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Potentiometric determination of ibuprofen

Diploma thesis
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I declare I processed this thesis on my own. All bibliographic sources and other materials that I used for this work are listed in the references and cited properly.

Hradec Králové, 15th May 2010 Ivana Šrámková

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1. INTRODUCTION

Pharmacy is a medical branch Chat deals scientifically and practically with the medication and drugs. The functions of pharmacy involve research, obtaining of scientific knowledge about drugs, evaluating and drug quality control among others. The aim of all these pharmacy functions is to obtain a safe drug of high quality, dispense it in a required time and amount, so the drug can work as a preventive, diagnostic or therapeutic means. Drug control is a branch of pharmacy that ensures the quality, safety and effectiveness of the drugs [1].

In drug control, many techniques are used to follow its aim such as spectral, radiometric, electrochemical, separation methods. Although the methods of separation are too disclosed in most pharmaceutical control laboratories, the spectrophotometric and electrochemical ones are the most widely used. The electrochemical methods can be classified into two major groups:

A) methods based on the electrode reapction (potenciometry, polarography, amperometry and and others),

B) methods, where the electrode reaction is not decisive (conductometry) [2].

Potentiometry is a technique based on a measurement of the potential difference between an indicator electrode and a reference electrode in solution, while the current is held at zero [3]. The potential obtained can be correlated with the activity of the ion to be measured [3].

Among the different types of electrodes indicators, it could be considered the ion selective electrodes (ISE´s) that are concentration cells.

Special kind of indicator electrode are ion-selective electrodes (ISEs). They are made usually by a membrane selective to an ion and, through a process of electronic transduction the chemical potential difference is converted in an electrical potential.

Nowadays, ISE´s are successfully applied in environmental, clinical, pharmaceutical analysis and also in monitoring process field.

This work aimed to develop an ibuprofen selective electrode for its potentiometric determination in pharmaceutical products.

Ibuprofen is a non-steroidal anti-inflammatory drug with analgesic and antipyretic properties. It has been extensively used in the treatment of many diseases like rheumatoid arthritis, degenerative joint disease, ankylosing spondylitis and acute gouty arthritis [4].

Ibuprofen has a monograph in European Pharmacopoeia and thus in Czech Pharmacopoeia, too.

2. AIM OF THE WORK

The aim of this work is to develop an ibuprofen selective electrode for its potentiometric determination in pharmaceutical products. Several membranes will be prepared, containing different ionophores as well as liphophilic species as additives and several mediator solvents. The ratio between the individual components will be changed in order to develop the membranes with better working characteristics. The electrodes will be evaluated in different conditions. The electrodes with the proper characteristics will be chosen for analytical applications. As a result, an environment friendly, low cost, fast and simple method should be proposed as an alternative to the more tedious generics methodologies.

3. THEORETICAL PART

3.1. ELECTROANALYTICAL METHODS

Electroanalytical methods are the second most used techniques in analytical chemistry, following the spectral methods. This type of analysis is based on relationships between electrical magnitudes (electrode potential E, current I, conductivity χ and others) and an amount of analyzed substance in a solution characterized by weight m, concentration c or volume V. [2]

IUPAC divides the electroanalytical methods into four groups [5] :

1) Potentiometry and related techniques *(Techniques Involving Electrode Reactions and Employing Constant Excitation Signals)*
    a) potentiometry,
    b) differential potentiometry
    c) potentiometric titration,
    d) differential potentiometric titration
    e) chronopotentiometry, controlled current coulometry and others)

2) Amperometric and related techniques (*Techniques Involving Electrode Reactions and Employing Constant Excitation Signals)*
    a) amperometry,
    b) amperometric titration,
    d) chronocoulometry,
    e) electrogravimetry,
    c) chronoamperometry,
    f) electrography
    g) controlled potential coulometry
    h) controlled potential electrogravimetry

3) Voltametric and related techniques *(Techniques Involving Electrode Reactions and Variable Excitation Signals of Large Amplitude)*

a) linear sweep voltammetry, stationary electrode voltammetry or chronoamperometry with linear potential sweep

b) hydrodynamic voltammetry

c) polarography

d) tast polarography

e) single drop sweep polarography

f) tringular wave voltammetry

g) cyclic voltammetry, cyclic tringular wave voltammetry

h) normal pulse polarography

i) double potential step chronoamperometry

j) differential pulse polarography

k) AC-polarography

l) Square-wave polarography

m) alternating voltage chronopotentiometry

4) Impedance or conductance and related measurements (*Techniques in which Neither the Electrical Double Layer Nor Any Electrode Reaction Need Be Considered*)

a) conductometry

b) conductometric titration

c) high frequency conductometry

d) high frequency conductometric titration

e) dielectrometry

f) dielectrometric titration .

3.2. POTENTIOMETRY

As mentioned above, determination of a substance in a sample using potentiometry is based on a measurement of the potential difference between an indicator electrode and a reference electrode in solution, while the current is held at zero. Concentration of hydrogen cations (pH) or concentration measurement of other ions (if ion-selective electrode is used) is provided in this way frequently [2].

3.2.1. REFERENCE ELECTRODES

An electrode that maintains a virtually invariant potential under the conditions prevailing in an electrochemical measurement, and that serves to permit the observation, measurement, or control of the potential of the indicator (or test) or working electrode [6]. Reference electrode and indicator electrode create two halves of an electrochemical measuring cell.

The most common reference electrodes are mercury–mercury (I) chloride (calomel), silver–silver chloride electrode.

Calomel electrode consists of inner element-mercury covered by Hg_2Cl_2, filling solution-potassium chloride saturated solution and a part for forming liquid junction-the only connection between the electrode system and the analyzed solution. Usually fritted disc, ground glass sleeve or a ceramic plug is used to create the connection. This condition must be accomplished, as the concentration of potassium chloride solution cannot change.

Silver-silver chloride electrode is another type of reference electrode. It consists of a silver wire covered by silver chloride. The filling solution represents an alkaline chloride solution or hydrochloric acid.

3.2.2. INDICATOR ELECTRODES

Indicator electrode is an electrode that serves as a transducer responding to the excitation signal (if any) and to the composition of the solution being investigated, but that does not affect an appreciable change of bulk composition within the ordinary duration of a measurement [6].

As indicator electrode, glass, silver or platinum electrodes are connected to create the measuring cell with the reference electrode.

Glass electrode serves to measure pH, as it answers to the activity (concentration) of hydrogen cations in a solution. It is a potentiometric sensor made from glass of specific composition which works as a selective membrane. The bulb is filled up with a solution, usually a buffer (constant pH), in which an electrode is immersed. Before working as a pH electrode, the surface of a glass membrane must be hydrated. After immersing the electrode into an aqueous solution, a thin solvated layer (gel layer) is formed on the glass surface. This applies to both the outside and inside of the glass membrane. Sodium cations and hydrogen cations from the glass bulb are exchanged - the glass is protonated/depronoted relatively to the inner solution. Due to this fact, an electrical potential develops. It is a function of the activity of hydrogen cations. Typically, pH electrode is a combined electrode which includes both reference and indicator electrode in one body and it is called pH combined electrode.

Silver electrode is an electrode constructed from pure silver. It is used in argentometry, as it response to the silver ions in the examined solution.

Platinum electrode has a use by all redox titrations. The electrode allows the electrons exchange between the oxidized and reduced form.

Ion-selective electrodes (ISEs) are special kinds of indicator electrodes.

An ISE is defined as an electroanalytical sensor with a membrane whose potential indicates the activity of the ion to be determined (the determinant) in a solution (the analyte). The membrane of ISE consists either of liquid electrolyte solution or of solid or glassy electrolytes that usually have negligible electron conductivity under the conditions of measurements [7]. ISEs allow the potentiometric determination of the activity of a certain ion in the presence of other ions [8]. Response of these sensors towards charged species is provided by ion-selective membranes, which can be prepared from different materials - glass (pH electrode), solid crystalline, ceramic or polymers [9]. Their selectivity is achieved either by means of material structure or by doping the membrane with specific ion-selective complexing agents.

The most successful ISE is a glass pH electrode, used when pH is controlled or adjusted. The most widely employed selective sensors are electrodes with polymer membranes. Their character enables to adjust the properties of the sensor. Nowadays it is possible to measure up to 50 different ions. The improvement can be easily done by the

change of membrane composition and electrode construction, or by new methodologies of data interpretation.

3.2.3. ION-SELECTIVE ELECTRODES WITH POLYMERIC MEMBRANES

A membrane is general term which refers to a continuous layer, usually consisting of a semi-permeable material, with controlled permeability covering a structure, such as carbon or an inert metal, or separating two electrolyte solutions [6].

ISEs classification according to IUPAC:

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

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

The membrane ion selective electrode is usually made up of a chemical recognition element, called *ionophore*, a *solvent mediator* that provides the plastic membrane characteristics and a *polymer* that physically supports these elements of the membrane. In some cases the membranes also include compounds that act as *lipophilic ionic additives* and shape the operating characteristics of the electrodes. The composition and relative proportion of membrane constituents influence the response selective electrodes.

Polymer matrix

Polymer matrix provides the mechanical stability of the membrane. The choice of polymer depends on the requirements, such as biocompatibility, physiological fluids sample etc.

One of the primary requirements of polymers creating the selective membrane is that their glass transition temperature should be below the room temperature [10]. There are some polymers that are used in potentiometry and meet this requirement, i.e. silicon rubber [11], several methacrylates [12], polyurethanes [13]. The most widely used polymer poly(vinylchloride) (PVC) must be plasticized.

Plasticizer

Plasticizers are solvents giving the convenient viscoelasticity to the membrane. In the ISE membrane, the plastisizer acts as a membrane solvent and also plays a role in the membrane selectivity and in the limit of detection. It can influence both extraction of an ion into organic phase and also the complexation with the ionophore [14,15,16]. The requirements that a plactisizer has to meet are: to be compatible with the polymer and to be a solvent for other membrane constituents. For PVC, 2-nitrophenyl octyl ether (o-NPOE, polar) and bis(2-ethylhexyl)sebacate (DOS, apolar) are commonly used as plasticizers.

Lipophilic ionic sites

Lipophilic ionic sites are additives that play an important role in electrode selectivity. Chemically, lipophilic ionic site is a salt of non-exchangeable lipophilic anion/cation and exchangeable counter ion. They provide electroneutrality of the membrane with neutral ionic carriers, so that no significant amount of counter ions can be co-extracted into the membrane together with primary ion. Therefore, the membrane is permeable only for ions of the same charge sign as primary ion [17].

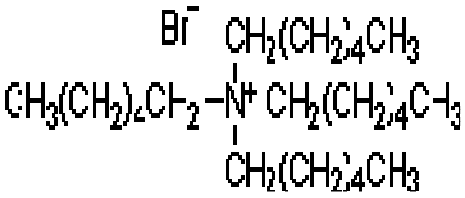
ISE membrane can eventually work without the additive, but there are some advantages on using the additive. The lipophilic ionic site in the ISE membrane keeps constant total concentration of the measuring ion in the membrane phase and can contribute to the membrane selectivity in the case that no ionophore is used or its present amount is insufficient.

The addition of lipophilic salt without ion-exchanger properties, or lipophilic inert electrolyte, was initially suggested in order to reduce electrical resistance of ISE

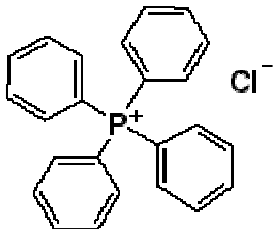
membranes [18]. Later it was found that the addition of tetradodecylammonium tetrakis(4-chlorophenyl)borate also improves the selectivity of divalent over monovalent ions if membranes of low polarity and low site concentration are used [19].

Examples of some lipophilic additives in use are shown in Fig.1.

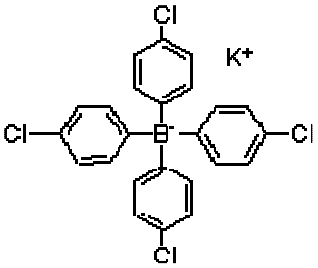
Fig. 1: Structure of some lipophilic ionic sites



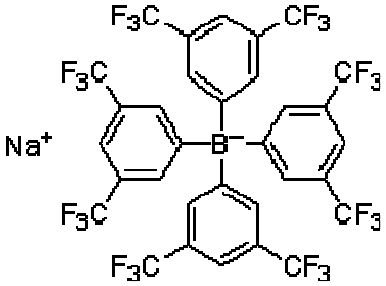
tetrahexylammonium bromide



tetraphenylphosphonium chloride



potassium tetrakis-(4-chlorophenyl) borate

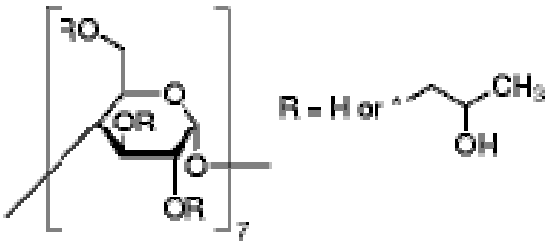


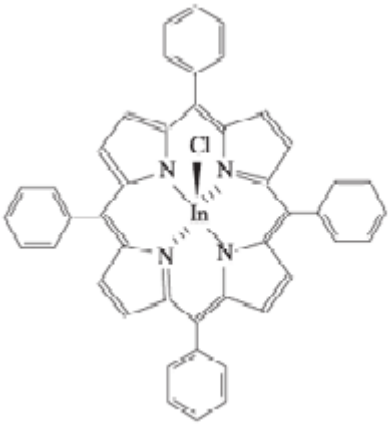
sodium tetrakis -{3,5-bis(trifluormethyl)phenyl}borate

Ionophore

Ionophore is a component of the ISE membrane that has a crucial role in the membrane selectivity. According to [9], ionophore is a compound that can carry a specific ion through a membrane. It forms a complex with the analyzed ion. The binding must be strong but reversible. In the ideal case, the ionophore binds only with the target ion - then we can say that the membrane is selective to this ion. It is important that the ionophore must also bind to the polymer matrix, so it can not be washed away from the membrane but it must be retained within the structure. To meet this requirement, an ionophore must have a) binding centre to form the complex with the detected ion, b) lipophilic groups to bind to the matrix. Structures of several substances used to construct an ISE are listed in Fig. 2.

Fig.2: Structures of several substances used to compose an ISE membrane





hydroxypropyl-β-cyclodextrine

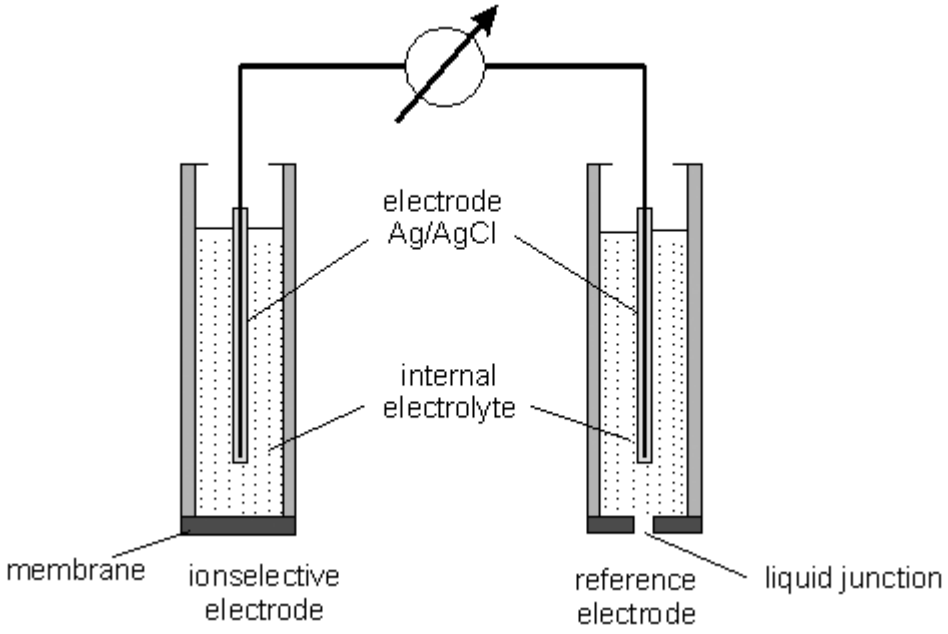
chloro(5,10,15,20-tetraphenylporphyrinato) indium (III)

3.2.4. RESPONSE MECHANISM OF ION-SELECTIVE ELECTRODES

Electrical response for ion-selective electrodes

Electrochemical measuring cell (Fig.3) is a device used in potentiometry measurements. It converts the chemical energy into the electrical one when a chemical reaction occurs in the cell. It consists of two galvanic half-cells. The half cell is representing by the electrode and a surrounding electrolyte. Indicator electrode and reference electrode, immersed in the solution of the analyte, are consider to be the two half cells of the electrochemical measuring cell.

Fig.3: Schematic diagram of an ISE measuring cell



The total potential difference (electromotive force, EMF) rising between the ISE and reference electrode can be measured by millivoltameter or a multi-channel measuring station. The total potential consists of more contributing potentials, rising at each electrochemical interface:

$$EMF=E_{const} + E_M + E_{D,ref}$$

E_{const} refers to potentials that can be kept constant. E_M (membrane potential) and $E_{D,ref}$ liquid-junction or diffusion potential) depend on the sample.

Liquid junction potential – rises because of different mobilities of ions in the sample and in the bridge electrolyte of the reference electrode. This potential can be kept constant by using concentrated bridge electrolytes with similar mobilities of cations and anions or can be prevented by using a double junction reference electrode.

Membrane potential – involves the phase boundary potentials at both interfaces and the diffusion potential within the ion-selective membrane. The diffusion potential depends on the sample, whereas the potential at the interface can be kept constant. The diffusion potential is significant if a considerable concentration gradients of ions with different

mobilities arise in the membrane [17]. Otherwise the diffusion potential is zero, which is often the case for membranes that show theoretical Nernstian response.

The phase boundary potential (part of membrane potential) – arises from a charge separation caused by the non-uniform distribution for ionic species between the organic membrane and the aqueous phase [17].

The equation describing the membrane potential is the *Nernst equation* :

$E_M = E_I^0 + (RT/z_I F).\ln a_{I,s}$

E_I^0 - the standard potential when the activity of the main ion is equal to 1.
R - molar gas constant (8,31441 J mol $^{-1}$ K^{-1})
T - absolute temperature (K)
z_I - charge of the ion I
F - Faraday constant (96484.56 C mol^{-1})
a - activity of the ion I

The potential of ISE selective towards ion I can be written as:

$E_I = EMF - E_{D,ref} = E_I^0 + s_I \log a_{I,S}$

$s_I = 2{,}303\ RT/\ z_I F = 0.059/z_I$
Graphically, E_I is a linear function with the slope s_I. If the ion has a charge -1 (the case of ibuprofen), the theoretical slope of the linear function is 59mV.

Response mechanism

Ionophores in junction with lipophilic ionic sites are responsible for the ISE response.

If the membrane contains no ionophore, but only lipophilic additive, extraction of ions from the sample to the membrane phase is the only potential determining process [17].

If the sufficient amount of ionophore is present in the membrane, complexation of the target ion by the ligand and membrane-solution ionic exchange determines membrane

selectivity [17]. If there are more ions complexed with the ionophore, the difference between the stability constants of ion-ionophore characterizes the selectivity of the ISE.

3.2.5. CHARACTERIZATION OF AN ION-SELECTIVE ELECTRODE

SELECTIVITY

It is one of the most important characteristics of an ISE. Selectivity is an expression of the specificity of the membrane towards the primary ion in the presence of other ions in the solution that can potentionally interfere with the primary ion. It determines the applicability of the ISE for certain measurements. Quantitatively, selectivity is characterized by selectivity coefficient $K_{A,B}^{pot}$, which is a direct function of the difference of the individual potentials extrapolated to 1 M activity of ions A and B [18]. It is usually expressed as a logarithm of $K_{A,B}^{pot}$. Negative values indicate a preference of the ISE for the target ion relative to the interfering ion. Positive values of log Kxy show the preference of an electrode for the interfering ion. At $K_{A,B}^{pot} = 1$ the electrode responses equally to both ions. $K_{A,B}^{pot}$ can be used to predict response functions in mixed samples [19].

IUPAC recommends these methods to measure the selectivity coefficient:

SSM *(separate solution method)* – the potential is measured first in the solution of the possibly interfering ion (E_B), and then in the solution of the primary ion (E_A). The logarithm of the electivity coefficient is then:

$$\log K_{A,B}^{pot} = (E_B - E_A)z_A F / 2.303RT + (1 - z_A/z_B)\log a_A$$

Advantages:

- speed and ease of determination,
- can determine a large array of interfering ion-selectivity coefficients very quickly,
- is used for simple flow-injection potentiometry applications (simple and well defined systems).

Disadvantages:

- does not account for any error due to multiple ion interaction,

• overly simplistic method for real solutions, often giving very different coefficients than other methods [20].

FIM *(fixed interference method)* – The emf (electromotoric force) of a cell comprising an ion-selective electrode and a reference electrode (ISE cell) is measured for solutions of constant activity of interfering ion, a_B, and varying activity of the primary ion. The emf values obtained are plotted vs. the logarithm of the activity of the primary ion a_A. The intersection of the extrapolated linear portions of this plot indicates the value of a_A which is to be used to calculate $K_{A,B}^{pot}$ from the Nikolsky-Eisenman equation [21] :

$$K_{A,B}^{pot} = a_A / a_B^{zA/zB}$$

Advantages:

• accurate for a larger variety of systems than separate solutions,

• relatively simple to perform for a reasonable set of potential interfering ions of interest,

• method gives good (reasonable) data for most real world systems,

• coefficients translate fairly well to many observed application selectivity performance.

Disadvantages:

• does not account for all multiple ion-ion interactions, only interfering ion analyte interference [20]

MSM *(mixed solutions method)*

Advantages:

• accurate for almost all stable systems, even if complex,

• more accurate than fixed interference solutions,

• method gives very good data for complex systems.

Disadvantages:

• very cumbersome to perform if the system has any variance of the ionic background,

• laboratory technique and uncertainties of measurement are of great importance [20].

SLOPE

It is the gradient of the line formed by plotting the electrode response in milivolts against the logarithm of the activity (or concentration) of the measured ion. The theoretical Nernstian slope at 25°C is 59.16 mV per decade for monovalent ions and 29.58 mV/dec for bivalent ions. In practice the slope is usually lower than the theoretical value, which calculates with ideal conditions that are not met in practice. The slope of an electrode can be determined by measuring the mV response in two standard solutions with concentrations (activities) of a_1 and a_2 and then calculated with:

$m = (E1-E2) / ((Log(a_1))-(Log(a_2)))$

or can be calculated from the calibration curve.

THE pH RANGE OF AN ISE

It is a range over which a change of pH will not cause a significant change in the measured voltage. The range of pH can be obtained from a graph of pH against potential, constructed at constant activity of the determined ion. The pH range is of an ISE is the plateau on this graph. Outside this pH area, a change in pH may cause a significant change in the measured mV. Consequence of this is the need of adjustment of pH of the sample with a buffer in the case that it differs from the pH of the standard solution.

DETECTION LIMITS

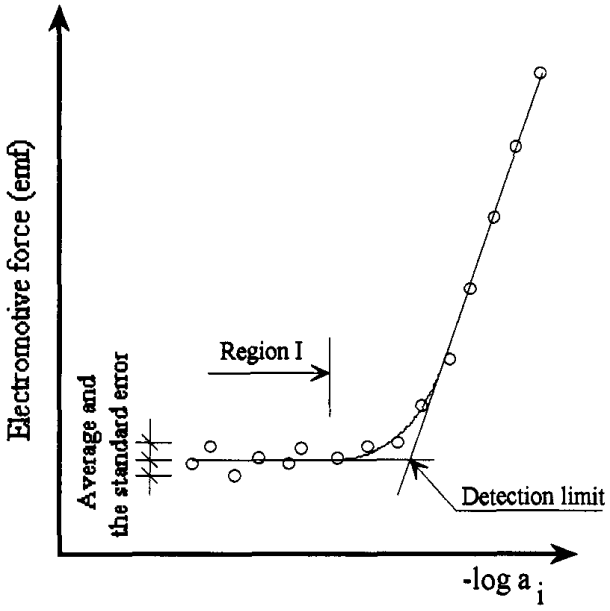
The response of an ISE is characterized by an upper and a lower detection limit. These values limit the analytical range of the electrode where the ISE shows Nernstian response. According the IUPAC recommendation, the detection limit is defined by the cross-section of the two extrapolated linear parts of the ion-selective calibration curve.

Upper detection limit is a consequence of coextraction process of the primary ion and interfering ion from the sample to the membrane, thereby leading to a loss of membrane permselectivity [17]. Due to this process, the calibration curve of the response to the increase of the primary ion concentration has lower then Nernstian slope.

The lower detection limit occurs as the loss of Nernstian response slope at low primary ion activity. Depending on the type of ionophore, the ISE usually present detection limits of about $10^{-5} – 10^{-6}$ mol.L^{-1}.

Figure 4 represents a typical potentiometric response of a cation. Region I represents a response of the electrode to the concentration of an ion that is under the lower detection limit.

Fig.4: The definition of the lower detection limit according to IUPAC recommendation



RANGE OF LINEAR RESPONSE

This region corresponds to the range of linear response for the electrode. It is limited by the practical detection limits of the electrode. That range of concentration (or activity) over which the measured potential difference does not deviate from that predicted by the slope of the electrode by more that ± 2 mV [22].

RESPONSE TIME

The length of time necessary to obtain a stable electrode potential when the electrode is removed from one solution and placed in another of different concentration [22]. There are many factors affecting the response time: the electrode type, the magnitude and direction of the concentration change, the temperature, if interfering ions are present, if the sample is stirred when the potential is measured or the measurement is performed in static conditions. For ISE, it's generally quoted as less than 10 seconds.

LIFETIME OF ISE

It is a time after which the sensor starts to lose its characteristics. It is caused by leaching of the membrane constituents into the solution. The life span can be also affected by the concentration of the solution, the ionic strength and the environment conditions, such as the temperature, O_2, CO_2, the light intensity, etc.

3.2.6. ADVENTAGES AND LIMITATIONS OF ISE

ADVANTAGES:

1. Linear response- over 4 to 6 orders of magnitude of A – a very low concentration of the analyte can be measured.
2. Non-destructive- no consumption of analyte.
3. Environment-friendly.
4. Short response time - in sec. or min., which makes these sensors appropriate for application in flow conditions systems.
5. The response is not affected by color or turbidity of the sample.
6. Simply device.

LIMITATIONS:

1. Precision is rarely better than 1%.
2. Electrodes can be fouled by proteins or other organic solutes.
3. Interference by other ions.
4. Electrodes respond to the activity of uncomplexed ion, so it has to be ensured that there is a free ion in the solution (masking the ligands).

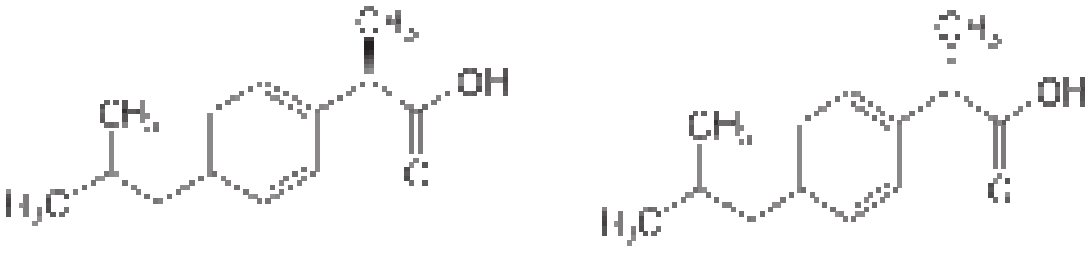
3.3. IBUPROFEN

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID). Ibuprofen was derived from propionic acid by the research arm of Boots Group during the 1960´s [23] and patented in 1961.

3.3.1. THE CHEMISTRY OF IBUPROFEN

Ibuprofen (Fig.5) is a derivative of propionic acid. The systematic (IUPAC) name is (RS)-2-(4-(2-methylpropyl)phenyl)propanoic acid. Ibuprofen is a white powder only slightly soluble in water, less than 1 mg of ibuprofen dissolves in 1 ml water (< 1 mg/mL). The solubility increases in the mixture of water and ethanol. Like in other propionic acid derivatives (including ketoprofen, flurbiprofen, naproxen), the carbon in α-position creates a stereocentre, so there are two enantiomers with different chemical and biological properties. The pharmaceutical effect is bound to the S-isomer. There were tendencies to prepare a single enantiomer product, because a half on a pharmaceutical formulation seemed to be inactive and useless. As further experiments revealed, there is an enzyme - isomerase ("2-arylpropionyl-CoA epimerase") which converts the inactive R-isomer to the active S-form. Nowadays, racemic mixtures are applied in practice. Ibuprofen is a relatively weak acid with pKa=4.4. The melting point is 74-78°C [24].

Fig.5: Ibuprofen enantiomers



R-ibuprofen S-ibuprofen

3.3.2. THE PHARMACOLOGY OF IBUPROFEN

MECHANISM OF ACTION OF NSAID

As it was said above, ibuprofen belongs to the non-steroidal anti-inflammatory drugs (NSAIDs).

Though the mechanism of action of non-steroidal anti-inflammatory drugs is apparently multifactorial, the crucial role plays their property to inhibit the cyclooxygenase, the key enzyme which limits the speed of prostanoids synthesis [25]. Physiologically, prostanoids have many roles in human body. In pathological state, they act as mediators of pain, fever and inflammation.

Cyclooxygenase (COX) converts the arachidonic acid into $PGG_{2,}$ a mediator of nociception and inflammation, and then to PGH_2. PGH_2 is then converted into prostanoids (prostacyklines, prostaglandins, leucotriens) by tissue-specific enzymes.

COX has more isoforms. Cyclooxygenase 1 is known as a constitutional form of cyclooxygenase. Prostanoids which synthesis is catalyzed by this enzyme, are responsible for homeostatic and physiological function of the body. The second isoform, cyclooxygenase 2, acts as a catalyzator of prostanoids that act as mediators of pain, fever and inflammation.

The effect of NSAID is given by the inhibition of COX-2. The synthesis of prostaglandins in peripheral tissues is then reduced. Though it is not the only mechanism. Some NSAIDs act as free radicals scavengers, reduce the transport of leucotriens and macrophages into the place of inflammation, influence the producing of cytokines and the activity of cell adhesive molecules, inhibit the releasing of histamine from mast cells [25].

NSAIDs can be divided into several groups according to the specificity towards COX-isomer.

A. non-selective COX inhibitors – influence both COX-1 and COX- 2 synthesis

B. COX-2 preferring inhibitors- inhibit COX-2 100 times more than COX-1

C. COX -2 selective inhibitors- do not inhibit COX-1 neither in the maximum therapeutic doses [25].

Ibuprofen belongs to the non-selective COX inhibitors that means that it inhibits the production of both groups of prostanoids - the one that exhibits homeostatic and physiological function and the one that acts in the case of infection, trauma or other pathological state. From this fact, the side effect of ibuprofen can be deduced.

SIDE EFFECTS

Very common side effect after ibuprofen administration (affect more than 1 user in 10) is nausea, vomiting, diarrhea, constipation, flatulence, heartburn.

Epigastric pain is a common side effect, it affects 1-10 users in 100.

As uncommon, vertigo and headache are described. These appear in 1-10 users in 1000.

Rare side effects are statistically present in 1-10 users in 10000. Problems in gastrointestinal tract (erosion, ulceration, perforation) can be caused by blocking the synthesis of cytoprotectively acting prostanoids PGE_2 and PGI_2. Patients long term treated with acetylsalicylic acid, elderly patients or patients with bleeding or ulcers in anamnesis are under higher risk of these side effects.

There is also a risk of bronchoconstriction after ibuprofen administration, as it was seen after the administration of acetylsalicylic acid. The bronchoconstriction is caused by leucotriens. As the synthesis of prostanoids is blocked, the synthesis of leucotriens increases.

Other rare cases of adverse reaction may include allergic reaction, disturbance of vision and color perception or hepatic functions disorders.

In trombocytes, the blockage of tromboxane A_2 can be followed by platelet synthesis inhibition and therefore bleeding can be caused [25]. This effect develops in 1 user in 10000. Retention of fluid, sleeplessness, depression, emotional lability, changes in blood pressure may also occur. As the prostanoids produced by both COX-1 and COX-2 play role in the autoregulation of renal function, another potential adverse effect is deterioration of renal function.

Ibuprofen should be taken with caution in patients who are under higher risk of side effects, such as:

-patients with gastrointestinal hemorrhage in history

-patients suffering asthma who has not taken NSAID before

-elderly patients

-patients with hepatic, renal or cardiac impairment.

CONTRAINDICATION

Ibuprofen administration is contraindicated in several cases:

-at hypersensitivity to ibuprofen or acetylic acid or NSAID presented as bronchial asthma or urticaria

-at active or recurrent ulcer of the stomach or duodenum

-at bleeding or perforation in gastrointestinal tract

-at disorders of blood formation and disorders of blood clotting

-at severe heart failure [26]

- third trimester of pregnancy

PREGNANCY AND BREAST FEEDING

Ibuprofen is contraindicated in the last trimester of pregnancy as it can cause closure of the foetal ductus arteriosus, foetal renal impairment, inhibition of platelet aggregation and may delay birth.

In the first and second trimester, ibuprofen can be taken only if the benefit for the mother and the foetus is higher than the risk.

Ibuprofen can be used during breastfeeding. It appears in the breast milk, but the concentration is very law. It does not have any undesirable effect on the breast fed child.

3.3.3. THE PHARMACOKINETICS OF IBUPROFEN

Ibuprofen, as well as other non-steroidal anti-inflammatory drugs, is available in peroral, rectal and parenteral dosage forms. There are many formulations for local treatment on the market.

The absorption of ibuprofen is rapid and complete when given orally [25]. The food does not affect the volume of absorption. The free fraction of ibuprofen reaches only 1%, the rest is bound to the blood proteins. As a consequence of this fact, interaction with other drugs can be expected.

Ibuprofen penetrates both haematoencephalic barrier and placenta and can undesirably affect the foetus. The concentration in breast milk is low, as mentioned above. Ibuprofen is eliminated following biotransformation to glucuronide conjugate metabolites that are excreted in urine, with little of the drug being eliminated unchanged. The excretion of conjugates may be tied to renal function and the accumulation of conjugates occurs in end-stage renal disease. Hepatic disease and cystic fibrosis can alter the disposition kinetics of ibuprofen [27].

Ibuprofen demonstrates significant interaction to acetylsalicylic acid, methotrexate and cholestyramine.

4. EXPERIMENTAL

4.1. REAGENTS AND APPARATUSES

4.1.1. CHEMICALS

-distilled and deionised water (conductivity less than 1 µS/cm) and analytical chemicals were used without further purification.
-ibuprofen, Acofarma, Madrid, Spain
-tetrahydrofuran (THF), Riedel-de Haën Laborchemikalien GmbH & Co. KG, Seelze, Germany
-sodium hydroxide (NaOH), Riedel-de Haën Laborchemikalien GmbH & Co. KG, Seelze, Germany
-sodium hydrogen phosphate, Fluka Chemika GmbH, Steinheim, Germany
-ibuprofen sodium salt, Fluka Chemika GmbH, Steinheim, Germany
-hydroxypropyl-ß-cyclodextrine (HP-β-CD), Fluka Chemika GmbH, Steinheim, Germany
-polyvinyl chloride (PVC), Fluka Chemika GmbH, Steinheim, Germany
-2-fluor-2- nitrophenylether, Fluka Chemika GmbH, Steinheim, Germany
-tetrahydroxyammonium bromide (THAB), Fluka Chemika GmbH, Steinheim, Germany
-tetraphenylphospohorium chloride (TPPC), Fluka Chemika GmbH, Steinheim, Germany
-dibutylsebacate, Fluka Chemika GmbH, Steinheim, Germany
-sodium tetrakis-{3,5-bis(trifluormethyl)phenyl} borate, Fluka Chemika GmbH, Steinheim, Germany
-potassium tetrakis(p-chlorphenyl) borate, Fluka Chemika GmbH, Steinheim, Germany
-water purified via reverse osmosis.

4.1.2. APPARATUSES

Crison 2002 potentiometer was used to measure the potential difference between the reference electrode and the ibuprofen selective electrode. The decimillivoltmeter was connected with a home made switcher. A double junction electrode (AgCl/Ag Orion Thermo) was used as reference. When necessary a Crison GLP 22 pH meter was used to measure and adjust pH of the solutions. A Keep & Zonen recorder was also used for collecting the results.

The sensor cocktails and buffer solutions were homogenized using a VWR ultrasonic bath.

4.1.3. LABORATORY GLASS AND TOOLS

-beakers, volumetric flasks, volumetric pipettes, pipette filler, automatic micropipettes, measuring cylinders, laboratory spoon, strick, stoppers

4.2. SOLUTIONS

4.2.1. BUFFERS

Two types of buffers were used during the procedures.

$H_2PO_4^-/OH^-$ buffer of the pH= 7.5 was prepared from $H_2PO_4^-$ and OH^- mother solutions. $H_2PO_4^-$ mother solution 0.05M was prepared by weighing approximately 3.45 g of NaH_2PO_4 (Mr=137.99), dissolving the powder in water and filling up the 500 mL volumetric flask to the mark.

OH^- mother solution 0.05M was prepared by weighing about 1.00 g of NaOH (Mr=40) dissolving in water and filling up the 500 mL volumetric flask to the mark.

The buffer of pH=7.5 was achieved by mixing the $H_2PO_4^-$ mother solution and OH^- mother solution in ratio circa 4:3.

The buffer of pH 9.14 was prepared from $H_2B_4O_7$ mother solution 0.01 $mol.L^{-1}$ and NaOH mother solution 0.01 $mol.L^{-1}$. About 0.31 g of $H_2B_4O_7$ (Mr=61.83) was weighed, transferred to the 500 mL volumetric flask and water was added to the mark.

NaOH mother solution 0.01 $mol.L^{-1}$ was prepared by transferring of about 0.1g of the substance to the 250 mL volumetric flask and filling up with water.

Buffer of acquired pH arised by mixing the above mentioned mother solutions in ratio circa 2:1.

4.2.2. IBUPROFEN SOLUTIONS

The ibuprofen stock solution was prepared every day by weighing about 0.21g of ibuprofen (Mr=226.28), adding 4.0 mL of NaOH (0.3 $mol.L^{-1}$) in the 100 mL volumetric flask and adding $H_2PO_4^-/OH^-$ buffer (0.05 $mol.L^{-1}$) to the mark. This was followed by sonication for approximately 5 min for complete dissolution. A solution of 10 or 100x lower concentration was prepared by transferring 10.0 or 1.0 mL, respectively, of the primary solution to 100 mL volumetric flask and filling up the flask with the buffer.

NaOH served as a co-solvent, as ibuprofen is only slightly soluble in water at pH 7.5.

The ibuprofen sodium salt stock solution was prepared everyday by weighing about approximately 0.26 g of ibuprofen sodium salt (Mr=260.29) and adding the buffer solution

($H_2B_4O_7$ /NaOH with concentration c=0.01 mol.L^{-1} and pH adjusted to 9.14) into the 100 mL volumetric flask to the mark. Solution of lower concentration was prepared by diluting the stock solution prepared in the way described above.

4.3. MEMBRANE PREPARATION

The membranes for electrode were prepared by mixing different amount of the metalloporphyrine, or HP-β-CD, respectively, with solvent mediator and an additive, showed in table 1. The corresponding amounts (percentage) of the different constituents of the potentiometric membranes prepared and evaluated shows Table 2. PVC was dissolved in THF and then added to the mixture of other components. The sensor membrane was dropped in more layers on the conductive surface of the electrodes as shown in Fig.6. The membranes were left to dry for 24 hours. After drying they were ready for use.

Tab.1: Membrane composition (mg) of ion-selective electrodes under study

Type	TPP IN(III)	HP-β-CD	DBS	FNDPE	PVC	NaTFPB	THAB	KTpClPB	TMBP	TPPC
	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg
B	11,1		361,0		170,6		5,7			
C	6,7		362,0		170,6	5,1				
D		10,4		403,1	180,2			1,5		
E		10,3		402,5	180,2		1,6			
F		10,7		405,6	179,8					2,3
G		21,9		810,4	361,2					3,2
H		11,5		405,6	181,0					4,9
I		10,15		403,70	180,30		2,34			
J		10,25		403,14	180,22				1,33	
M		11,6		407,44	180,44		1,24			

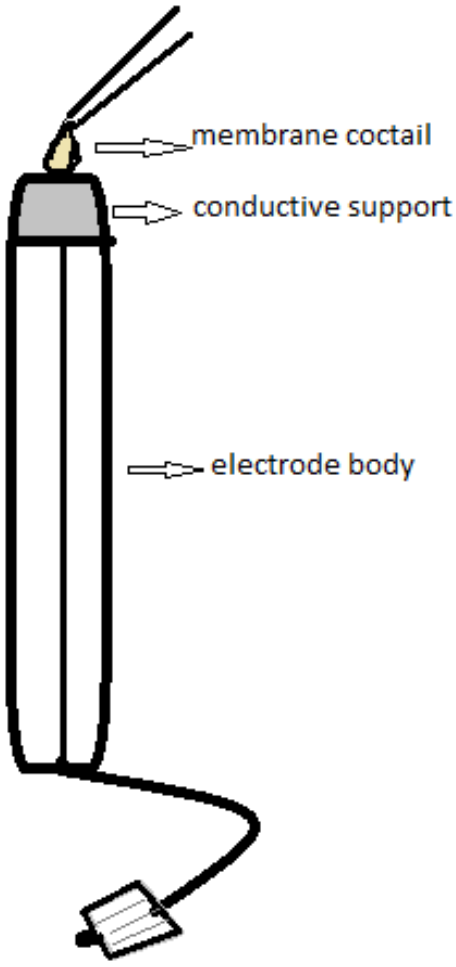
Tab.2: membrane composition (%) of ion-selective electrodes under study

Type	TPP IN(III)	HP-β-CD	DBS	FNDPE	PVC	NaTFPB	THAB	KTpClPB	TMBP	TPPC
	%(w/w)	%(w/w)	%(w/w)	%(w/w)	%(w/w)	%(w/w)	%(w/w)	%(w/w)	%(w/w)	%(w/w)
A	1,0		66,0		33,0					
B	2,0		65,8		31,1		1,0			
C	1,2		66,5		31,3	0,9				
D		1,8		67,7	30,2			0,3		
E		1,7		67,7	30,3		0,3			
F		1,8		67,8	30,1					0,4
G		1,8		67,7	30,2					0,3
H		1,9		67,3	30,0					0,8
I		1,7		67,6	30,3		0,4			
J		1,7		67,8	30,3				0,2	
M		1,9		67,8	30,0		0,2			

Abbreviations

TPP IN (lll) - 5,10,15,20-tetraphenylporphyrinato indium (III)
HP-β-CD - hydroxypropyl-ß-cyclodextrine
DBS - dibutylsebacate
FNDPE - 2-fluorophenyl-2-nitrophenyl ether
PVC -polyvinyl chloride
NaTFPB - sodium tetrakis-{3,5-bis(trifluormethyl)phenyl}borate
THAB - tetrahexylammonium bromide
KTpClPB - potassium tetrakis (p-chlorophenyl)borate
TPPC - tetraphenylphosphonium chloride
TMBP - 4-(1,1,3,3-tetramethylbutyl)phenol

Fig.6: electrode preparation



4.4. PROCEDURES

4.4.1. pH INFLUENCE EVALUATION

The influence of the pH on the answer of the electrode was evaluated between 11.56 and 5.6. The reference electrode in the junction with the pH electrode were immersed into approximately 40 mL of ibuprofen solution, c=10^{-3} mol.L^{-1} in the beaker. NaOH or H_2SO_4 concentrated solution were added drop by drop to change the pH by the unchanged volume of the solution. pH electrode was calibrated before the trial. Potential of the pH electrode was measured. The potential was plotted as a function of pH.

The same trial was provided with the 10x more concentrated ibuprofen solution.

The second trial for the same purpose was provided in the pH range 11.58-6.65 with the ibuprofen solution of concentrations 6.8x10^{-3} and 6.8x10^{-4} mol.L^{-1}.

The aim of these measurements was to find the pH value at which the difference between the potentials measured in ibuprofen solutions with one decade concentration difference at similar value of pH is not higher then 5mV, it means to find the pH range within which the change of pH does not affect significantly the measured potential.

4.4.2. SENSOR CALIBRATION

The base for the membrane composition and the experiment set-up were the data reported in [28].

The evaluation was carried out by obtaining several calibration with the ibuprofen solution in the $H_2PO_4^-/OH^-$ buffer at pH=7.5. The ionic strength was adjusted to 0.1mol.L^{-1}. 25 mL of the buffer was measured into the beaker and put on the magnetic stirrer. The reference electrode and the indicator electrode with the membrane dropped on the conductive surface were immersed into the buffer. The ibuprofen solution concentration varied from 7.9x10^{-7} mol.L^{-1} to 4.95x10^{-3} mol.L^{-1}. The variation of the solution was arranged by adding an exact volume of the ibuprofen solution by the micropipettes to the 25 mL of the buffer in the beaker. 500 µL of 10^{-3} mol.L^{-1} ibuprofen solution was added little by little, then 25000 µL of 10^{-2} mol.L^{-1} ibuprofen solution to achieve the concentration variation in the analyzed solution. The potential was recorded when the signal became stable (within ± 0.5 mV from the final equilibrium potential).

The electrodes were conditioned by immersing into the standard ibuprofen solution (10^{-2} mol.L^{-1}) before every measuring for approximately 15 minutes.
The potential response was plotted as a function of the logarithm of the ibuprofen concentration.

Every calibration was repeated (at least) three times for each sensor. The sensor characteristics were calculated as the average of values obtained from each calibration.

4.4.3. SENSOR IMPROVEMENT

As first, membrane A composed of 1% TPP In(lll), 66% DBS and 33% PVC was tested.

To improve the sensors characteristics, electrode with the membrane containing additives were prepared.

Membrane B contained 1% of THAB, a positive charged additive.
For membrane C, a negative charged additive - 0.9% of NaTFPB was used.

The evaluation of these sensors was carried out by means of the same setup as for the TPP In(lll) based sensors.

As cyclodextrines have wide application in pharmaceutical analysis, trials with HP-β-CD - based membranes (D-M) were also performed. The membranes were prepared in a way described above. According to the results with membrane based on TPP In(lll), the composition of the selective membrane also included an additive, such as tetraphenylphosphonium chloride, 4-(1,1,3,3-tetramethylbutyl)phenol, tetrahexylammonium bromide. The complete composition see in Tab.1.

Other changes were provided in the used buffer. $H_2B_4O_7$ /NaOH buffer was used instead of $H_2PO_4^-$/OH^-. pH of the ibuprofen solution was adjusted to 9.1 using this buffer. The change was done in order to improve the sensor characteristics and it followed the results of the second evaluating of the pH influence (performed in the same way as the previous measurements). The buffer was also used to condition the electrodes. They were immersed into the buffer insetad of the ibuprofen standard solution for approximately 15 min before every measuring.

Ibuprofen sodium salt instead of ibuprofen was used to prepare the standard solution, as the solubility in water of sodium salt is better than ibuprofen solubility.

4.4.4. SENSOR SELECTIVITY

Selectivity of the membrane E was studied according the IUPAC recommendation. SSM (single solution method) was used. First, a calibration with the standard ibuprofen solution in the $H_2PO_4^-/OH^-$ buffer of the pH= 7.5 was done. After this, the calibration with individual ions (Br^-, Cl^-, F^-, I^-, HPO_4^{-2}, $H_2PO_4^-$) of the same concentration as ibuprofen was done. After calibration with the suspectively interfering ion the calibration with ibuprofen was performed. The concentration varied between 7.99x10$^-$6 and 5x10$^-$3 mol.L^{-1}. The interference coefficient was calculated according to the equation

$$\log K = (E_B - E_A)/\text{slope}$$

K interference coefficient
E_B – potential of the interfering ion
E_A – potential of the ibuprofen

The solutions of the interfering ions were prepared in the same buffer as the ibuprofen standard solution.

5. RESULTS AND DISCUSSION

5.1. IBUPROFEN STANDARD SOLUTION PREPARATION

Ibuprofen standard solution was prepared by dissolving the appropriate amount of ibuprofen and dissolving it in the $H_2PO_4^-/OH^-$ buffer solution (0.05M) at pH=7.5 .

The process of solubilization of the substance was difficult and not satisfactory.

An improvement in the solubilization was achieved by adding 4.0 mL NaOH solution (0.3 $mol.L^{-1}$) to 96.0 mL of the ibuprofen solution (when preparing the solution of c=0.01 $mol.L^{-1}$). As ibuprofen standard solution, the solution with NaOH was used for evaluation of all membranes at pH=7.5.

Another improvement in ibuprofen solubilization was achieved by raising the pH of the standard solution to 9.14 and using ibuprofen sodium salt instead of ibuprofen.

As buffer, $H_2B_4O_7$ / NaOH (0.01 $mol.L^{-1}$) was used. This ibuprofen standard solution was used for the evaluation of sensors E, I, J and M at pH 9.14.

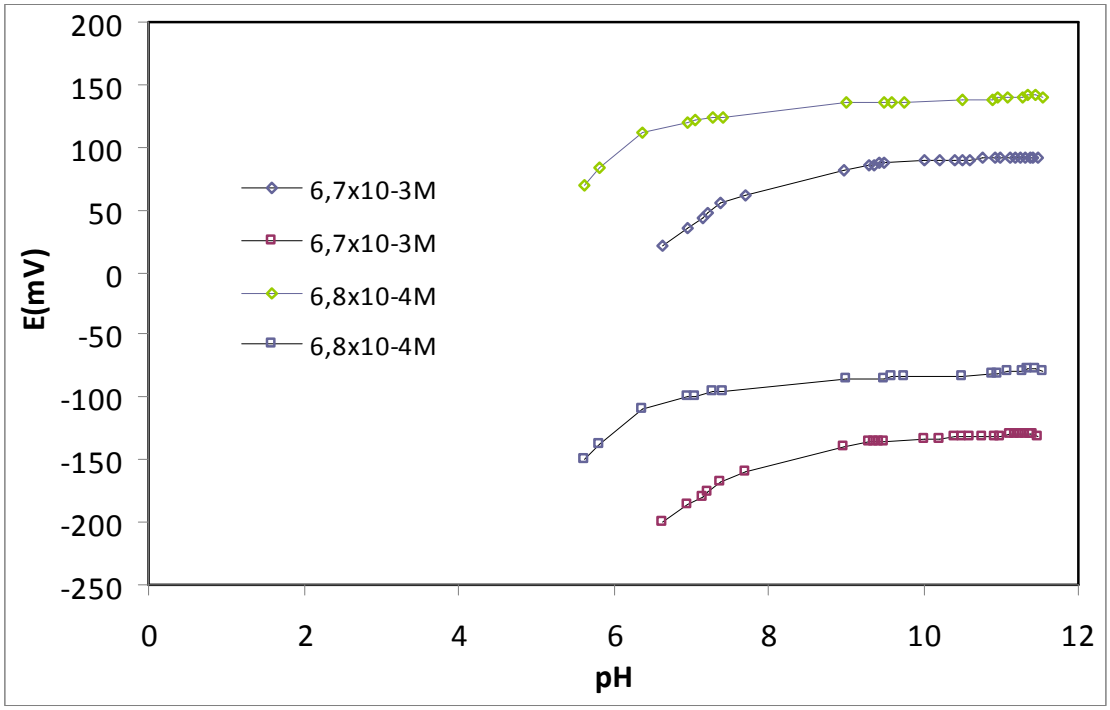
5.2. INFLUENCE OF THE pH ON THE SENSORS ANSWER

The influence of the pH on the sensors answers was evaluated in the way described above. The first experiment revealed the range of pH 6.8-7.9 as the range in which the difference of pH does not affect the answer significantly. The chosen pH 7.5 lies in this range.

The second experiment exhibited different results. The pH range in which the change of pH does not change significantly the answer of the sensor is 8.7-10.2.

The pH range was calculated according the set up described above from the diagram (Reilley diagram) in fig.7.

Fig. 7: Reilley



5. 3. SENSORS EVALUATION

Membranes with different composition were prepared. Electrodes with (TPP) In(III) and HP-β-CD as ionophores, different types of additives and solvent mediators were under study. The results of this study are summarized in the Tab.3 and 4.

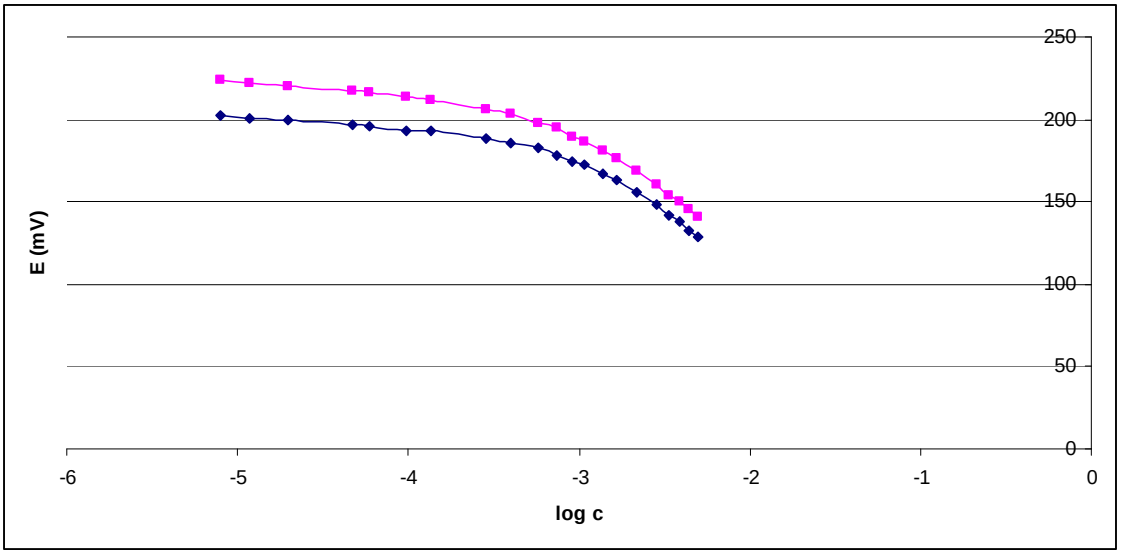
5.3.1. MEMBRANES BASED ON (TPP) In(III)

All membranes based on (TPP) In(III) were evaluated at pH = 7.5.

The membrane A composed from 1% of the ionophore, 66% DBS and 33% PVC, without a cationic lipophilic species, shows a Nernstian response with the slope of (-37.1 ±2.5) mV/dec. The lower limit of linear range (LLLR) was (1.6±1.3)x10^{-4} mol.L^{-1}. The slope (absolute value) was much lower than the theoretical -59mV.

Membrane B with the presence of 1% of cationic lipophilic species (tetrahexylammonium bromide), containing 2 % of the ionophore, 65.8 % DBS and 31.1% PVC shows a Nernstian response. It revealed an increase of sensitivity in comparison with membrane A without the additive, as described in [26]. The calibration curve see in Fig. 8. The slope of the calibration curve was (-69.9 ± 3.3) mV/dec and the LLLR (9.7 ± 2.0) x 10^{-4} mol.L^{-1} . Though this sensor showed the Nernstian response, the results were not better than in [26], where a slope (-53±1) mV/dec and LLLR 4.2x10^{-6} mol.L^{-1} is reported.

Fig.8: Calibration curves of electrode B



Membrane C, based also on (TPP) In(III) (1.2% in the composition), containing 0.9% of negative charged additive (sodium tetrakis-[3,5-bis(trifluormethyl)phenyl]borate),66.5% DBS and 31.3% PVC showed no response.

5.3.2. MEMBRANES BASED ON HP-β-CD

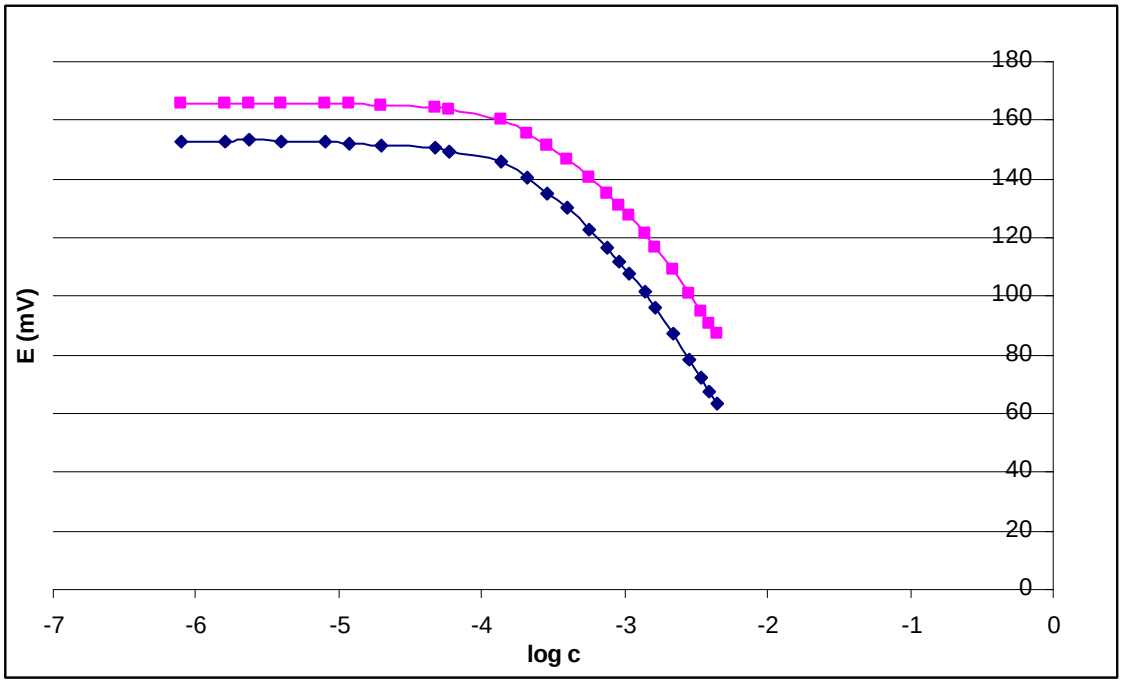
HP-β-CD as an ionophore was used to prepare following membranes: D, E, I, J, M.

The portion of HP-β-CD in the evaluated membranes varied from 1.7 to 1.9%. 2-fluorophenyl-2-nitrophenylethere (FNDPE) was used as a solvent mediator.

As additive, potassium tetrakis (p-chlorophenyl)borate was under study. Membrane D, containing 0.3% of this lipophilic specie, 1.8% of the ionophore, 67.7 % of the solvent mediator and 30.2% of the polymer did not response to ibuprofen.

Membrane E brought an improvement. As the trials with the (TPP) In(III) exhibited, a lipophilic specie of opposite charge sign as ibuprofenate ion must be used. Membrane consists of 1.7% HP-β-CD, 67.7% FNDPE, 30.3% PVC, 0.3% of the additive - tetrahexylammonium bromide. The slope of the calibration curve showed in Fig.9 was (-63.6 ±6.5)mV/dec and LLLR = (4.6±1.0)x10^{-4} mol.L^{-1}.

Fig.9: Calibration curves of membrane E at pH=7.5



The slope differs approximately 4.5 mV/dec from the theoretical -59mV/dec. The LLLR is though not satisfactory and required an improvement. The shape of the curve around the point of LLLR also does not response the expectations and can be theoretically caused by the ions present in the solution.

The change in the composition was done in the used lipophilic salt. Tetraphenylphosphonium chloride (TPPC) was used for membranes F, G, H instead of THAB. The same ionophore (1.7-1.8%), solvent mediator (67.7-67.8%) and the polymer (30.1-30.3%) as in the membrane E were used.

Membrane F containing 0.4% of the lipophilic specie showed no Nernstian response.

A little decrease in the portion of the additive to 0.3% caused improvement in the electrode answer. The characteristics of the electrode G were not satisfactory. The slope of the calibration curve was (-37.3±4.5) mV/dec. Also LLLR reached high value of $(2.2\pm0.0)x10^{-3}$ $mol.L^{-1}$.

The effort to improve the characteristics led to the increased amount of the additive in membrane H to 0.8%. This step was not successful, as the sensor showed no response.

Three more electrodes were evaluated. As the sensor E containing THAB as the additive showed the best answer, membranes I, J and M have the same constituents and differ in the their portions.

Membrane I was composed from: 1.7% HP-β-CD, 67.6% FNDPE, 30.3% PVC and 0.4% THAB. The sensor exhibited Nernstian response. The slope calculated from the calibration curve was (76.6±4.0) mV/dec and the lower limit of linear range $(1.1\pm0.0)\ x10^{-3}$ $mol.L^{-1}$, so it did not meet the expectation, as the usual value is two-three decades lower.

For membrane J, 1.7% of the ionophore, 67.7% FNDP, 30.3% PVC and 0.2% THAB was used. At pH = 7.5 the electrode shows the Nernstian response with the slope = (-55.6±2.4) mV/dec and LLLR= $(8.0\pm0.0)x10^{-5}$ $mol.L^{-1}$.

Membrane M was also evaluated at pH 7.5. The portion of the additive did not change from the previous one- 0.2% . 1.9% HP- β-CD, 67.8% FNDPE and 30.0% PVC was used to prepare this membrane. The results was: Nernstian response, but too high value of the slope, which was (-43.4±1.3) mV/dec. LLLR : $(1.0\pm0.0)\ x10^{-4}$ $mol.L^{-1}$.

Results of the evaluation of all membranes evaluated at pH 7.5, based on both (TPP) In(III) as well as HP- β-CD are summarized in Tab.3.

Tab.3: Major characteristics of ibuprofen electrodes based on TPP IN (lll) and HP-β-CD at pH=7.5

Membrane type	Slope (mV/dec)	LLLR (mol.L^{-1})
A	-37.1±2.5	(1.6±1.3)x10^{-4}
B	-69.9±3.3	(9.7±2.0)x10^{-4}
E	-63.6±6.5	(4.6±1.0)x10^{-4}
G	-37.3±4.5	(2.2±0.0)x10^{-3}
I	-76.6±4.0	(1.1±0.0)x10^{-3}
J	-55.6±2.4	(8.0±0.0)x10^{-5}
M 1	-80.0±4.0	(3.4±0.0)x10^{-4}

All sensors answering to ibuprofen had short response time (< 10s).

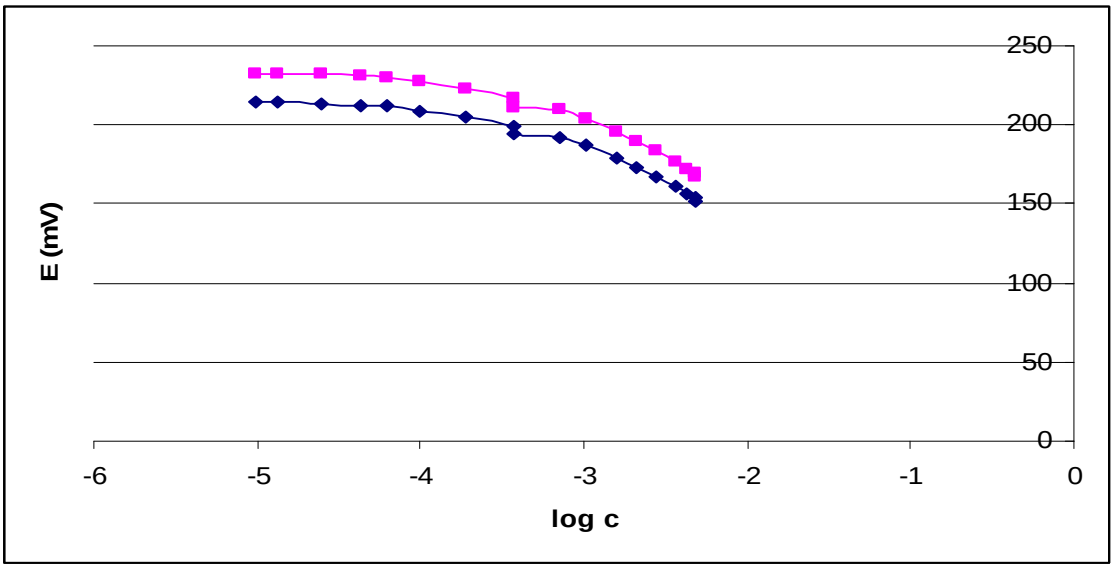
Though the sensors tested at pH 7.5 did not display the best reproducibility as it can be seen when looking at the standard deviations. The results of membranes E, I, J, M with the same qualitative composition revealed the importance of the appropriate choice of the ratio between the portion of the ionophore and lipophilic sites.

The membranes E, I, J, M were also evaluated at pH = 9.14. The trials were provided with the ibuprofenate standard solution using ibuprofen sodium salt for its preparation in the way described above.

This experiment displayed following results:

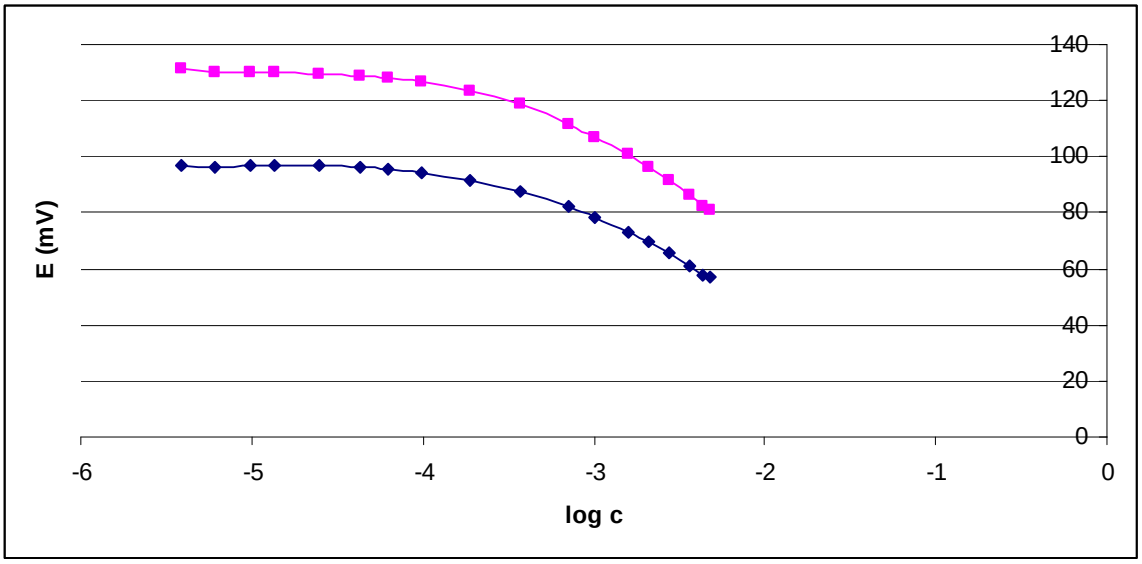
For membrane E, (calibration curve in Fig.10) the slope reached the value (-49.2±3.6) mV/dec and LLLR was (1.9±0.6) x10^{-4} mol.L^{-1} and it did not improve the electrode characteristics.

Fig.10: Calibration of membrane E at pH=9.14



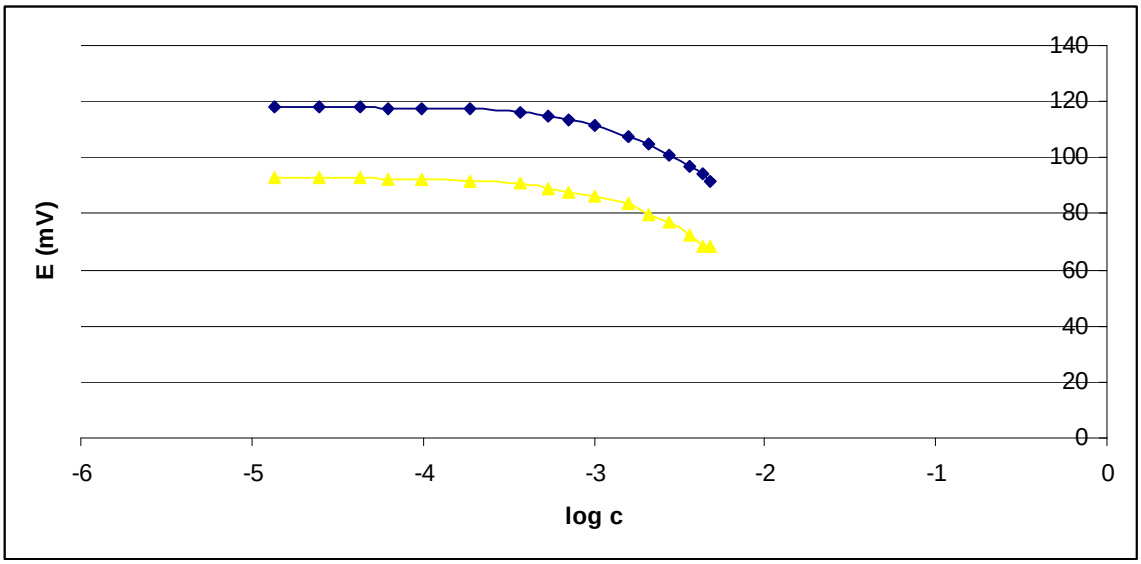
The slope of the calibration curve (see in Fig. 11) of the membrane I exhibited an increase to (-36.2±5.5) mV/dec. LLLR was (3.8±1.5) x10^{-4} mol.L^{-1}.

Fig.11: Calibration curve of the membrane I at pH=9.14



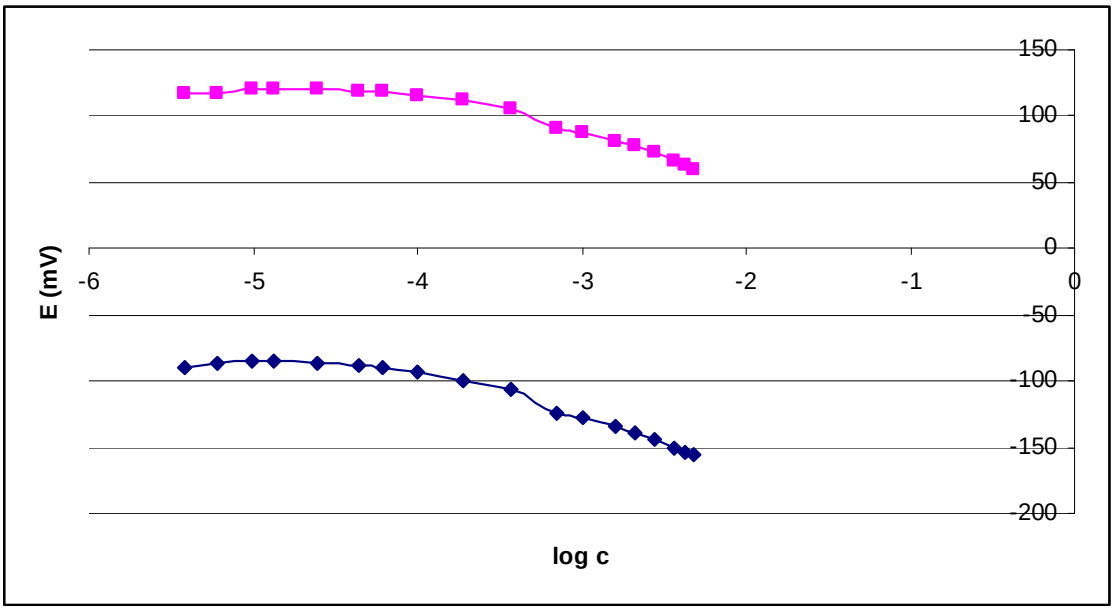
For the next membrane J, the experiment at pH 9.14 brought these results: slope-(-30.8±4.8) mV/dec and LLLR (9.7±2.0) x10^{-4} mol.L^{-1}. Calibration curve is displayed in Fig. 12.

Fig.12: Calibration curve of the membrane J at pH=9.14



The slope calculated from the calibration curve of electrode with membrane M (Fig.13) was (-38.9±1.7) mV/dec, lower limit of linear range was (6.7±1.6) x10^{-5} mol.L^{-1}, what was the best results at pH=9.14.

Fig.13: Calibration curve of the membrane M at pH 9.14



Results of the evaluation of membranes E, I, J and M at pH= 9.14 are shown in Tab.4.

Table 4: Major electrode characteristics of ibuprofen electrodes based on HP-β-CD at pH=9.14

Membrane type	**E**	**I**	**J**	**M**
Slope (mV/dec)	-49.2±3.6	36.2±5.5	-30.8±4.8	38.9±1.7
LLLR (mol.L^{-1})	(1.9±0.59)x10^{-4}	(9.7±2.01)x10^{-4}	(5.3±0.78)x10^{-4}	(6.7±1.62) x10^{-5}

5.4. SELECTIVITY

Selectivity towards different ions present in the analyte is the most important characteristic of any ion-selective sensor, expressed in the terms of the potentiometric selectivity coefficient.

Selectivity of ibuprofen-selective sensor was determined under the conditions described in section 4.4.4.

Single solution method was used.

Results of membrane E evaluation at pH 7.5 exhibited the response to ibuprofen which was closest to the Nernstian response.

Selectivity to ibuprofen of sensor E towards following ions was tested: F^-, Cl^{-1}, Br^{-1}, I^{-1}, HPO_4^{-2}, $H_2PO_4^-$.

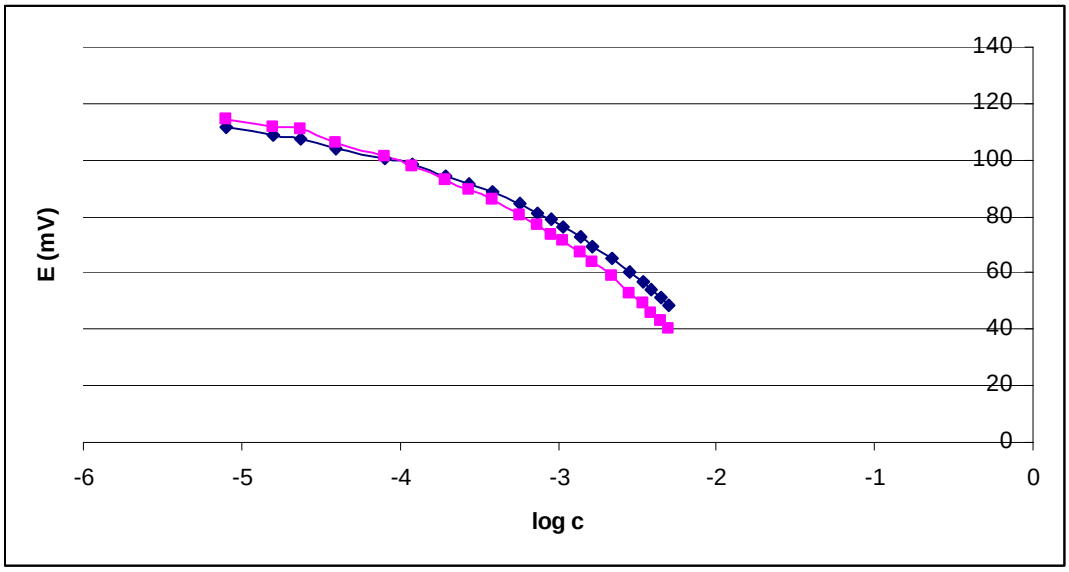
The results are summarized in Tab.5. The selectivity coefficient showed that the sensor is 10x more selective to ibuprofen towards most of the examinated ions. The graph of calibration done with chloride solution is showed in Fig.10.

These data show that the sensor selectivity is not better than the selectivity of sensor described in [28] or [29].

Tab.5: Potentiometric selectivity coefficient for ibuprofen HP-β-CD membrane sensors using single solution method (SSM); N=ion

Ion	F^-	Cl^-	Br^-	I^-	HPO_4^{2}	$H_2PO_4^-$
$K^{pot}_{Ibu,N}$	$2.58x10^{-1}$	$7.78x10^{-1}$	$4.37x10^{-1}$	$1.03x10^{+1}$	$5.79x10^{-1}$	$3.15x10^{-1}$

Fig.14: Graph of calibration with chloride solution at pH=7.5

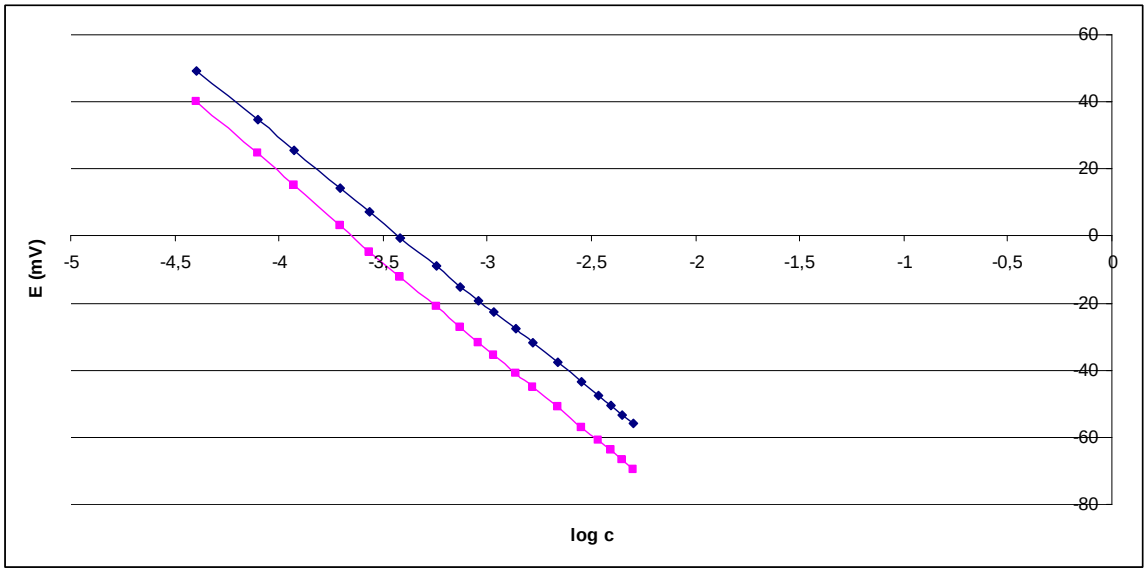


The studied sensor revealed higher selectivity to iodide. The selectivity coefficient is 10.3, what means that the sensor is ten times more responsive to iodide than to ibuprofen (see Fig. 11).

As can be seen in the graph, the curve has a very good linearity, with the correlation coefficient 0.9998.

The selectivity to iodide towards chloride ions was calculated. The selectivity coefficient is 8.33×10^{-3}, what attracts the attention and gives an offer to further studies.

Fig. 15: Graph of calibration with iodide solution at pH = 7.5



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6. CONCLUSION

The literature dealing with ion-selective electrodes and ibuprofen selective electrodes was studied.

The pH influence on the sensor was tested and the best pH range for ibuprofen was found.

Sensors with two different ionophores were evaluated. The electrodes with different additives were under study. The portion of the membrane components was changed in order to improve the sensor characteristics.

The appropriate choice of the molar ratios of ionophore and lipophilic ionic species revealed to be important to get high sensitivity and linear range.

Sensor E based on HP-β-CD containing tetrahexylammonium bromide as a ionophore and 2-fluorophenyl -2-nitrophenyl ether as a solvent mediator showed a Nernstian response -63.6 (±6.5) mV/dec and LLLR = $(4.6\pm1.0)\times10^{-4}$ mol.L^{-1} with a short response time at pH 7.5.

The lower detection limit was improved to $(6.7\pm1.6)\times10^{-5}$ mol.L^{-1} with membrane M at pH=9.14.

The selectivity test with membrane E revealed that the proposed sensor might be selective to iodide. The selectivity coefficient (iodide towards chloride) 8.33×10^{-3} makes this sensor very interesting for further study, as it can represent a new iodide-selective sensor suitable for iodide determination in some pharmaceutical products or in waters.

The study of constructed sensors did not bring better sensor characteristics than those of existing sensors and it offers a space for further experiments.

The development of ibuprofen-selective electrode continues and several membranes are under study in order to improve the selectivity and detection limit and to evaluate other sensor characteristics.

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ABSTRACT

Potentiometric determination of ibuprofen

Thesis

Ivana Šrámková

A development of a selective electrode for potentiometric determination of ibuprofen in pharmaceutical formulations was aimed in this work. Several sensors were constructed. Their response to the ibuprofen was under study. It was revealed that a choice of appropriate membrane components as well as the molar ratio of them is of high importance for an ibuprofen-selective sensor. Some of the tested sensor's response is close to the Nernstian response. The best results were achieved using HP-β-CD with the function of an ionophore, 2-phenyl-2-nitrophenyl ether as a solvent mediator and tetrahexylammonium bromide as lipophilic specie. It was found that one of the evaluated electrodes is potentially iodide-selective. The ibuprofen-selective electrode with the best features is still under study.

SOUHRN

Potenciometrická detekce ibuprofenu

Diplomová práce

Ivana Šrámková

Cílem práce bylo nalézt vhodné složení sensoru ibuprofen-selektivní elektrody pro potenciometrickou detekci ibuprofenu ve farmaceutických produktech. Bylo navrženo a připraveno několik sensorů, u kterých se zjišťovalo, jestli vykazují odpověď na analyt podle Nernstovy rovnice. Ukázalo se, že pro membránu ibuprofen-selektivní elektrody je důležitý jak výběr komponentů, tak poměr látkovéhomnožství jednotlivých složek. Několik navržených sensorů vykazuje odpověď blízkou teoretické odpovědi dle Nernstova vztahu. Nejlepší výsledky byly dosaženy s elektrodou s hydroxypropyl-β-cyklodextrinem s funkcí ionoforu, 2-fluorphenyl -2-nitrophenyl etherem jako korozpouštědlem a tetrahexylammonium bromidem jako additivem. Zjistilo se, že jedna ze zkoušených elektrod je potenciálně jodid-selektivní elektrodou. Nalezená ibuprofen-selektivní elektroda s nejlepšími charakteristikami je stále studována.