.6_{n=3}$
– between sensors	$-63.3 \pm 1.2_{n=3}$	$-77.4 \pm 1.5_{n=3}$
R^2	$0.9936 \pm 0.0007_{n=9}$	$0.9945 \pm 0.0015_{n=9}$
LOD (mol/l)	$(1.07 \pm 0.01) \times 10^{-5}_{n=3}$	$(7.29 \pm 0.12) \times 10^{-5}_{n=3}$
LLLR (mol/l)	$(1.11 \pm 0.00) \times 10^{-5}_{n=3}$	$(8.10 \pm 0.00) \times 10^{-5}_{n=3}$

According the results it is possible to conclude that apolar solvent mediator doesn´t produce better results than 2-NPPE for membrane with anionic additive. Slope showed more Nernstian value (– 60.5 ± 4.7 mV/dec), but lower than slope of membrane 2, and also values of LOD and LLLR slightly increased.

Figure 29: Calibration curves of sensors with membrane 4.2.

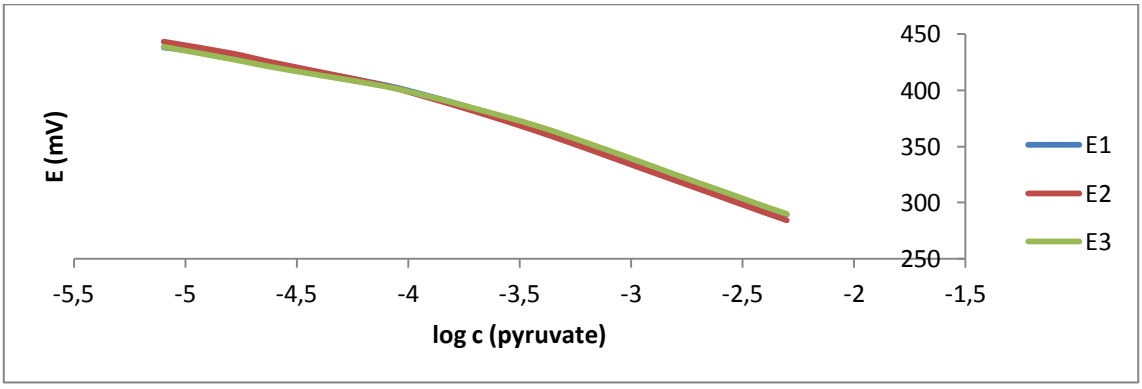


Table 12: Composition and characteristics of pyruvate sensors under study.
LOD – limit of detection, LLLR – lower limit of linear range.

Sensor:	4.3	3.2	5.3
Membrane composition:			
MnTPPBr [a)] (wt. %)	0.99	0.97	0.94
KTpClPB [b)] (mol %)	–	–	–
TOABr [c)] (mol %)	20.37	19.46	20.10
2-NPPE [d)] (wt. %)	–	66.96	–
DBP [e)] (wt. %)	66.21	–	–
2-FNDPE [f)] (wt. %)	–	–	68.56
DBS [g)] (wt. %)	–	–	–
PVC [h)] (wt. %)	32.80	32.07	30.50
Performance:			
Slope (mV/dec)	$-68.3 \pm 3.1_{n=9}$	$-64.5 \pm 3.0_{n=6}$	$-71.4 \pm 1.8_{n=9}$
Reproducibility:			
– between calibrations	$-66.4 \pm 3.2_{n=3}$	$-65.0 \pm 3.2_{n=3}$	$-72.2 \pm 1.8_{n=3}$
– between sensors	$-66.5 \pm 3.7_{n=3}$	$-63.0 \pm 0.8_{n=2}$	$-72.5 \pm 2.2_{n=3}$
R^2	$0.9959 \pm 0.0013_{n=9}$	$0.9966 \pm 0.0018_{n=6}$	$0.9983 \pm 0.0016_{n=9}$
LOD (mol/l)	$(1.03 \pm 0.01) \times 10^{-4}{}_{n=3}$	$(5.26 \pm 0.48) \times 10^{-5}{}_{n=3}$	$(1.59 \pm 0.05) \times 10^{-4}{}_{n=3}$
LLLR (mol/l)	$(1.05 \pm 0.17) \times 10^{-4}{}_{n=3}$	$(9.50 \pm 1.33) \times 10^{-5}{}_{n=3}$	$(1.98 \pm 0.00) \times 10^{-4}{}_{n=3}$

Apolar solvent mediator (DBP) showed more less the same value of slope as basic membrane but also no improvement of detection limits. Polar solvent mediator (2-FNDPE) showed improvement of slope and increasing of LOD and LLLR.

According the results it is possible to conclude that both apolar and polar solvent mediator did not produce better results than 2-NPPE for membrane with cationic additive.

Figure 30: Calibration curves of sensors with membrane 4.3.

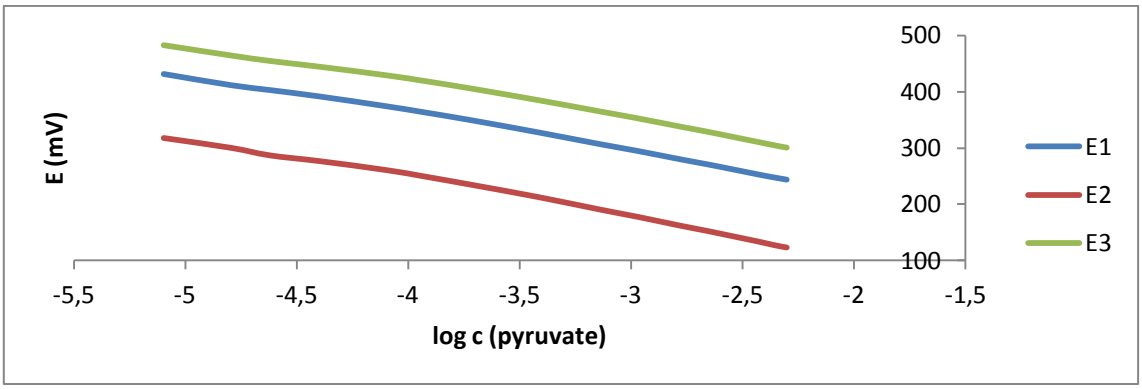
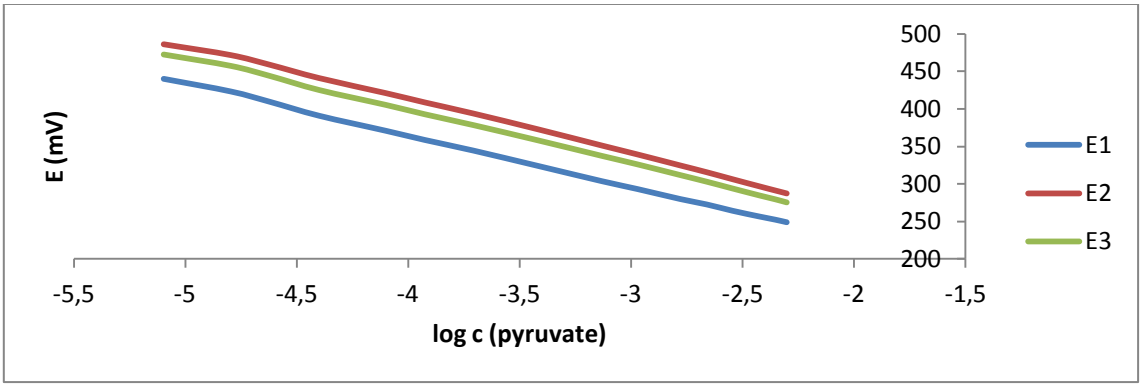


Figure 31: Calibration curves of sensors with membrane 5.3.



Another measurement with membrane 4.1, 4.3, 5.1 and 5.2 was done. It was recorded how the potential is changing during time. Finally it was found that it takes a long time to reach equilibrium and probably the values of LOD and LLLR are incorrect. Because of that these membranes probably will not be used for next testing.

During calibrations of membranes with new solvent mediators (4.1, 4.2, 4.3, 5.1 and 5.3) was recognized that aqueous solution after hydration and cleaning of the electrodes was colored. Light green color of solution may be caused by washing out of metalloporphyrin, because DBP is colorless and 2-FNDPE is yellow. Probably we can use it as explanation why the values of slope were varying during calibrations of sensors. In next steps will be necessary make the evaluation of life time of all prepared membranes.

5.2. Evaluation of the selectivity of the potentiometric sensors

Selectivity of ISE is one of the most important characteristic expressed by potentiometric selectivity coefficient, $K_{A,B}^{pot}$. Used fixed interference method is described in chapter 4.3.3.

$K_{A,B}^{pot}$ defines the ability of any ion-selective electrode to distinguish a particular ion, i.e., primary ion from the others. The smaller the value of $K_{A,B}^{pot}$, the greater the electrode´s preference for the primary ion, A (5).

The selectivity of sensors to pyruvate was studied with membrane 1, 2 and 3. The anions (Cl^-, SO_3^-, SO_4^-, HPO_4^{2-}, acetate, tartrate, citrate) presented in wine (50–52) was chosen. Results are summarized in Table 13.

Table 13: Potentiometric selectivity coefficients for pyruvate using fixed interference method. Sensors with membrane 1, 2 and 3.

INTERFERENT	$K^{pot}_{Pyruvate, Interferent}$		
	Membrane 1.1 (without additive)	**Membrane 2** (anionic additive)	**Membrane 3** (cathionic additive)
Sodium Chloride	$5.44x10^{-1}$	$4.10x10^{-1}$	$5.58x10^{-1}$
Sodium Sulfite	$2.20x10^{-2}$	$1.97x10^{-2}$	$2.37x10^{-2}$
Sodium Sulfate	$2.42x10^{-3}$	$2.10x10^{-3}$	$4.61x10^{-3}$
Sodium Carbonate	*	$1.89x10^{-2}$	$1.84x10^{-2}$
Sodium Phosphate Dibasic	$2.03x10^{-2}$	$1.04x10^{-2}$	$1.12x10^{-2}$
Sodium Acetate	$1.87x10^{-1}$	$1.62x10^{-1}$	$1.84x10^{-1}$
di-Sodium Tartrate	$1.89x10^{-2}$	$1.99x10^{-2}$	$1.82x10^{-2}$
tri-Sodium Citrate	$5.57x10^{-3}$	$6.74x10^{-3}$	$6.94x10^{-3}$

* high interference, no value of K^{pot}

Carbonate showed very high interference for membrane 1, it wasn´t possible to quantify it from the graph. The selectivity coefficients obtained for all species except carbonate were lower than $5.58x10^{-1}$ that means absence of significant interferences.

6. Conclusion

This work describes development and evaluation of new potential pyruvate-selective electrodes.

Sensors with 5,10,15,20-Tetraphenyl-21*H*,23*H*-porphine manganese (III) chloride as the ionophore were prepared. Two lipophilic additives, cationic and anionic, and several solvent mediators, apolar and polar, were under study. Sensors with different composition of membrane were tested and proportions of membranes were changed to find sensors with optimal response characteristics.

Sensors that showed slopes closest to Nernstian response (-59 mV) were sensors with membrane 1 (– 56.8 ± 5.7 mV/dec), membrane 3.1 (– 59.7 ± 7.7 mV/dec), membrane 3.2 (– 64.5 ± 3.0 mV/dec) and membrane 4.2 (– 60.1 ± 4.0 mV/dec).

Membrane 2 with 2-Nitrophenyl phenyl ether as solvent mediator and Potassium tetrakis(2-chlorophenyl)borate as lipophilic additive showed the best detection limits: LOD (7.29 ± 0.12)x10^{-5} mol.dm^{-3} and LLLR (8.10 ± 0.00)x10^{-5} mol.dm^{-3}. Slope of this membrane was super-Nernstian (– 76.4 ± 1.2 mV/dec) and probably can be affected by appropriate pH adjustment.

The evaluation of selectivity with chosen anions didn´t show significant interferences except carbonate that showed very high interference for membrane 1. The selectivity coefficients (obtained by fixed interference method) were lower than 5.58x10^{-1} except carbonate where was not possible to quantify the selectivity coefficient for membrane 1 from the graph.

The pH influence was tested with membrane 1, 2 and 3 and in next steps will be necessary to find the best pH range for pyruvate determination.

The development of new pyruvate-selective electrode and new method for determination of pyruvate still continues. After this work the best electrode will be chosen to make an application in wine analysis. The potentiometric results will be validated against a reference method.

7. Závěr

Tato práce popisuje vývoj a testování nových elektrod, potenciálně selektivních pro pyruvát.

Připravené senzory obsahovaly jako ionofor chlorid 5,10,15,20-tetrafenyl-21*H*,23*H*-porfin manganitý. Byla testována dvě lipofilní aditiva, anionické a kationické, a několik nepolárních i polárních rozpouštědel. Složení jednotlivých membrán testovaných senzorů bylo upravováno tak, aby byly nalezeny elektrody s optimálními vlastnostmi.

Směrnice kalibračních křivek u elektrod s membránou 1 (-56,8 ± 5,7 mV/dec), membránou 3.1 (-59,7 ± 7,7 mV/dec), membránou 3.2 (-64,5 ± 3,0 mV/dec) a membránou 4.2 (-60,1 ± 4,0 mV/dec) byly nejblíže teoretické hodnotě směrnice kalibrační křivky (-59 mV) vycházející z Nernstovy rovnice.

Nejlepší detekční limity, LOD (7,29 ± 0,12)x10^{-5} mol.dm^{-3} a LLLR (8,10 ± 0,00)x10^{-5} mol.dm^{-3}, byly nalezeny u membrány 2, která jako rozpouštědlo obsahovala 2-nitrodifenylether a jako lipofilní aditivum tetrakis(2-chlorofenyl)borát draselný. Směrnice kalibrační křivky, která byla vyšší než teoretická hodnota -59 mV, může být dále ovlivněna vhodnou úpravou pH.

Během měření interferencí vybraných aniontů nebyly nalezeny významné interference s výjimkou uhličitanových aniontů pro membránu číslo 1. Koeficienty selektivity (získané použitím FIM) byly nižší než 5,58.10^{-1}, pouze u uhličitanových aniontů nebylo možné koeficient selektivity z grafu pro membránu číslo 1 vypočítat.

Vliv pH na byl testován s membránou 1, 2 a 3. V následujících krocích testování bude třeba určit optimální rozsah pH pro stanovení pyruvátu.

Vývoj nové pyruvát-selektivní elektrody a nové metody pro stanovení pyruvátu stále pokračuje. Po dokončení testování senzorů bude vybrán nejvhodnější pro aplikaci v analýze kontroly vína. Výsledky z potenciometrického stanovení budou validovány referenční metodou.

Attachment 1: Table 4 with composition and characteristics of pyruvate sensors under study.

LOD – limit of detection, LLLR – lower limit of linear range.

Sensor:	1	2	3
Membrane composition:			
MnTPPBr [a] (wt. %)	1.00	0.99	1.00
KTpClPB [b] (mol %)	–	10.22	–
TOABr [c] (mol %)	–	–	10.30
2-NPPE [d] (wt. %)	66.73	66.64	66.73
DBP [e] (wt. %)	–	–	–
2-FNDPE [f] (wt. %)	–	–	–
DBS [g] (wt. %)	–	–	–
PVC [h] (wt. %)	32.27	32.37	32.27
Performance:			
Slope (mV/dec)	$-56.8 \pm 5.7_{n=9}$	$-76.4 \pm 1.2_{n=9}$	$-47.2 \pm 2.0_{n=9}$
Reproducibility:			
– between calibrations	$-54.6 \pm 6.4_{n=3}$	$-77.4 \pm 1.6_{n=3}$	$-47.5 \pm 1.9_{n=3}$
– between sensors	$-52.5 \pm 4.3_{n=3}$	$-77.4 \pm 1.5_{n=3}$	$-46.3 \pm 0.5_{n=3}$
R^2	$0.9940 \pm 0.0015_{n=9}$	$0.9945 \pm 0.0015_{n=9}$	$0.9937 \pm 0.0032_{n=9}$
LOD (mol/l)	$(1.49 \pm 0.25) \times 10^{-4}{}_{n=3}$	$(7.29 \pm 0.12) \times 10^{-5}{}_{n=3}$	$(2.19 \pm 0.70) \times 10^{-5}{}_{n=3}$
LLLR (mol/l)	$(1.65 \pm 0.34) \times 10^{-4}{}_{n=3}$	$(8.10 \pm 0.00) \times 10^{-5}{}_{n=3}$	$(5.50 \pm 0.90) \times 10^{-5}{}_{n=3}$

a) 5,10,15,20-Tetraphenyl-21H,23H-porphine manganese (III) chloride

b) Potassium tetrakis(2-chlorophenyl)borate

c) Tetraoctylammonium bromide

d) 2-Nitrophenyl phenyl ether

e) Dibutyl phthalate

f) 2-Fluorophenyl 2-nitrophenyl ether

g) Dibutyl sebacate

h) Poly(vinylchloride)

Attachment 1: Table 4 with composition and characteristics of pyruvate sensors under study.

LOD – limit of detection, LLLR – lower limit of linear range.

Sensor:	2.1	2.2	3.1	3.2
Membrane composition:				
MnTPPBr [a] (wt. %)	0.94	0.98	0.95	0.97
KTpClPB [b] (mol %)	7.02	21.19	–	–
TOABr [c] (mol %)	–	–	4.99	19.46
2-NPPE [d] (wt. %)	67.87	66.38	68.18	66.96
DBP [e] (wt. %)	–	–	–	–
2-FNDPE [f] (wt. %)	–	–	–	–
DBS [g] (wt. %)	–	–	–	–
PVC [h] (wt. %)	31.19	32.64	30.78	32.07
Performance:				
Slope (mV/dec)	$-86.2 \pm 2.7_{n=9}$	$-85.7 \pm 5.1_{n=9}$	$-59.7 \pm 7.7_{n=9}$	$-64.5 \pm 3.0_{n=6}$
Reproducibility:				
– between calibrations	$-85.5 \pm 3.4_{n=3}$	$-87.7 \pm 6.2_{n=3}$	$-59.5 \pm 8.2_{n=3}$	$-65.0 \pm 3.2_{n=3}$
– between sensors	$-82.9 \pm 1.8_{n=3}$	$-92.1 \pm 3.3_{n=3}$	$-61.6 \pm 0.54_{n=3}$	$-63.0 \pm 0.8_{n=2}$
R^2	$0.9948 \pm 0.0009_{n=9}$	$0.9945 \pm 0.0015_{n=9}$	$0.9957 \pm 0.0006_{n=9}$	$0.9966 \pm 0.0018_{n=6}$
LOD (mol/l)	$(3.42 \pm 0.30) \times 10^{-5}{}_{n=3}$	$(2.22 \pm 0.13) \times 10^{-5}{}_{n=3}$	$(4.05 \pm 0.66) \times 10^{-5}{}_{n=3}$	$(5.26 \pm 0.48) \times 10^{-5}{}_{n=3}$
LLLR (mol/l)	$(5.66 \pm 1.73) \times 10^{-5}{}_{n=3}$	$(4.44 \pm 0.00) \times 10^{-5}{}_{n=3}$	$(9.31 \pm 1.72) \times 10^{-5}{}_{n=3}$	$(9.50 \pm 1.33) \times 10^{-5}{}_{n=3}$

a) 5,10,15,20-Tetraphenyl-21H,23H-porphine manganese (III) chloride

b) Potassium tetrakis(2-chlorophenyl)borate

c) Tetraoctylammonium bromide

d) 2-Nitrophenyl phenyl ether

e) Dibutyl phthalate

f) 2-Fluorophenyl 2-nitrophenyl ether

g) Dibutyl sebacate

h) Poly(vinylchloride)

Attachment 1: Table 4 with composition and characteristics of pyruvate sensors under study.
LOD – limit of detection, LLLR – lower limit of linear range.

Sensor:	4.1	4.2	4.3
Membrane composition:			
MnTPPBr [a] (wt. %)	0.99	0.97	0.99
KTpClPB [b] (mol %)	–	9.93	–
TOABr [c] (mol %)	–	–	20.37
2-NPPE [d] (wt. %)	–	–	–
DBP [e] (wt. %)	67.02	68.16	66.21
2-FNDPE [f] (wt. %)	–	–	–
DBS [g] (wt. %)	–	–	–
PVC [h] (wt. %)	31.99	30.87	32.80
Performance:			
Slope (mV/dec)	$-78.3 \pm 4.0_{n=9}$	$-60.1 \pm 4.0_{n=9}$	$-68.3 \pm 3.1_{n=9}$
Reproducibility:			
– between calibrations	$-78.1 \pm 4.3_{n=3}$	$-60.5 \pm 4.7_{n=3}$	$-66.4 \pm 3.2_{n=3}$
– between sensors	$-75.5 \pm 0.9_{n=3}$	$-63.3 \pm 1.2_{n=3}$	$-66.5 \pm 3.7_{n=3}$
R^2	$0.9958 \pm 0.0004_{n=9}$	$0.9936 \pm 0.0007_{n=9}$	$0.9959 \pm 0.0013_{n=9}$
LOD (mol/l)	$(6.64 \pm 0.32) \times 10^{-5}{}_{n=3}$	$(1.07 \pm 0.01) \times 10^{-5}{}_{n=3}$	$(1.03 \pm 0.01) \times 10^{-4}{}_{n=3}$
LLLR (mol/l)	$(8.10 \pm 0.00) \times 10^{-5}{}_{n=3}$	$(1.11 \pm 0.00) \times 10^{-5}{}_{n=3}$	$(1.05 \pm 0.17) \times 10^{-4}{}_{n=3}$

a) 5,10,15,20-Tetraphenyl-21H,23H-porphine manganese (III) chloride
b) Potassium tetrakis(2-chlorophenyl)borate
c) Tetraoctylammonium bromide
d) 2-Nitrophenyl phenyl ether
e) Dibutyl phthalate
f) 2-Fluorophenyl 2-nitrophenyl ether
g) Dibutyl sebacate
h) Poly(vinylchloride)

Attachment 1: Table 4 with composition and characteristics of pyruvate sensors under study.

LOD – limit of detection LLLR – lower limit of linear range.

Sensor:	5.1	5.2	6.1
Membrane composition:			
MnTPPBr [a)] (wt. %)	0.97	0.94	1.01
KTpClPB [b)] (mol %)	–	–	–
TOABr [c)] (mol %)	–	20.10	–
2-NPPE [d)] (wt. %)	–	–	–
DBP [e)] (wt. %)	–	–	–
2-FNDPE [f)] (wt. %)	67.00	68.56	–
DBS [g)] (wt. %)	–	–	65.9
PVC [h)] (wt. %)	32.03	30.50	33.09
Performance:			
Slope (mV/dec)	$-72.2 \pm 2.2_{n=9}$	$-71.4 \pm 1.8_{n=9}$	$-53.6 \pm 3.0_{n=9}$
Reproducibility:			
– between calibrations	$-71.7 \pm 2.3_{n=3}$	$-72.2 \pm 1.8_{n=3}$	$-54.9 \pm 2.9_{n=3}$
– between sensors	$-70.7 \pm 2.3_{n=3}$	$-72.5 \pm 2.2_{n=3}$	$-53.9 \pm 3.4_{n=3}$
R^2	$0.9957 \pm 0.0022_{n=9}$	$0.9983 \pm 0.0016_{n=9}$	$0.9950 \pm 0.0025_{n=9}$
LOD (mol/l)	$(1.87 \pm 0.31) \times 10^{-4}{}_{n=3}$	$(1.59 \pm 0.05) \times 10^{-4}{}_{n=3}$	$(5.92 \pm 0.46) \times 10^{-5}{}_{n=3}$
LLLR (mol/l)	$(2.31 \pm 0.94) \times 10^{-4}{}_{n=3}$	$(1.98 \pm 0.00) \times 10^{-4}{}_{n=3}$	$(8.08 \pm 0.00) \times 10^{-5}{}_{n=3}$

a) 5,10,15,20-Tetraphenyl-21H,23H-porphine manganese (III) chloride

b) Potassium tetrakis(2-chlorophenyl)borate

c) Tetraoctylammonium bromide

d) 2-Nitrophenyl phenyl ether

e) Dibutyl phthalate

f) 2-Fluorophenyl 2-nitrophenyl ether

g) Dibutyl sebacate

h) Poly(vinylchloride)

References

1. Čermáková L, Feltl J, Němcová I, Němec I, Štulík K. Analytická chemie 2 INSTRUMENTÁLNÍ ANALÝZA pro SPŠ skupiny studijních oborů technická chemie. Praha: SNTL - NAKLADATELSTVÍ TECHNICKÉ LITERATURY; 1980.

2. Microsoft PowerPoint - Lecture 7 - Lecture 7.pdf [Internet]. [cited 2013 Apr 16]. Available from: http://memo.cgu.edu.tw/hsiu-po/Analytical%20Chem/Lecture%207.pdf

3. Handbook of instrumental techniques for analytical chemistry. Upper Saddle River, NJ: Prentice Hall PTR; 1997.

4. Buck R. P, Lindner E. Recommendations for nomenclature of ion-selective electrodes (IUPAC Recommendations 1994). Pure Appl Chem. 1994; 66 (12): pp. 2527–2536.

5. Inczédy J, Lengyel T, Ure A. M. Compendium of Analytical Nomenclature (definitive rules 1997) - The Orange Book, 3rd edition [Internet]. Blackwell Science; 2012. Available from: www.iupac.org/publications/analytical_compendium/

6. Buchanan E. B, Seago J. L. Impregnated silicone rubber membranes for potential-indicating electrodes. Anal Chem. 1968 Mar; 40 (3): pp. 517–521.

7. Yoon I. J, Shin J. H, Paeng I. R, Nam H, Cha G. S, Paeng K. J. Potentiometric behavior of metalloporphyrin-based ion-selective electrodes: Use of silicone rubber matrix for serum chloride analysis. Anal. Chim. Acta. 1998; 367: pp. 175–81.

8. Shin J. H, Sakong D. S, Nam H, Cha G. S. Enhanced Serum Carbon Dioxide Measurements with a Silicone Rubber-Based Carbonate Ion-Selective Electrode and a High-pH Dilution Buffer. Anal Chem. 1996 (68 (1)): pp. 221–225.

9. Ambrose T. M, Meyerhoff M. E. Characterization of photopolymerized decyl methacrylate as a membrane matrix for ion-selective electrodes. Electroanalysis. 1996 Dec; 8 (12): pp. 1095–1100.

10. Qin Y, Peper S, Bakker E. Plasticizer-Free Polymer Membrane Ion-Selective Electrodes Containing a Methacrylic Copolymer Matrix. Electroanalysis. 2002 Nov; 14 (19-20): pp. 1375–1381.

11. Maertens C, Detrembleur C, Dubois P, Jérome R. Synthesis and Electrooptic Properties of a New Chromophore Dispersed or Grafted in a Carbazolyl Methacrylate Matrix. Chem Mater. 1998; 10 (4): pp. 1010–1016.

12. Liu D, Meyerhoff M. E, Goldberg H. D, Brown R. B. Potentiometric ion- and bioselective electrodes based on asymetric polyurethane membranes. Anal. Chim. Acta. 1993 Mar 1; 274 (1): pp. 37–46.

13. Cosofret V. V, Erdosy M, Raleigh J. S, Johnson T. A, Neuman M. R, Buck R. P. Aliphatic polyuretane as a matrix for pH sensors: effects of native sites and added proton carrier on electrical and potentiometric properties. Talanta. 1996 Jan; 43 (1): pp. 143–151.

14. Malinowska E, Niedziólka J, Meyerhoff M. E. Potentiometric and spectoscopic characterization of anion selective electrodes based on metal (III) porphyrin ionophores in polyuretane membranes. Anal. Chim. Acta. 2001 Mar 22; 432 (1): pp. 67–78.

15. Hisamoto H, Siswanta D, Nishihara H, Suzuki K. Anion selective polymeric membrane electrodes based on metallocenes. Anal. Chim. Acta. 1995 Mar 30; 304 (2): pp. 171–176.

16. Oh K. Ch, Kim K. A, Paeng I. R, Baek D, Paeng K. J. Anion-selective membrane electrodes based on polymer-supported metalloporphyrins. J. Electroanal. Chem. 1999 May 17; 468 (1): pp. 98–103.

17. Suzuki H, Nakagawa H, Mifune M, Saito Y. Triiodide Ion-Selective Electrode Based on Manganese(III)-Tetraphenylporphine. Anal. Sci. 1993 Jun; 9: pp. 351–354.

18. Santos E. M. G, Araújo A. N, Couto C. M. C. M, Montenegro M. C. B. S. M. Potentiometric behaviour of ion selective electrodes based on iron porphyrins: The influence of porphyrin substituents on the response properties and analytical determination of diclofenac in pharmaceutical formulations. J. Pharm. Biomed. Anal. 2006; 42: pp. 535–542.

19. Kataky R, Parker D. Sensitive and specific electrochemical sensors for charge-diffuse cations: use of lipophylic cyclodextrins and an enzyme relay for the determination of acetylcholine. Analyst. 1996; 121: pp. 1829–1834.

20. Amorim C. G, Araújo A. N, Montenegro M. C. B. S. M, Silva V. L. Cyclodextrin-based potentiometric sensors for midazolam and diazepam. J. Pharm. Biomed. Anal. 2008 Dec 1; 48 (4): pp. 1064–1069.

21. Cunha C. O, Silva R. C. R, Amorim C. G, Severino A. Júnior, Araújo A. N, Montenegro M. C. B. S. M, et al. Tetracykline Potentiometric Sensor Based on Cyclodextrin for Pharmaceuticals and Waste Water Analysis. Electroanalysis. 2010 Dec; 22 (24): pp. 2967–2972.

22. Totu E, Josceanu A. M, Covington A. K. Improved potassium-selective membrane using valinomycin as ionophore for ion-selective microdevices. Mater. Sci. Eng. C. 2001 Dec; 18 (1-2): pp. 87–91.

23. Bobacka J, Ivaska A, Lewenstam A. Plasticizer-free all-solid-state potassium-selective electrode based on poly(3-octylthiophene) and valinomycin. Anal. Chim. Acta. 1999 Apr 5; 385 (1-3): pp. 195–202.

24. Ceresa A. Ion-Selective Polymeric Membrane Electrodes for Potentimetric Trace Level Measurements. Dissertation. Zürich; 2001.

25. ISE_1.gif (GIF obrázek, 510 × 356 bodů) - Měřítko (0%) [Internet]. [cited 2013 Apr 18]. Available from: http://web.nmsu.edu/~kburke/Instrumentation/ISE_1.gif

26. Šrámková I. Potentiometric determination of ibuprofen. Diploma thesis. Hradec Králové; 2010.

27. Glossary of ISE terms and calculations. [Internet]. [cited 2013 Mar 1]. Available from: http://www.nico2000.net/datasheets/glossary.html

28. UMEZAWA Y et. al. Potentiometric selectivity coefficients of ion-selective electrodes. Part I. Inorganic Cations. Pure Appl Chem. 2000 IUPAC; 72: pp. 1851–2082.

29. Umezawa Y, Umezawa K, Sato H. Selectivity coefficients for ion-selective electrodes: recommended methods for reporting Kpot(AB) values. Pure Appl Chem. 1995 IUPAC; 67 (3): pp. 507–518.

30. Pyruvic Acid - PubChem [Internet]. [cited 2013 Mar 2]. Available from: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=1060

31. pyruvic acid | C3H4O3 | ChemSpider [Internet]. [cited 2013 Mar 2]. Available from: http://www.chemspider.com/Chemical-Structure.1031.html

32. pyruvate | C3H3O3 | ChemSpider [Internet]. [cited 2013 Mar 2]. Available from: http://www.chemspider.com/Chemical-Structure.96901.html

33. 127-17-3 CAS MSDS (Pyruvic acid) Melting Point Boiling Point Density CAS Chemical Properties [Internet]. [cited 2013 Mar 4]. Available from: http://www.chemicalbook.com/ChemicalProductProperty_US_CB5257557.aspx

34. Ledvina M, Stoklasová A, Cerman J. Biochemie pro studující medicíny. Praha: Karolinum; 2009.

35. Eiteman M. A, Chastain M. J. Optimization of the ion-exchange analysis of organic acids from fermentation. Anal. Chim. Acta. 1997; 338: pp. 69–75.

36. Mato I, Suárez-Luque S, Huidobro J. F. Simple determination of main organic acids in grape juice and wine by using capillary zone electrophoresis with direct UV detection. Food Chem. 2007; 102: pp. 104–112.

37. Mato I., Suárez-Luque S., Huidobro J. F. A review of the analytical methods to determine organic acids in grape juices and wines. Food Res. Int. 2005; 38: pp. 1175–1188.

38. Biesaga M, Pyrzyńska K, Trojanowicz M. Porphyrins in analytical chemistry. A review. Talanta. 2000; 51 (2): pp. 209–224.

39. 640px-Chlorophyll_c1_c2.svg.png (PNG obrázek, 640 × 513 bodů) - Měřítko (0%) [Internet]. [cited 2013 Mar 6]. Available from: http://upload.wikimedia.org/wikipedia/commons/thumb/d/de/Chlorophyll_c1_c2.svg/640px-Chlorophyll_c1_c2.svg.png

40. 543px-Heme_b.svg.png (PNG obrázek, 543 × 600 bodů) - Měřítko (0%) [Internet]. [cited 2013 Mar 6]. Available from: http://upload.wikimedia.org/wikipedia/commons/thumb/b/be/Heme_b.svg/543px-Heme_b.svg.png

41. 598px-Porphyrin.svg.png (PNG obrázek, 598 × 600 bodů) - Měřítko (0%) [Internet]. [cited 2013 Mar 6]. Available from: http://upload.wikimedia.org/wikipedia/commons/thumb/5/5c/Porphyrin.svg/598px-Porphyrin.svg.png

42. Górski L, Malinowska E, Parzuchowski P, Zhang W, Meyerhoff M. E. Recognition of Anions Using Metalloporphyrin-Based Ion-Selective Membranes: State-of-the-Art. Electroanalysis. 2003 Sep; 15 (15-16): pp. 1229–1235.

43. Oh K. Ch, Lim S. M, Paeng I. R, Paeng K. J. The potentiometric behavior of polymer supported metalloporphyrins as anion-selective electrodes. J. Electroanal. Chem. 2001 May 1; 506(1): pp. 42–47.

44. Gao D, Gu J, Yu R. Q, Zheng G. D. Substituted metalloporphyrin derivatives as anion carrier for PVC membrane electrodes. Anal. Chim. Acta. 1995 Feb 20; 302 (2-3): pp. 263–268.

45. 5,10,15,20-Tetraphenyl-21H,23H-porphine manganese(III) chloride 95% | Sigma-Aldrich [Internet]. [cited 2013 Apr 21]. Available from: http://www.sigmaaldrich.com/catalog/product/aldrich/254754?lang=en&region=CZ

46. Potassium tetrakis(4-chlorophenyl)borate Selectophore^{TM}, ≥98.0% | Sigma-Aldrich [Internet]. [cited 2013 Apr 21]. Available from: http://www.sigmaaldrich.com/catalog/product/fluka/60591?lang=en&region=CZ

47. CAS # 14866-33-2, Tetraoctylammonium bromide, Tetra-n-octylammonium bromide [Internet]. [cited 2013 Apr 21]. Available from: http://www.chemblink.com/products/14866-33-2.htm

48. Dielectric Constants of Various Materials [Internet]. [cited 2013 Apr 21]. Available from: http://www.deltacnt.com/99-00032.htm

49. Katsu T, Ido K, Kataoka K. Poly(vinyl chloride) membrane electrode for a stimulant, phemtermine, using tris(2ethylhexyl) phosphate as a solvent mediator. Sensors Actuators. 2002; B 81: pp. 267–272.

50. Analysis of anions in wine by Ion Chromatography [Internet]. [cited 2013 Jan 21]. Available from: http://www.showa-denko.com/index.php?id=35&tx_ttnews%5btt_news%5d=63&tx_ttnews%5bbackPid%5d=36&cHash=49c48f7b67

51. Analysis of Anions = Analysis of Anions in Wine (VH-anion 4D) [Internet]. [cited 2013 Jan 21]. Available from: http://www.hplc.com/Shodex2/english/dc070535.html

52. Analysis of Inorganic and Organic Anions by Capillary Zone Electrophoresis (5965-5744E) - 59655744.PDF [Internet]. [cited 2013 Jan 21]. Available from: http://www.vscht.cz/ktk/www_324/lab/cze/CE/PDFs/ap-notes/59655744.PDF