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VÝVOJ OPTICKÉHO SENSORU PRO SALICYLÁT

Diplomová práce

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Porto, Praha 2009

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DEVELOPMENT OF OPTICAL SENSOR FOR SALICYLATE

Diploma work

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Porto, Prague 2009 Marie Soukupová

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I hereby declare I have worked on this project solely by my own with the use of referenced literature.

Marie Soukupová

Abstract

We dealt with the development of optical sensor for salicylate. Sensing layer of sensor was based on metalloporphyrins. Metalloporphyrins embody great optical features. Metalloporphyrins are commonly employed as ionophores in ion-selective electrodes (ISEs). Observations from work with ISEs are applied to optical sensors. At first, membrane was constructed, based on tetraphenylporphyrin copper(II) (Cu(TPP)) and it did not show significant sensitivity towards salicylate. Further there were membranes created from tetraphenylporphyrin manganese(III)chloride (Mn(TPP)) and gallium(III)phthalocyanine chloride (Ga(PC)). Reactivity of Mn(TPP) was comparable with reactivity of Cu(TPP). Membrane from Ga(PC) displayed considerable reactivity and sensitivity towards salicylate. Later the membrane's capability was tested to detect valproate. We discovered that the limit of detection for valproate is much lower than for salicylate. Due to great reactivity and variability in membrane response in spectrophotometric assay we were not able to set the working conditions for application to flow system and to dissolution as well. Ga(PC) has thus very good potential in use for detection of valproate. Valproate is one of the most used antiepileptic drugs.

Abstrakt

V této práci jsme se zabývali vývojem optického sensoru pro salicylát. Snímající část sensoru byla založena na metaloporfyrinech. Metaloporfyriny vykazují výborné optické vlastnosti. Běžně jsou využívány jako ionofory v iontově-selektivních elektrodách (ISEs). Poznatky z práce s ISEs jsou aplikovány do optických sensorů. Nejdříve byla zkonstruována membrána, která byla založena na tetrafenylporfyrinu mědi(II) (Cu(TPP)) a nevykazovala signifikantní citlivost k salicylátu. Dále byly vytvořeny membrány z tetrafenylporfyrinu manganu(III)chloridu (Mn(TPP)) a gallia(III) ftalocyaninu chloridu (Ga(PC)). Reaktivita Mn(TPP) byla srovnatelná s reaktivitou Cu(TPP). Membrána z Ga(PC) vykazovala značnou reaktivitu a citlivost směrem k salicylátu. Později byla zkoušena i její schopnost detekovat valproát. Ukázalo se, že limit detekce pro valproát je mnohem nižší než pro salicylát. Kvůli velké reaktivitě a variabilitě v odpovědi membrány při spektrofotometrickém měření jsme nebyli schopni nastavit podmínky pro aplikaci této membrány do průtokového systému a později do disoluce. Ga(PC) má pravděpodobně velký potenciál ve využití v praxi pro detekci valproátu jako jednoho z nejpoužívanějších antiepileptik.

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1 Introduction

1.1 Context

Nowadays, societies are concerned with quality of environment around them and utensils or tools they resort in their every days life. When suddenly problems arise, analytical control comes into play giving the facts, helping to diagnose the problems and in its resolution. However, this way of thinking is quickly changing and pharmaceutical industry is one of leader players. In fact, instead of promoting the control of pharmaceuticals through representative sampling and analysis in produced lots, they are looking to continuously monitor the several productions steps. Through this concept, if a production step develops without any problems final control of the resulting product becomes of secondary importance, since problems are now detected upstream in the production pathways. Complete chemical characterization though screening tasks are impracticable and the common solution is the resort to sensor technology for the most important physical and chemical parameters. The number of chemical sensors available are relatively short and research efforts are consequently needed.

1.2 Aim of this project

The research project was concerned with the development of an optical sensor for valproate aiming the control of this active principle in pharmaceutical formulations and in studies of the last dissolution profiles. To accomplish these aims, three complementary tasks were devised, namely: 1) development of a membrane recognizing membrane, 2) study of its response characteristics under flow conditions and 3) method validation and samples assays.

My work plan was mainly centered on the first task. In it, PVC polymeric membranes containing a Ga(III) phthalocyanine as ionophore were prepared and spectroscopy properties evaluated. Main factors for membrane optimization were identified and some previous experiments with valproate, salycilate and chloride performed.

To accomplish the second task, a setup was devised based on the use of 20 W halogen-tungsten lamp as radiant source, a flow through acrylic homemade cell, a

monochromator, a mechanical chopper and a lock-in amplifier connected to a silicon semiconductor detector, according the schematic representation presented down:

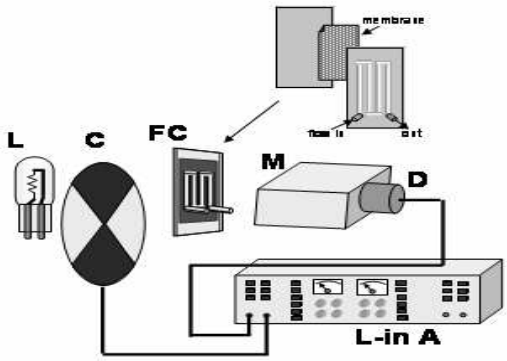


Fig. 1.: Schematic setup for optrode assay. L- radiant source; C-mechanic chopper; FC – flow cell; M – monochromator; D – detector and L-inA – lock-in amplifier.

Several experiments were also performed with this setup, but at that time any useful answer was obtained due to absence of membrane enough optimization. In the experiments, buffer solution was propelled into the flow cell and signals compared with the ones obtained by pumping salicylate buffered solutions. After optimizing the chopper rotating speed to 325 Hz, where the lowest noise from environment light was noticed, also no significant answer was obtained in for initial developed membranes.

In the third task, after having an optimized recognizing membrane, the validation procedure for valproate quantitative assessment is envisaged concerning evaluation of useful analytical range, limit of detection and quantification, main interferences, useful lifetime, repeatability and intermediate reproducibility. Parallel assay of pharmaceutical formulations by the proposed procedure and the pharmacopoeia standard procedure will enable to establish accuracy.

2 Theoretical part

2.1 Porphyrins, metalloporphyrins

Porphyrins are naturally occurring macroclyclic compounds composed of four pyrrole rings linked via methine bridges as shown in Fig. 2 (red is pyrrol cycle and blue is the methane bridge). Frequently, porphyrins coordinate a metal atom in the middle of the porphyrine ring, thus being designated as metalloporphyrins. Among the most known naturally occurring belong heme, chlorophyll and vitamin B12 (Fig. 3). Nowadays, porphyrins can be divided in two groups: natural and synthetic.

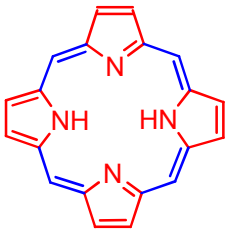


Fig. 2.: Porphyne - the simplest porphyrin

2.1.1 Natural porphyrins

Heme contains as central metal the iron cation. It has an essential role in several living functions either in isolated form as in the oxygen/carbon dioxide transport in blood or as enzyme cofactor in cytochromes involved ion oxidation/detoxification pathways. Heme can be connected to different substituents on porphyrin core, thus being known fifteen isomers, but of only one is biologically active.

Chlorophyll appears in all green plants and it coordinates magnesium as central metal atom. The chlorophyll molecule is capable to absorb sun light photons through transition of π-electrons in conjugated double bonds of the porphyrin structure.

Vitamin B12, whose ring framework is contracted by the absence of one methine bridge is called corrin although capable to coordinate a cobalt atom. Vitamin B12 is very important for good haematogenesis, participates on synthesis of DNA and ATP and is indispensible for neural system.

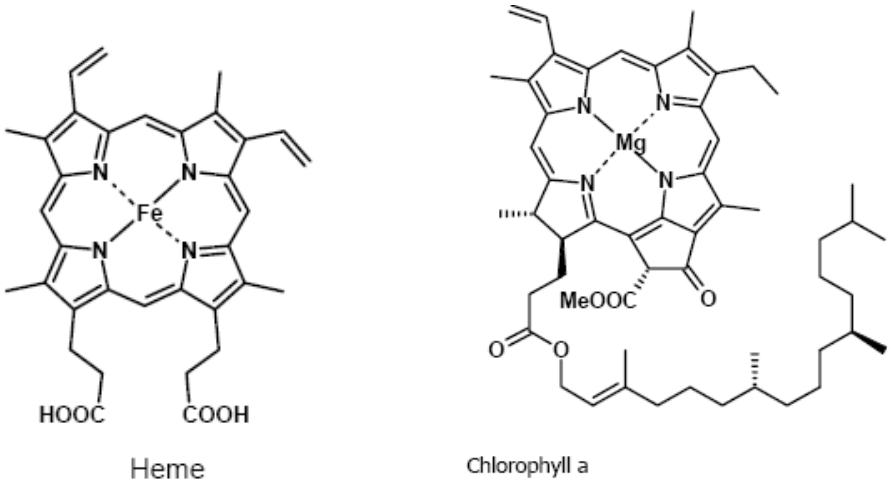


Fig. 3.: Examples of natural porphyrins

2.1.2 Synthetic porphyrins

In a similar way to what happens with numerous substances occurring in nature porphyrins could be obtained by synthetic means aiming to improve the accomplishment of different applications and uses. Thus, nowadays are synthesized many substituted ring types with different central metallic ions (or side substituents), the both changing significantly the features of porphyrin. As the first one to be described in this class one could find tetraphenylporphyrin (TPP) described by Rothmund in 1936 (Fig. 4) [1]. TPP structure includes the simplest porphyrin porphyne and four phenyl groups in meso-positions of the macrocyclic ring. By the substitution of hydrogens in meso-position has also been prepared a plenty of symmetrical and asymmetrical porphyrin's compounds. Symmetrical porphyrins are more easily synthesized than asymmetrical. Their synthesis is based on condensation of pyrrol and aldehyde whereas various reaction conditions or methods are available [1].

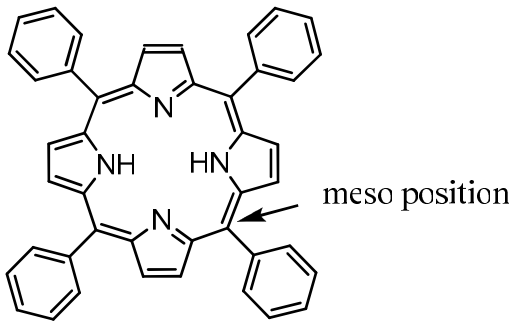


Fig. 4.: TPP

2.1.3 Analogues of porphyrin

By the modifying the position of methine bridge various species of porphyrins can be also derived, as is seen in Fig.5.

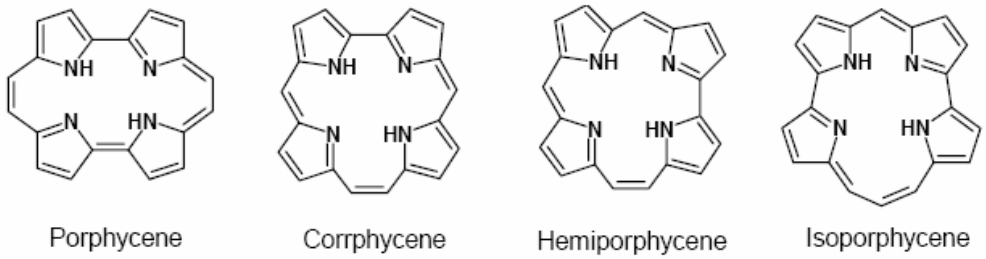


Fig. 5.: Analogues of porphyrins

Through the changing of nitrogen atom position from a pyrrol ring inverted, confused and fused porphyrins are enable (Fig. 6). In the same fashion contracted and expanded porphyrins were also reported [1].

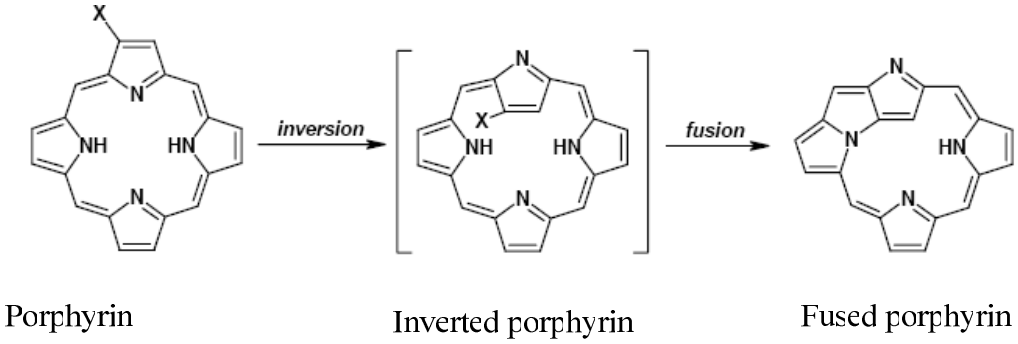


Fig. 6.: Inverted and fused porphyrins [1]

2.1.4 Physical and optical properties of porphyrins and metalloporphyrins

Porphyrin structure is planar and fully aromatic, because it contains conjugated double bonds. This extensive conjugation leads to the decrease of excitant energy to promote π-electrons and to the increase of the transition probability. That is why porphyrins and metalloporhyrins are intensively coloured. The size of tetrapyrrolic ring is good to bind almost every metal ion. Metal cation takes the position in the middle of the macrocyclic ring by coordination linkage. Coordinate means, that the metal ion has ligands than correspond to its oxidate number. Ligands create donor-acceptor binding, providing

electrons to free d-orbitals of metal. In the present case electrons come from pyrrols nitrogen atoms.

Furthermore than being coloured absorb light very strongly in the visible region of electromagnetic spectrum. The absorbance at about 400nm is characteristic and denoted as Soret band. Another typical absorption area is one or several smaller peaks at higher wavelengths called Q bands. Absorbtion spectrum can thus be used for identification of metalloporphyrins.

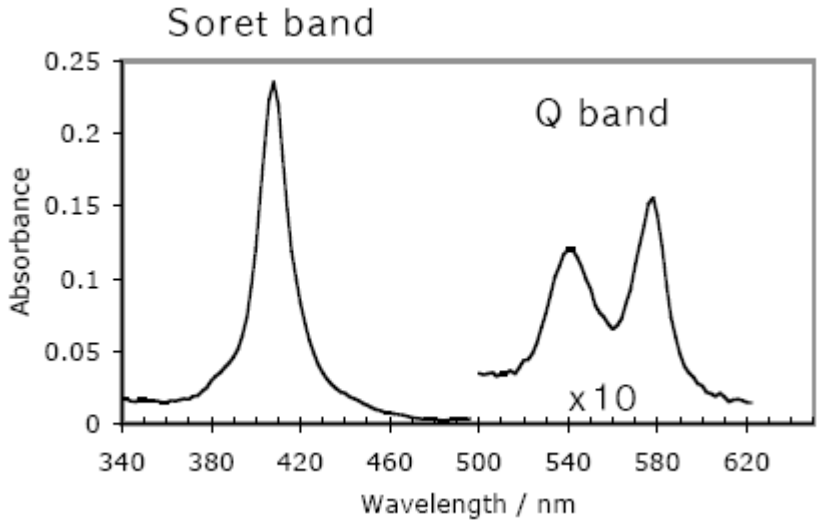


Fig. 7.: UV-VIS spectrum of Sc(III)[OEP]OH in $CHCl_3$ [2]

2.1.5 Applications

Several applications were reported in field of analytical chemistry. The application scope embraces their use as ionophores in sensors development, or as complexing agents in HPLC for the determination of transition metals and as stationary phases in immobilized metal ion chromatography [3]. Porphyrins were found utilization in medicine in therapy of cancer. Photodynamic therapy takes advantage of porphyrins as energetic anthenas, because they can be efficiently excitated by light of specific wavelength. After excitation, drugs get back to basic energetic state and transfer the absorbed quantum of energy to oxygen, which becomes very reactive against any nearby molecules. This is the beginning of apoptosis or necrosis processes of the cells. In this method have been used porphyrin's derivates, phthalocyanins and other compounds with similar features.

2.2 UV-VIS spectrometry

UV-VIS spectrometry is one of most simple and resorted spectral technique in chemical analysis. It excels with its exactness, sensitivity and experimental modesty. It is based on the measurement of intensity of radiation which is absorbed by the solution of substance. Absorption depends on wavelength of radiation, thickness of layer, structure and concentration of substance.

UV-VIS spectrometry is exploited for identification and concentration determination, further as a detector for HPLC and it can be used in automated equipments such as flow systems.

2.2.1 Transmittance and absorbance

During the absorption measuring for a fixed wavelength the radiation flow I which passed through the sample cell is compared with the intensity I_0 emerging from a reference cell.The rate of these radiation flows, respectively I and I_0 is called transmittance T. Transmittance takes values ranging from 0 to 1. T = 1 means, that the sample is totally transparent for the radiation. T = 0 shows, that the sample is opaque.

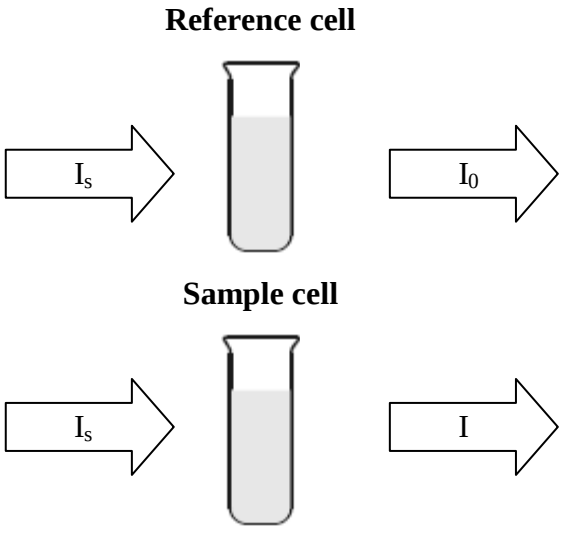


Fig. 8.: Schematic representation of the UV-VIS absorption spectrometry principle; I_s – source radiation flow; I_0 - radiation flow from the reference and I- radiation flow from the sample

$$T = \frac{I}{I_0} \qquad A = -\log T$$

Absorbance is defined as negative logarithm of transmittance and it varies from 0 to ∞. If the dependence of absorbance on the wavelength is registered, an absorption spectrum is obtained like the one shown in Fig.9.

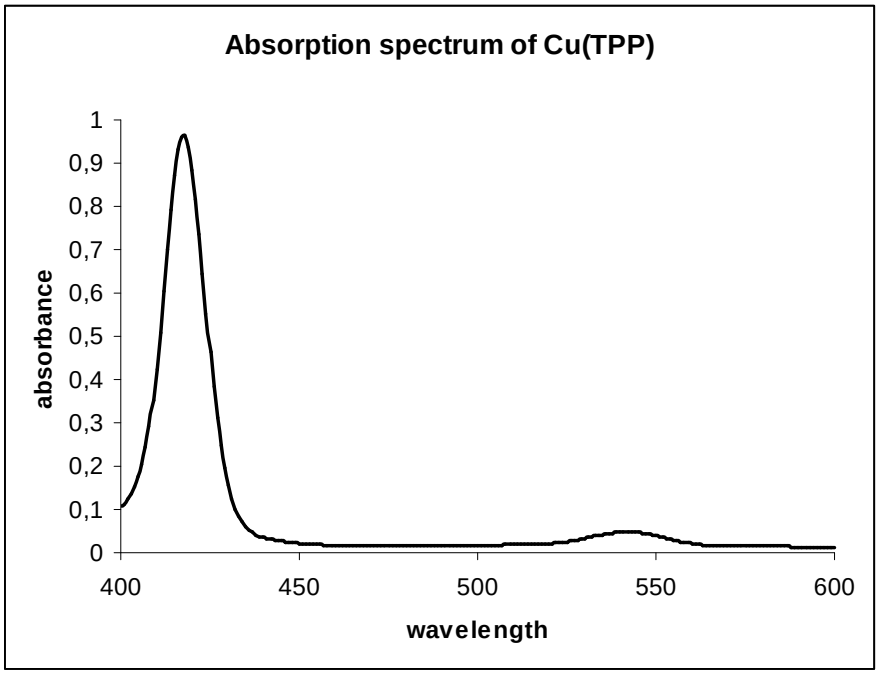


Fig. 9.: Absorption spectrum of 5,10,15,20-Tetraphenyl-21H,23H-porphine copper(II) in DMSO

2.2.2 Lambert-Beer principle

For quantitative determination of substances is used Lambert-Beer law, which is defined by the equation:

$$A = \varepsilon \times c \times l$$

where: A – absorbance of measuring solution

ε - molar absorbance factor (characteristic for given substance)

c – concentration of absorbed substance in mol/L

l – optical path or thickness of measured layer in cm

The formula of Lambert-Beer principle shows, that the absorbance increases with increasing concentration and length of the solution. Lambert-Beer principle remains valid, if A< 1-2 and under the constant conditions like temperature, monochromatic source of light, stability of substance in solution. Above those values negative

derivations are usually observed due to refractive index variation induced by concentration and loss of the independent character of absorption by the substance molecules.

2.2.3 Spectrometry in ultraviolet (UV) and visible (VIS) region

Absorption spectra in UV (190-350nm) and visible region (350-760nm) are also called electron's spectra. The electromagnetic radiation in these wavelengths causes excitation of electrons to higher energetic levels. Π- electrons are liable to excitation easily. These electrons are included in groups with double or triple bonds, so-called chromophores, and in conjugated linkages (Fig. 10).

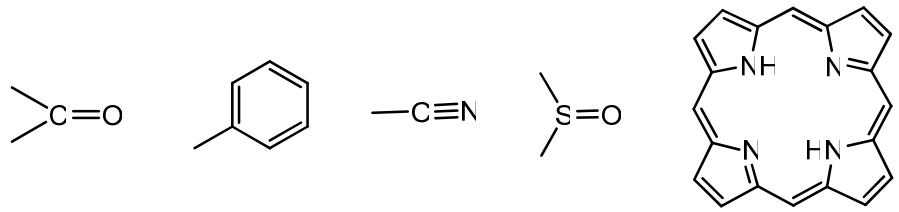


Fig. 10.: Chromophores and conjugated double bonds in porphyne structure

The position and intensity of electrons absorptive zones depends on the type and amount of chromophores and cojugated binds in substance. If the substance has enough conjugated binds, the absorption will be shifted from ultraviolet to visible area of spectrum. Other possible but less intense transitions involve electron transfer from heteroatom orbitals to molecular orbitals, between semi-occupied non-degenerate orbitals of an atom in the molecule, or intermolecular or extramolecular photoredox transitions usually known as charge transfer absorption.

2.2.4 Experimental assay

2.2.4.1 Spectrophotometer

The basic arrangement of a single beam spectrophotometer is shown in Fig 11 and contains a:

- continuous source of light
- monochromator
- sample in the cell
- detector

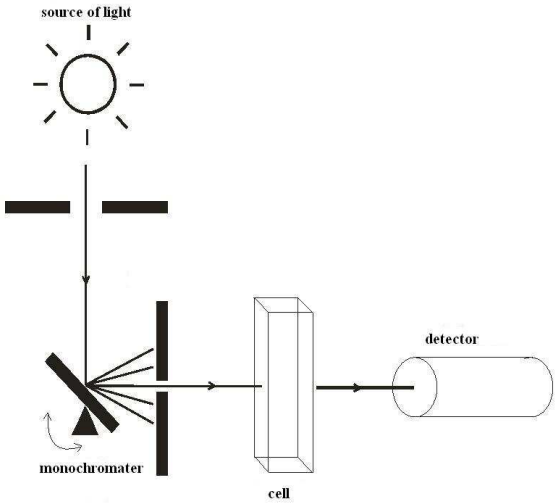


Fig. 11.: Basic arrangement of spectrophotometer [4]

2.2.4.2 Source of light

A tungsten/halogen lamp is used as visible radiant source while UV requires the use of lamps with electrical discharge on hydrogen or deuterium vapour

2.2.4.3 Monochromator

Nowadays is usually employed optical grating. With the inclining of optical grating is changed wavelength fluently. These devices present high transparency for the isolated radiation and enable regulate the exit slit operture, thus providing to isolate more or less monochromatic radiation beams. Other simpler monochromator devices are used in photometers, namely interferential or colour filters.

2.2.4.4 Material for cell

Sample fused silica glass cells are usually required for measures in UV region, although one can find nowadays disposable plastic transparent cells. For VIS region, glass or plastic material cells are more commonly resorted.

2.2.4.5 Detector

Detector enables transduction of radiant power in electrical signal by photoelectric effect. Produced current intensity is evaluated by the electric system and compared with blank, by this process is got absorbance.

Different spectrophotometric arrangements are also found with diode-array detector. Optical grating is behind sample and decomposes the light, which passed the sample and is headed to plenty of photodiodes (Fig. 12).

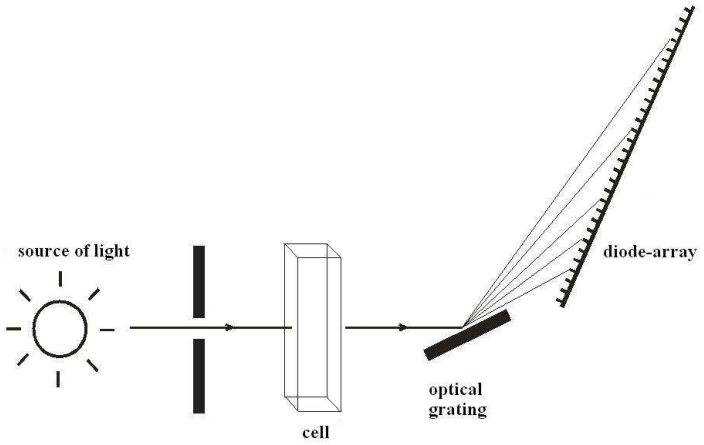


Fig. 12.: Scheme of spectrophotometer with diode-array detector [4]

There are two types of spectrophotometers: mono-beam or double-beam. With double-beam spectrophotometer sample is in real time compared with blank, because equipment has two detectors in two parallel optical ways.

2.3 Sensors, optical sensors

Chemical sensors are miniaturized devices that can deliver real time and on-line information on the presence of specific compounds or ions in even complex samples [5]. Sensors are classified in 3 groups:

1. *physical sensors*- for measuring physical quantities such as temperature, weight, pressure, electricity, movement;
2. *chemical sensors*- which measure chemical substances by using chemical or physical signals to quantitative or qualitative determination of analyte;
3. *biosensors*- employ biological sensing element to identify chemical substances.

Sensor is composed of two main parts: recognition part and physical-chemical transducer. Physical-chemical transducers give signal, which is further processed (Fig.13). According to processed magnitude transducers are divided into several groups, see Table 1:

Transducer	Measuring method
electrochemical	potenciometry, amperometry, voltametry, conductometry
optical	absorbance, fluorescence, luminiscence
piezoelectrical	
acustical	
calorimetrical	

Table 1.: Types of physical-chemical transducers

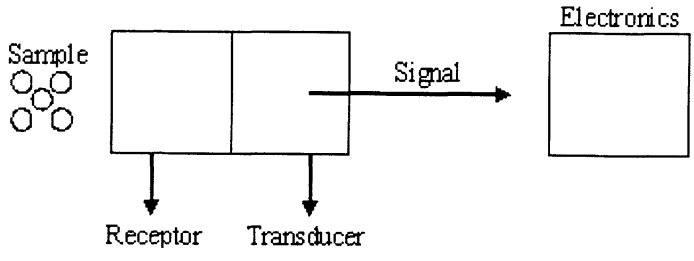


Fig. 13.: Schematic diagram of sensor [6]

An ideal sensor should be sensitive only to substance of interest, stable and should not be influenced by surroundings.

2.3.1 Properties of sensor devices and basic terms

2.3.1.1 Sensitivity

Sensitivity is usually defined as the change of output signal caused by unitary variation of the amount of analyte in the sample. Hence, useful sensors present signals that should be strong enough to be measured. Ideally, the sensitivity characteristics of a sensor should keep the same during the lifetime of sensor. In routine assay however, calibration is usually required before each metering.

2.3.1.2 Limit of detection

From the calibration curve it is possible to recognize the limit of detection and linearity range, where the sensor exactly distinguishes between particular concentrations. Limit of detection is often lower by unfavorable environment.

2.3.1.3 Lifetime

Sensitivity and limit of detection often become worst with aging of sensor of for that reasons commonly resort as its indicators. The lifetime of sensor generally depends on the weakest part, commonly the recognition part. To increase the lifetime, it is usually important to attend good conditions of storage such as humidity and temperature.

2.3.1.4 Selectivity

Selectivity is one of the most key features of a sensor device. Other compounds present in the screened samples, can induce a signal response by the sensor but the signal given in the presence of analyte should be the most intensive if sensor presents selectivity. A common way to define quantitatively the selectivity is based on the ratio between the amounts of interfering compound and the analyte that determine equal response of the sensor.

2.3.1.5 Electrical noise

Electrical noise comes from devices and network system around. It can be improved by suitable connection of devices and electric screen of sensor.

2.3.1.6 Background

Background is the signal in absence of sample, usually taken away from measured signal S=S(measured signal)-S(background) [7].

2.3.2 Optodes

An optode or optrode (similarly to the designation *elec*trode) is a miniaturized optical device consisting of a selective layer (membrane), a transducer (converts radiation energy to electrical energy) and a reading device. Optodes for chemical purposes are nowadays a subject of intense research in many laboratories; their popularity is growing due to the low-cost and easiest miniaturization enabled by growing knowledge in micro-mechanics, microelectronics, data communication protocols, etc. Other important aspects are the possibility of far-distances screening, absence both of electrical noise and of a signal comparison reference (demanded in electroanalytical techeniques) and on-line monitoring in human hazardous environments.

Like other chemical sensors, one of the most common classes of optical sensors is that based on ionophores – organic and inorganic compounds that selectively bind to ions. Among the ionophore-based sensors, the most widely employed forms of transduction (except potenciometry) are just the same optical principles used in absorbance and fluorescence methods [8]. Reflectance and luminescence are applied as well. Work with optodes covers different regions of spectra (UV, VIS, IR, NIR) and allows the measurement of intensity of light, refractive index, scattering, diffraction and polarization [9].

2.3.2.1 Optical fibre

Optical fibres (waveguide) are exploited for construction of optrodes, once they provide a simple means for probe based monitoring. Optical fibers are designed having a high refractive index core, a low refractive index cladding and an external protection (jacket) layers. Core is made from various sorts of step-index or graded index glass or plastic. Light diffuses inside of fiber by total internal reflections on interface between core and cladding almost without intensity loss even considering lengths of some km (Fig. 14).

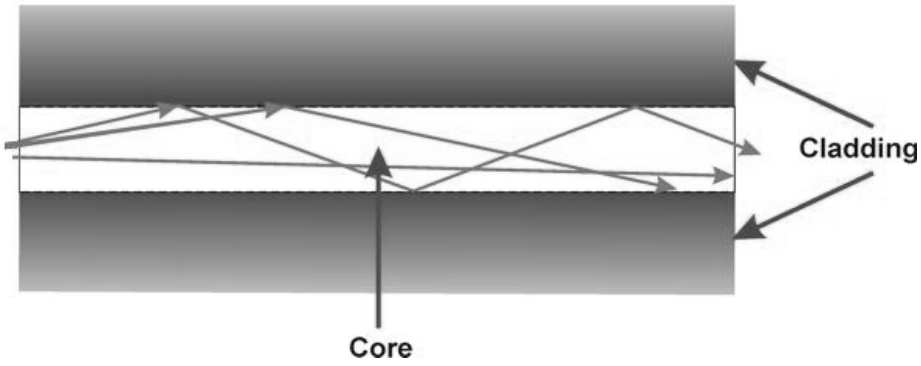


Fig. 14.:Scheme of optical fiber

2.3.2.2 Extrinsic and intrinsic mode

There are two basic sensing configurations depending on the position of optical fibre and sensor recognizing layer. Extrinsic mode means, that the sample is directly irradiated by light traveling in the optical fibre and the interactions occur at the end of the waveguide. Intrinsic mode exploits the fibre itself as transduction element [9]. In this case optical conductor is not only for wiring of light, but the chemical changes in the surrounding of waveguide are observed due to the alterations in conditions for guiding of light inside. This principle known as evanescent wave is based on the fact that electromagnetic fields of light penetrate slightly on the recognizing layer during reflection, thus being absorbed or starting a photoluminescent process. Basic scheme of these two types are shown in Fig. 15.

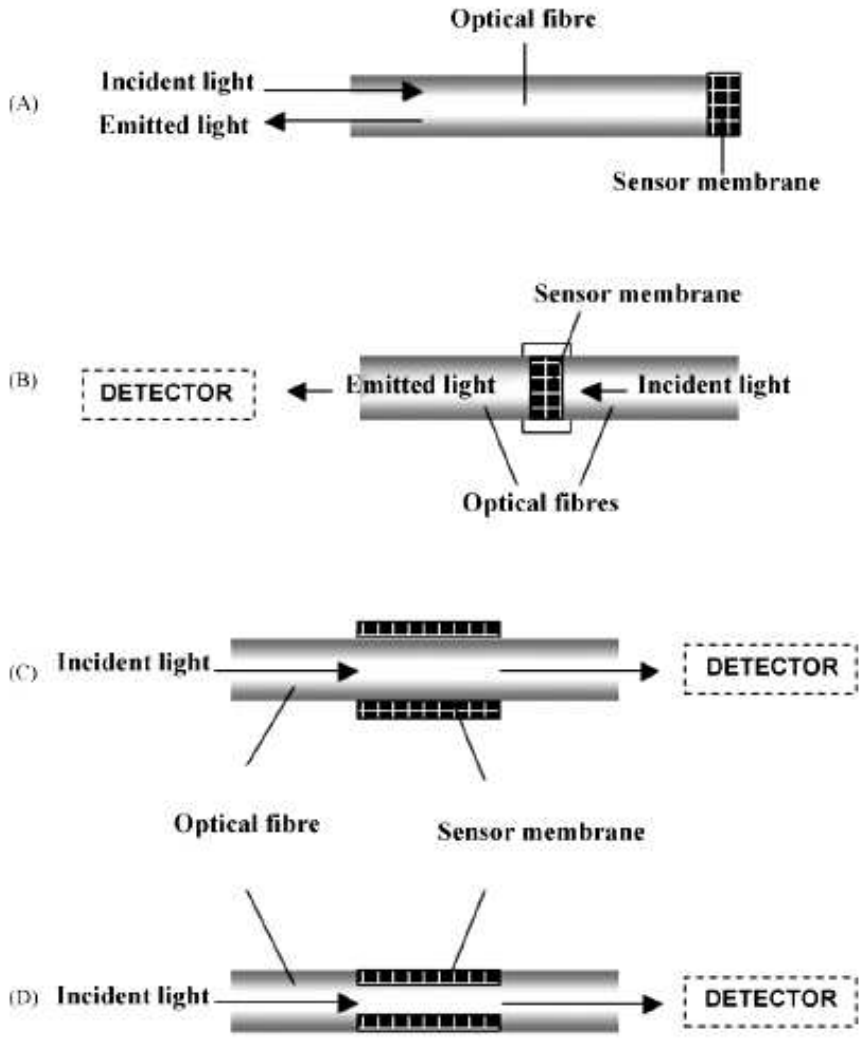


Fig. 15.: Types of configurations of optical fibres. Extrinsic mode: A and B, Intrinsic mode: C and D. On D optical fibre cladding was firstly removed and the recognizing layer was placed instead [9].

2.3.3 Ion-selective optodes

Concerning recognizing layers, ion-selective optodes are compositionally very similar to ISEs (ion-selective electrode) [8]. They consist of a polymeric organic layer with optimized proportions of:

- plasticizer
- lipophilic ionic additive
- ionophore
- chromoionophore.

Commonly, it is the chromoionophore that enables to distinguish the ISEs membrane from that used in ion-selective optodes. Being much older in research activities, experience acquired from the work with ISEs is initially applied to develop useful ion-selective optodes.

2.3.3.1 Polymeric membrane and plasticizer

The most common material is poly(vinylchloride) (PVC), because of the good mechanic characteristics, low price and easy plasticization. If the PVC is not suitable, other polymeric substances as silicone rubber or polyurethanes (PU) are also employed, but producing significant changes in the layer final response as can be seen in Fig. 16.

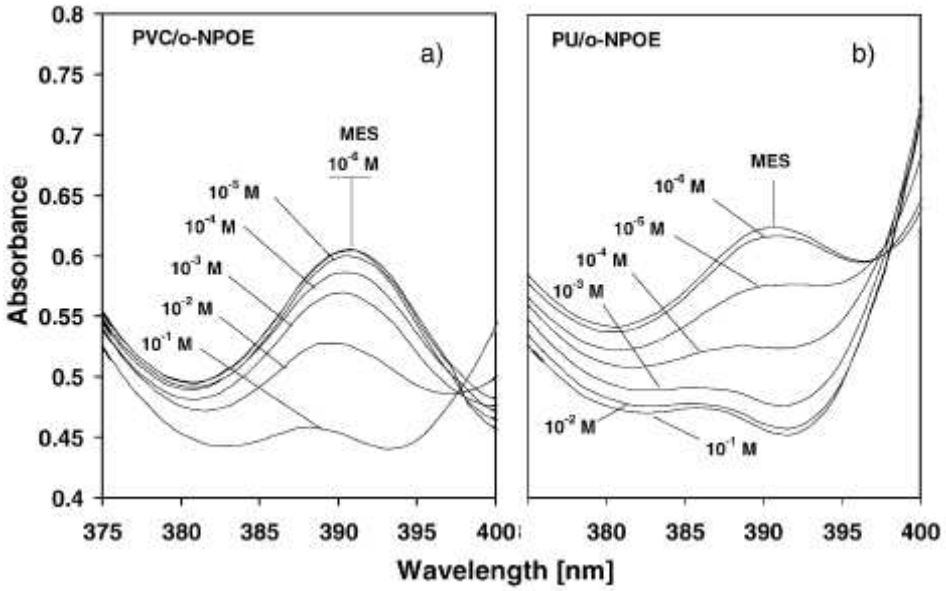


Fig. 16.: The influence of polymeric matrix in spectra of film with In(III)[OEP]Cl and 20% mol of additive, conditioned in aqueous solutions of chloride anions in MES buffer, pH=5,5; a) PVC and b) PU [10]

The membrane is usually prepared by dissolving the monomer of oligomer giving the polymeric net in an organic solvent as tetrahydrofuran (THF). Further the solution of polymer and solvent is poured into a glass form or used to cast support materials by using dip-coating or the spin-coating techniques. In the first, the material to be coated is removed from the PVC solution at a constant speed which mainly determines the final film thickness (about 1μm thick). In the spin-coating procedure a small volume of PVC solution is uniformly spread over the support spinning at a constant speed, usually producing very thin films (less than 1μm thick).

Plasticizers addition to PVC membranes is essential and has direct influence on the final performance of ISEs and optrodes, once it allows modulate ductile characteristics of PVC. By increasing the proportions of the plasticizer (also called as mediator solvent) the obtained polymeric net becomes malleable, enabling to adopt different shapes

without breaking or cracking. The usual weight ratio for ISEs is about 1:2 on PVC/plasticizer with which a highly viscous membrane is obtained. It means that the amount of plasticizer is two times higher than polymer and plasticizer acts as solvent for other components in membrane [8]. It was recognised that the nature of plasticizer also can have an effect on the selectivity of response of ISEs and optodes. For instance, with the most commonly used plasticizers ortho-nitrophenyl octyl ether (o-NPOE) and dioctyl sebacate (DOS) it was evidenced, that cation-exchanger ability of membranes with o-NPOE is higher for divalent cations than the membrane with DOS [8]. As a consequence other figures of merit like sensitivity or detection limit could be in this way modulated (see Fig. 17), although the predominant factor of selection of plasticizer depends on the compatibility with ionophore and aim of application [8].

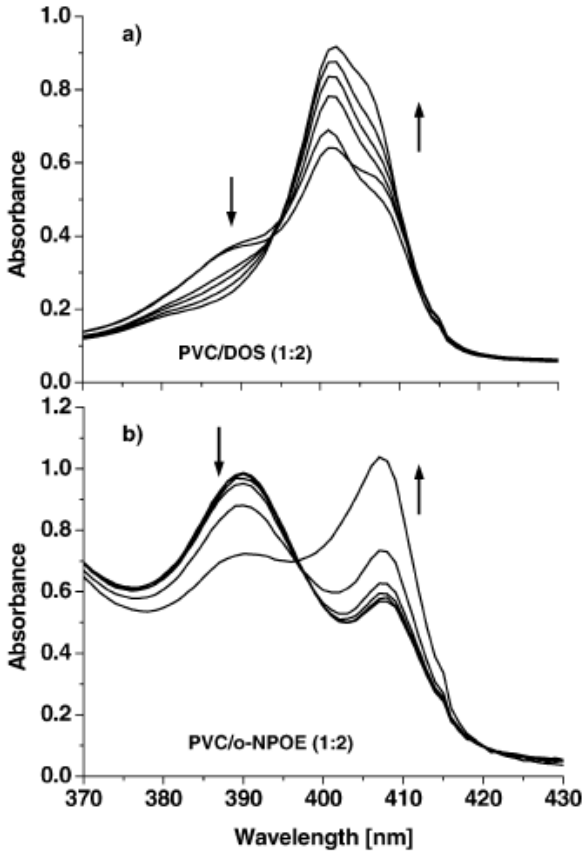


Fig. 17.: Changes in spetra in PVC film consists of a) DOS or b) o-NPOE, under conditions of chloride aqueous solutions of different concentrations; MES buffer, pH=5,5 [10]

2.3.3.2 Lipophilic ionic additives

Another important component of a sensor membrane resides on its composition regarding the ionic additive. Ionic additives should be sufficiently lipophilic to remain in the membrane during the contact with the aqueous solutions and are of the cationic or anionic type (in common terms a membrane is said to contain anionic or cationic sites). Anionic tetraphenylborate derivates are employed where the ionophores serve as positive charged carriers and in this case anionic sites are used to preserve the electro neutrality conditions in the membrane phase. In second group are included tetraalkylamonium salts. They are needed where the ionophores operate as neutral carriers, by ensuring cationic analyte extraction into the membranes phase. Optimal selectivity of the sensor is thus dependent on the appropriate choice of ionic additive. Their effect have been explained by the stabilization of ion-ionophore complexes and by ensuring permselectivity, it means, that only required ions are extracted from the solution without important amount of a counter ions [8].

Basic mechanisms between ionophore and ionic additive are schematically displayed in Fig. 18. When the ionophore is used as neutral carrier, the charge of ionic additive is opposite from the ion of interest (Fig. 18a and 18b). When the ionophore is employed like charged carrier, the ionic additive has the same charge as the ion of interest (Fig. 18c and 18d).

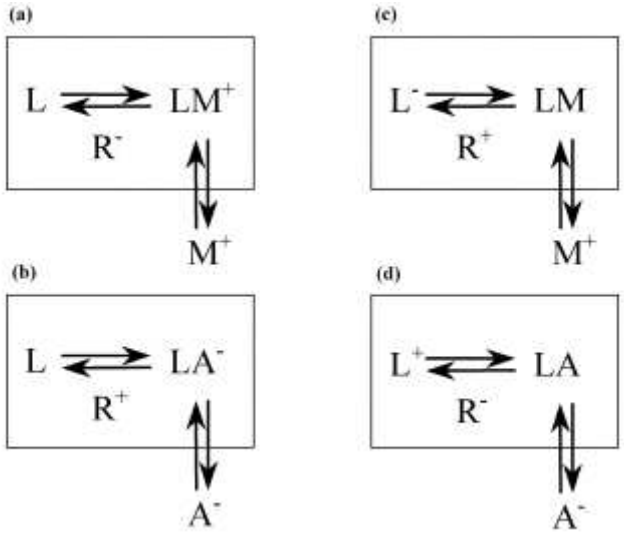
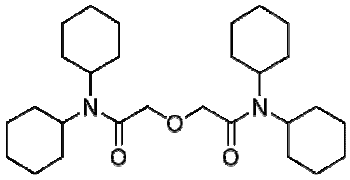


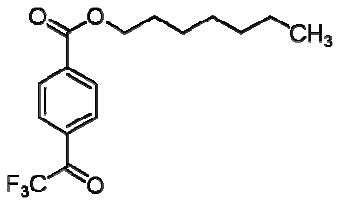
Fig. 18.: Basic schemes of procesess in the membrane; R-lipophilic additive, M^+ - cation and A^--anion from the sample, L is neutral ionophore and L^- or L^+ is charged ionophore [8]

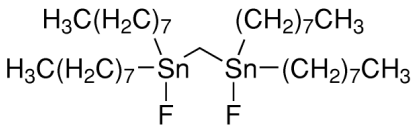
Optodes usually don't need to be permselective, however the ionic additives are still being used, because they support the total ionic strength and electronetrality in membrane.

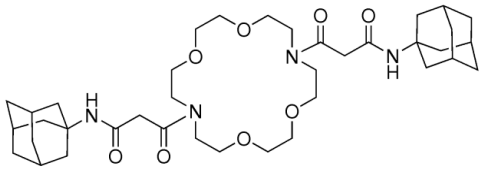
2.3.3.3 Ionophore

The main component of membrane (recognizing layer) is the ionophore. It is the recognition component, which more or less selectively binds ions. There are many species of ionophores and they are commercially available for ISEs and optodes also (Fig. 19). Though, there are so many kinds of ionophores, we can observe similar general principles and structure features, which can be used in targeting designing and developing of ionophores.

a) 

b) 

c) 

d) 

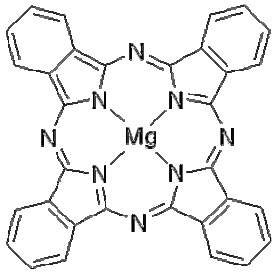
e) 

Fig. 19.: Examples of commercially available ionophores for ISE from Sigma-Aldrich company; a)Ionophore for Ca^{2+} b)Ionophore for carboxyl and hydroxide groups c) Ionophore for determination of F^- d) Ionophore for Mg^{2+} e) Ionophore for CN^- based on phthalocyanin

2.3.3.3.1 General characteristic of ionophores for sensors

A selected target ion has to be extracted from aqueous phase to organic lipophilic phase (usually the mediator solvent supported by PVC) in order to ion-selective optodes or ISEs function properly. In the absence of the ionophore membrane ion-extraction is governed only by the lipophility character of ions established by the so called "Hofmeister selectivity series" [8]:

Cations: $Cs^{+} > Ag^{+} > K^{+} > NH_4^{+} > Na^{+} > Li^{+} > Ca^{2+} > Pb^{2+} > Cu^{2+}$
Anions: $ClO_4^{-} > SCN^{-} > I^{-} >$ salicylate $> NO_3^{-} > Br^{-} > NO_2^{-} > Cl^{-} > HSO_3^{-} >$ acetate $> SO_4^{2-} > HPO_4^{2-}$.

Process of complexation between ionophore and analyzed ion has to be fast enough to use compound as ionophore and generally introduces a unique selectivity pattern that differs from the Hofmeister series. Formed complexes should be sufficiently stable but involved in a dynamic equilibrium to guarantees the sensor reversible response. If ion is fixed to strongly, reaction gets irreversible and sensor can be used only to perform a single measure. Furthermore, a limiting factor is that the ionophore must be lipophilic to stay as long as possible in membrane phase.

2.3.3.3.2 Methods to prepare selective ionophores

There are two different ways to prepare a proper ionophore: by using rational or biomimetic approaches. In rational procedure are included features as structure flexibility, molecule topology, charge, π – electrons interactions, H-bridges, bindingsite geometry. Biomimetic designing exploit similar parameters as rational, but based on natural example. Selectivity of ionophore is mainly studied after incorporation potential compound to sensor [8].

2.3.3.3.3 Cation-binding and anion-binding ionophores

Cation-selective ionophores are mostly macrocyclic compounds (Fig. 19d) as calixarenes, crownetheres. Acyclic cation-binding ionophores are employed also (Fig. 19a). These molecules are very flexible, have "chelate effect" and long chain to easy

adapt molecule of cation. Cation-binding ionophores are more often than anion-binding, because anions are geometrically more different.

Anion-binding sensors are based on these structures: porphyrins and phthalocyanins (will mention later), organometallic compounds, hydrogen-bonding and covalent binding (see Fig. 19b, c, e).

2.3.3.4 Chromoionophore

In many examples it is the chromoionophore which differentiates ion-selective optodes from ISEs. These optodes contain additional chromoionophore to provide optical activity to the recognizing ionophore chemistry. In some cases it can play as ionophore optical active itself. But usually chromoionophore is separate part from selective ionophore. As other components chromoionophore has to be lipophilic to stay in membrane phase. Prefix "chromo" is connected with the concepts like coloured, dyes and colour and indeed chromoionophores are coloured compounds. They must exhibit changes in UV-VIS spectral region or fluoresce.

Ionophore mediates the extraction of the ion of interest into the film a leaves H^+ proton. Chromoionophore binds H^+ proton and shifts itself colour's features. Thanks to these procedures the necessary neutrality in film keeps preserved. To maintain neutrality of membrane mechanisms of optode are based on ion-exchange of two cations or anion or co-extraction of two counter charge ions. In equations below display the mechanisms of equilibrium governing ion exchange in the optode membrane:

a) $\mathbf{CH^+ + L + I^+_{(aq)} \Leftrightarrow C + LI^+R^- + H^+_{(aq)}}$

b) $\mathbf{C + L^+R^- + H^+_{(aq)} + X^-_{(aq)} \Leftrightarrow CH^+R^- + LX}$

Fig. 20.: Mechanisms in optode membrane, where C is the chromoionophore, R^- is anionic site, L is the ionophore, I^+ and X^- are ions of interest [2]

In this figure is illustrated proton response of pH-dependent chromoionophores. The film could represent cross-sensitivity to pH. This problem is solved by using of suitable buffer in sample phase [2,8].

Several general chromoionophores are commercially available to be resorted in evaluating a particular membrane with optical transduction (see Fig. 21).

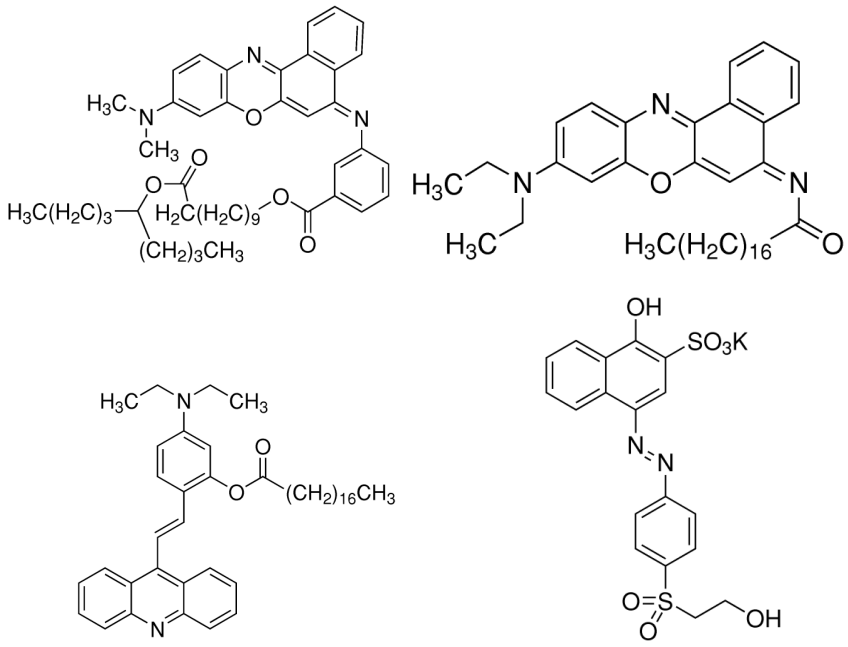


Fig. 21.: Examples of commercial chromoionophores based on benzophenoxazine (a,b), azo group (d) and acridin (c)

2.3.4 Anion-selective optodes based on metalloporphyrins

It is very important to monitor and determine anions in many fields of analysis e.g. clinical, environmental and food. The amount of anion-selective ionophores is lower than for cations. Thus molecule (for anion sensing) should be designed with well-advised of all factors to improve the selectivity and sensitivity.

Metalloporphyrins are some of few types of this class of ionophores species. They can bind anion to axial position toward their planar structure (see Fig. 22).

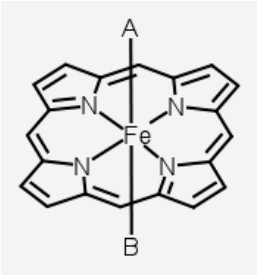


Fig. 22.: Porphyrin structure with iron as central metal cation and axial ligands are represented by A and B

Mn(III)-porphyrin was first employed as ionophore component in the polymeric membrane of ion-selective electrodes in 1980s. At that time was shown the utility to modify the traditional Hofmeister series in having enhanced selectivity towards chloride and salicylate [10]. Moreover, it was pointed out that the selectivity to anions arouses from the specific anion coordination to the central metal cation in the prophyrin ring. The improvement of selectivity, detection limit and working mechanism where afterwards achieved by setting conditions in membrane and in sample solution as well.

Every metalloporhyrin can be characterized by using its electron-absorption curve. As mentioned above, absorption curve is consisted of two typical maxima: the Soret band (around 400nm) and Q bands (in higher wavelengths). Position of λ_{max} and intensity of absorbance is dependent on central metal cation, axial ligand and structure of molecule.

2.3.4.1 Dimer-monomer equilibrium of metalloporphyrins

The response slope of ion-selective electrodes in potenciometry is called “Nernstian”, when the slope corresponds to 59 mV/ decade for single charged ions in the Nernst equation:

$$E = E^0 - \frac{RT}{zF} \ln \frac{a_{1(waterphase)}}{a_{1(organicphase)}}$$

Fig. 23.: Nernst equation where: E - potential, E^0- standard potential, R- universal gas constant, T- absolute temperature, z- numer of electrons, F- Faraday's constant, a - acitivities of ions (activity in mambrane phase is constant and buffered by the ionophore)

Super-Nernstian behavior of response was often observed with using metalloporphyrins (Super-Nernstian behavior- the slopes of calibration are much higher than is calculated by Nernst-equation). This feature was ascribed to ability of metallporphyrins to furthermore create dimer-monomer equilibrium, thus concomitantly changing the overall charge in the membrane [10].

In organic medium, molecules of metalloporphyrin dimerises, bounded through a hydroxide ion bridge (see Fig. 24). This equilibrium can be observed in UV-VIS spectrometry measurements since the changes between monomer and dimer structure produce batochromic or hypsochromic shifts in Soret band. Usually, the dimeric form leads to the Soret band absorption at lower wavelengths. When the anion in sample solution is extracted into the membrane it induces the cleavage of the hydroxide ion

bridge (Fig. 25) and consequently the amount of monomer will increase, while the amount of dimer decreases (Fig. 26). These variations are seen in absorption spectrum of metalloporphyrin. Hence, metalloporphyrins can function as ionophore and chromoionophore together. They are of advantageous usage, in contrast with application of two compounds for each action, since they could lead to simpler membranes with reduced components leaching problems.

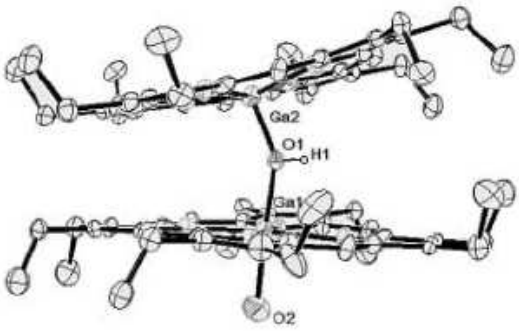


Fig. 24.: Proof of creation dimeric structure from X-ray crystallography, Ga(III)octaethylporphyrin dimer

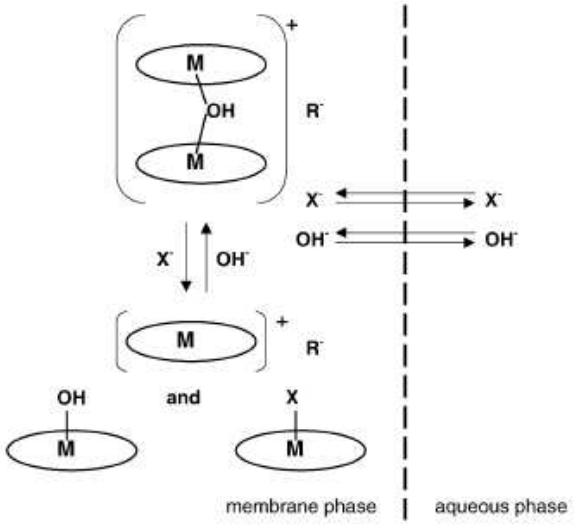


Fig. 25.: Equilibrium between dimeric and monoric forms, X^- - anion in sample solution break hydroxo dimer to monomers [10]

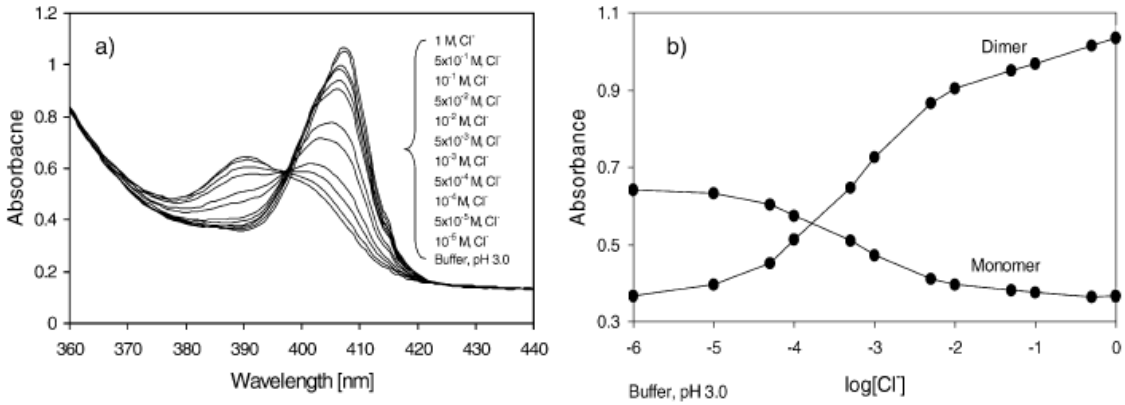


Fig. 26.: Spectra of optode film containing In(III)[OEP]OH at 1 wt% and anionic additive 45 mol% (relative to In[OEP]OH) as a function of bathing chloride ion concentration added to 0,05M phosphate buffer, pH 3,0 (a) and changes in absorbance of monomer (λ= 408nm) and dimer (λ = 390nm) as the function of chloride concentration (b) [10]

2.3.4.1.1 Improvement of dimer formation

There are few factors needing to be settled to ensure dimer formation of metalloporphyrins in the sensor layer. Firstly, appropriate choice of the mediator solvent is needed. Plasticizers with high dielectric constants create more hydrophobic environments thus ensuring extended amount of the dimeric form in membrane to keep low electroneutrality in the membrane. Organic solvent o-NPOE has dielectric constant ε=24, while DOS has ε=4,6. Though, DOS exploited as plasticizer ensured enough dimeric form of metalloporphyrin in membrane [10]. Secondly, through addition of lipophilic anionic sites up to 45 mol% (relative to metalloporphyrin), enables to stabilize dimers. For ISEs it is generally used 10-20 mol% [10]. Finaly, in the case of employing metalloporphyrin with large substituents in meso-position it can happen, that the steric hindrance (among substituents) will not allow dimer formation, albeit the lower leaching profile of metalloporphyrin achieved.

3 Experimental part

3.1 List of chemicals

MES, low moisture content (Sigma-Aldrich)

MES, sodium salt (Sigma-Aldrich)

Tetrahydrofuran (Fluka)

Methanol (Merck)

2-nitrophenyl-octylehter (Fluka)

Natriumsalicylat (Merck)

Sodium hydroxide (Fluka)

Poly(vinylchloride) high molecular (Fluka)

Natriumnitrit (Merck)

Sodium nitrate (Merck)

Natriumhydrogenocarbonat (Merck)

Gallium(III) phthalocyanine chloride (Sigma-Aldrich)

5,10,15,20- Tetraphenyl- 21H,23H- porphine copper(II), synthetic (Sigma-Aldrich)

5,10,15,20- Tetraphenyl- 21H,23H- porphine manganese(III) chloride, 95% (Aldrich)

2-propyl-pentanoic acid, sodium salt (Sigma)

Sodium benzoate (BDH Chemicals Ltd Poole England)

Tri-Natriumcitrat-2-hydrat (Riedel-de Haën)

Citric acid (Sigma)

Acetylsalicylic acid (Sigma)

Hydrochloric acid 32% (Panreac)

Dimethylsulfoxide (Sigma)

Ortho-Phosphoric acid 85% (Fluka)

Sodium chloride (Riedel-de Haën)

All reagents (analytical grade) were used without further purification.

3.2 The preparation of solutions

For the preparation of all solutions deionised water (Millipore Milli-Q RG system) was prepared just before use. Buffer solutions were prepared as stock solutions and stored at room temperature.

3.2.1 Buffers composition

Preparation of MES buffer solution (0.1M) (MES)

MES was prepared by dissolving MES acid (4.88g) in water and volume was filled in 250ml. After dissolution the pH was adjusted to 3.9 (with 0.1M MES sodium salt solution).

Preparation of citrate buffer solution (0.01M) (CitBS)

The series of citrate buffers was prepared with mixing citric acid solution 500ml 0.02M and sodium citrate 500ml 0.02M. Mixed volumes are in Table 2.

	Ml(citric acid 0,02M)	ml(sodium citrate 0,02M)	water
pH=3.0	46.50	3.50	ad 100ml
pH=3.5	38.50	11.50	ad 100ml
pH=4.0	33.00	17.00	ad 100ml
pH=4.5	26.75	23.25	ad 100ml
pH=5.0	20.50	29.50	ad 100ml

Table 2.: Volumes used to preparation series of citrate buffers

Preparation of glycin-phosphate buffer (0.05M) (GPBS)

GPBS was prepared by dissolving of glycin (0.9383g) in water and volume was made up to 250ml. Glycin solution was adjusted to pH 3.0 with phosphoric acid.

3.3 Preparation of PVC membrane with metalloporphyrin

Preparation of Ga(PC) membrane

The membrane was prepared from PVC and Ga(PC). PVC (1.44g, up to 100 w/w%, total mass was 2g) was dissolved 1 day in THF (30mL) in beaker. In the second beaker was dissolved Ga(PC) (60mg, 3 w/w%) with o-NPOE (0,5g, 25 w/w%) and THF (3mL). To better dissolving of this mixture was used ultrasonic cleanser. After dissolving of PVC the mixture with Ga(PC) was added to dissolved PVC. The second beaker was carefully washed by THF (3mL) and putted to beaker with PVC also. Mixture of PVC, Ga(PC), o-NPOE and THF was mixed very well for few minutes with glass rod. Thereafter mixing the blend was spread on Petri dish, which was washed with water, ethanol and methanol before. Then covered Petri dish was left 4 days in fume hood to evaporate THF at ambient temperature (22 ± 2°C). Prepared membrane is shown in Fig. 27. Membrane should have a thickness of 150 ± 2μm [11] and be kept in the dark place to minimize photoreaction and decomposition.

Preparation of Cu(TPP) membrane

Membrane with Cu(TPP) was prepared in the same way as Gallium(III) phthalocyanine chloride membrane, but in place of Ga(PC) 5,10,15,20- Tetraphenyl- 21H,23H- porphine copper(II) (Cu(TPP)) was used.

Preparation of Mn(TPP) membrane

Membrane with Mn(TPP) was prepared equally as Ga(PC) membrane, but in place of Ga(PC) 5,10,15,20- Tetraphenyl- 21H,23H- porphine manganese(III) chloride (Mn(TPP)) was used.

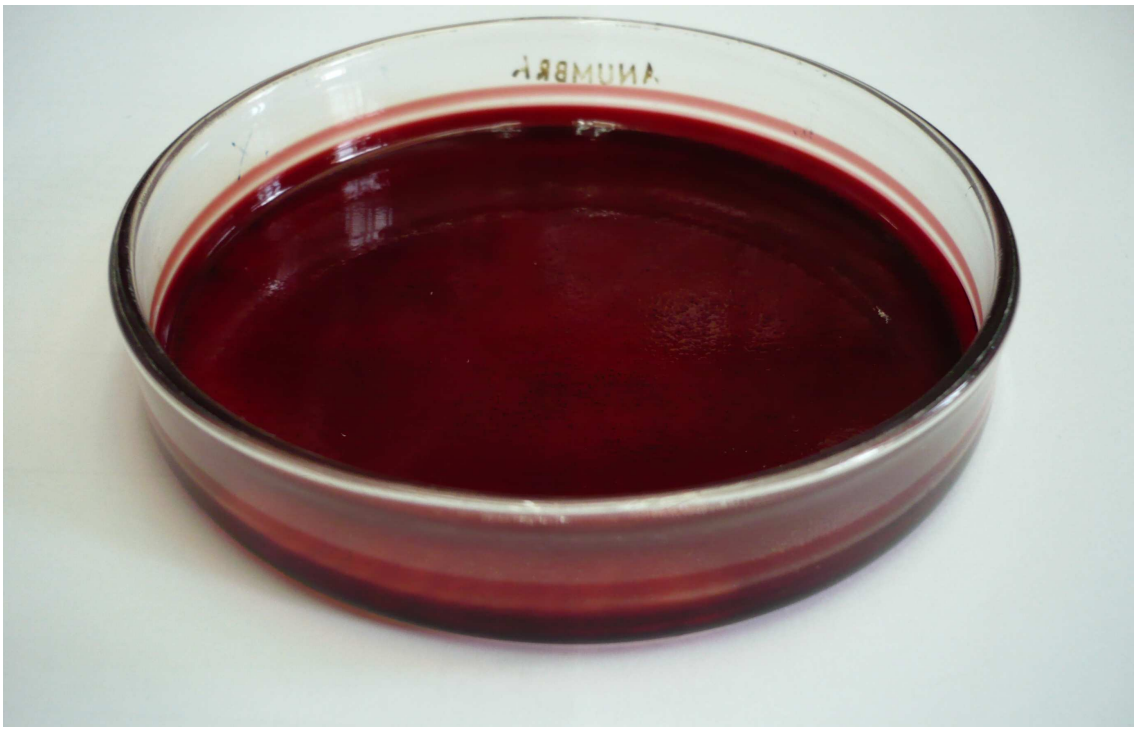


Fig. 27.: Prepared Cu(TPP) membrane in Petri dish

3.4 Apparatus

UV/VIS Spectrometer with UV Winlab 2.85.04 software (Perkin Elmer instruments, Lambda 45, USA)

Ultrasonic cleaner (VWR, USC100T, USA)

PH meter (GLP 22, Grison, Spain)

Millipore Milli-Q RG system (Millipore, Milford, MA, USA)

3.5 Optical assay

Before each spectrophotometric measurement, baseline was set by using appropriate buffer solution. Spectra were taken directly against sensing phase (PVC membrane with metalloporphyrin) in the cuvette with double-beam spectrometer. In referential cuvette was usually correspondent buffer solution. If it was necessary, membrane was fixated by clip to prevent moving in the cell. Plastic cuvettes were employed for all metering.

Absorption spectra were taken under conditions speed: 120nm/min and lower, slit: 2 or 4nm, smooth: 10.

Optical response for sample solution was assessed by determining the degree of decreasing/increasing dimer/monomer form of metalloporphyrin. Acquired spectra were substracted from the blank membrane (composited from: 0,2mg of metalloporphyrin, 0,5g of o-NPOE, 1g of PVC). Set of sample solutions was measured from the lowest concentration to the highest.

4 Results and discussion

4.1 Membrane - sensing phase- development and optimization

Development of gallium(III)phthalocyanine chloride membranes was headed with the objective to attain adequate transparency and reproducibility on spectrophotometric response.

4.1.1 Membrane incorporating 60 mg (3 w/w%) of gallium(III)phthalocyanine chloride

In a first attempt it was implemented a membrane which contained:

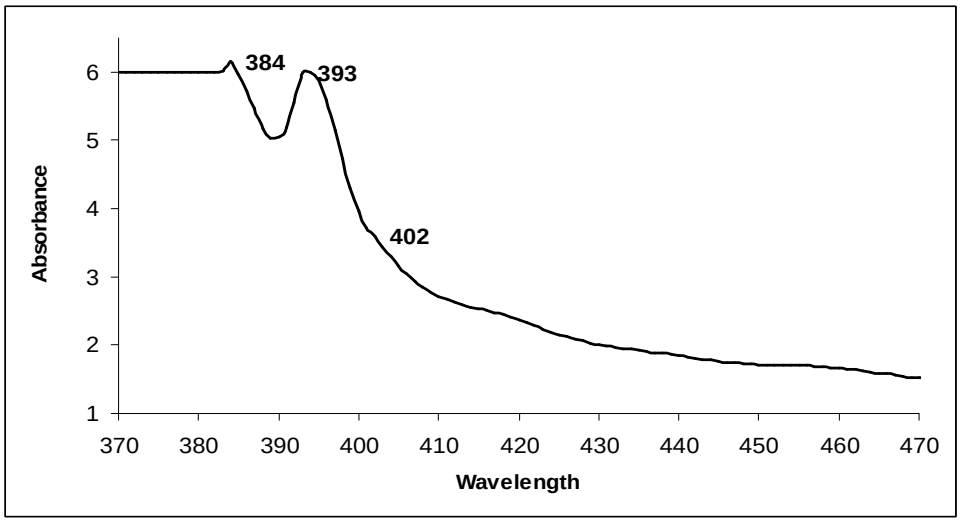
- 60 mg (3 w/w%) of Ga(PC)
- 1,44 g (72 w/w%) of PVC (membrane polymer)
- 0,5 g (25 w/w%) of o-NPOE (plasticizer).

After weighing the correct amounts, the ionophore was dissolved in the mediator solvent inside one closed amber glass vial. Afterwards, this solution was gently mixed with 3 mL of a solution of PVC in tetrahydrofuran and poured in a 10 cm Petri dish, then covered. The membrane was left to dry for about 48 h, after which a detachable circular membrane, with a tickness of about 0.8 mm was obtained. This membrane was very dark green (much darker than membrane with 10mg of Ga(PC) which is on Fig. 28). Membrane was very rigid and resistant to mechanic stress, probably due to the big amount of PVC used. Low transparency for spectrophotometric measurement and future optimization of working conditions were unfitting, but a lot of spectra were collected. High rigidity probably didn't allow good contact between anion and chromoionophore. In spite of this poorer characteristics, membrane with Ga(PC) seemed well reactive. Reproducibility of response was difficult.

4.1.1.1 Spectrophotometric assay

First, a rectangular 0.8x3 cm piece of membrane was placed inside a fused silica spectrophotometric cell and it was taken spectrum of the membrane immersed in 3 mL of MES buffer pH 3.4. Then spectrophotometric conditions settled were of: 120 nm/min for scan speed, 2 nm for monochromator slit with and 600-370 nm for wavelength scan range. It is worth of mention that adoption of a slow scan speed enabled to reduce

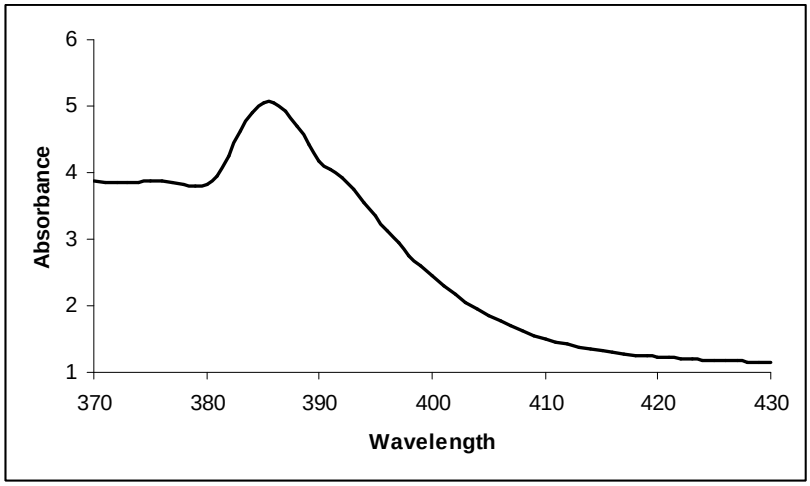
random noise errors observed for high absorbance values. We detected two absorption maxima at 384nm and 393nm and shoulder at 402nm (see Graph 1). In the beginning of measurement we presumed that all Ga(PC) was in the dimer form. Therefore, we indicated absorption maximum at 393 nm as dimer form and the shoulder at 402nm as the monomer form, based on the hypsochromic shift of the Soret band associated the dimerisation process. Use of MES buffer at low pH conditions was based on the presumption that hydroxyl- ions were in low concentration and thus not disturbing, by mass effect, the posterior reaction with valproate, salicylate and chloride. Other important aspects were the significant continuous raising in the absorption values below 470 nm probably due to turbidity of membrane, and the very high absorbance values associated the bands. In ordinary wet chemistries, high absorbance values should be avoid because Beer´s law is not obeyed either by significant contribution of stray light or by distortion of absorption characteristics of the ionophore. However in this prospective study, these aspects were overlooked since we were only trying to see significant changes in the spectrum pattern.



Graph 1.: Absorption spectrum of Ga(PC) membrane in MES buffer pH 3,4; two maxima at 384 and 393nm, shoulder at 402nm

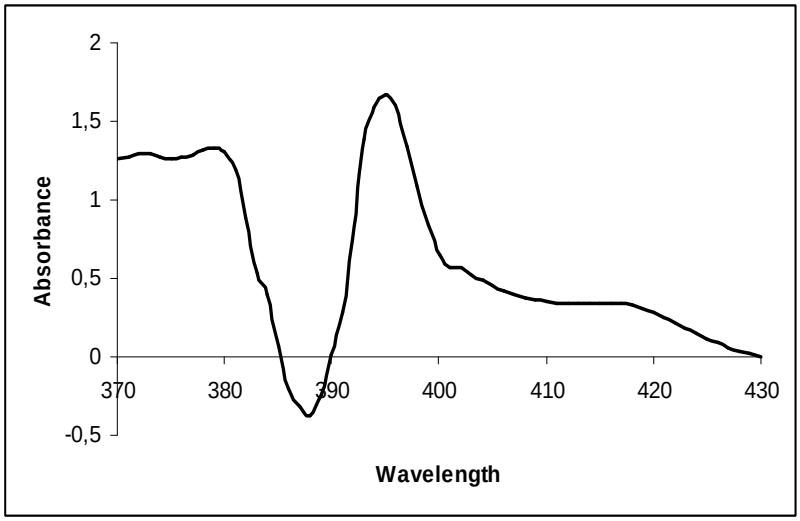
In order to consider the assumption of turbidity interference, a blank membrane was prepared considering a similar implementation procedure and with the following composition:

- 0.2 mg (11.8 w/w%) of Ga(PC)
- 1 g (58.8 w/w%) of PVC
- 0.5 g (29.4 w/w%) of o-NPOE



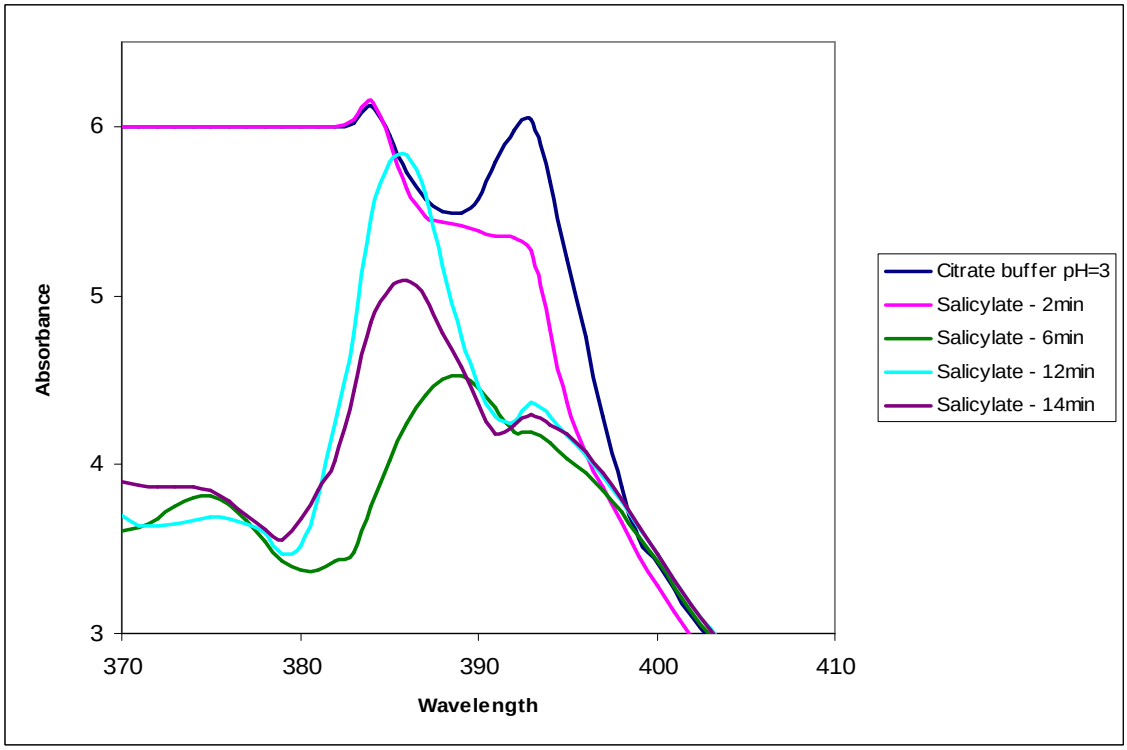
Graph 2.: Absorption spectrum of blank membrane with 0.2 mg of Ga(PC)

The spectrum obtained against buffer solution enabled to conclude a similar absorptive raising profile below 470 nm, confirmed with the translucent aspect by looking with naked eyes (difficult to observe in the dark colored membrane).

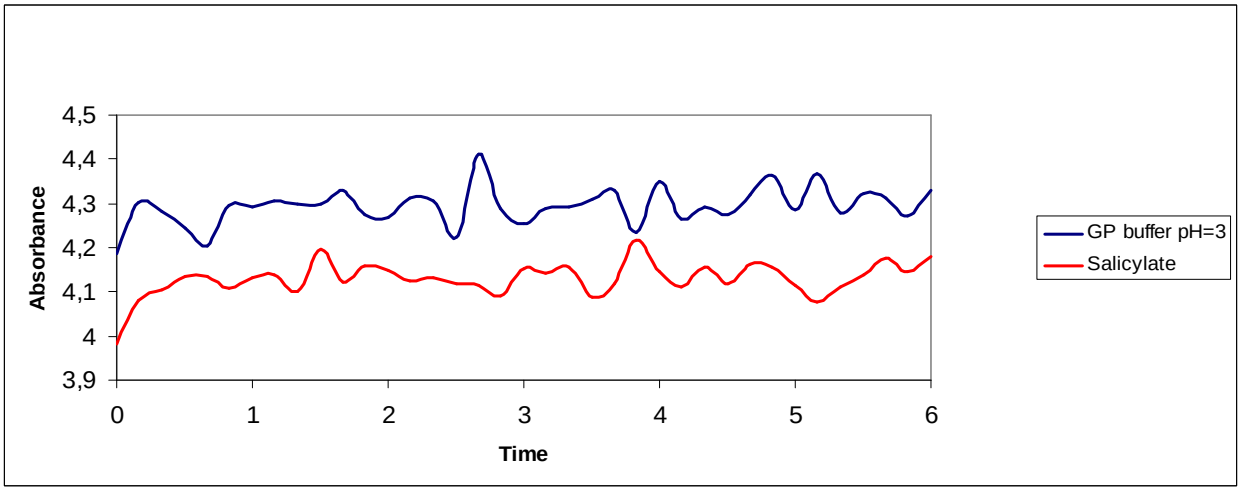


Graph 3.: Absorption spectrum of Ga(PC) membrane subtracted from the "blank" membrane, shoulder at 402nm is clearly visible

In the hope of definitive identification of bands corresponding to dimeric and monomeric forms, we performed two simple experiments. In the first experiment, we left the membrane soaked for two hours long in solution of NaOH (10^{-3} M) in order to displace the equilibrium towards the dimer form. After two hours we took the spectra of membrane immersed in NaOH. Further, we changed the solution in the cell for solution of HCl (10^{-3} M) to see increasing of monomer formed by axial binding of chloride anions plus displacement of hydroxyl- bridges at the lower pH solution. In a second experiment we placed inside the reference cell and also into the sample cell membranes with water and took the baseline. Then we transferred water for HCl (10^{-3} M) solution and waited for 30 minutes. We carried out the spectra with HCl (10^{-3} M) solution and saw a slight decrease of band with maximum absorption at the wavelength 384nm and an increase of the band at 393nm. Spectrum should look like sinusoid over 90 degrees shifted in ideal case. Experiments were not interfered by other anions. As main conclusion it was recognized that the dimer metalloporphyrin form for the band around the first maximum 384nm and the monomer form for the band around 393nm. Moreover, it means that since the beginning the amount of monomer was very high, or the molar absorptivity of the monomer band much higher than the corresponding to the dimeric form, how can be deduced from the corresponding bands seen on Graph 1. We tried to improve the ratio between dimer and monomer by immersing membrane in buffer solution for longer times. Membrane in buffer solution will becomes more hydrated and can thus enable hydroxyls extraction into organic phase and so more dimers form via hydroxyl bridging. Several tests exhibited this assumption. However, general reproducibility of this membrane was very poor considering both the different pieces tested and successive overlaid sprectra collected with one or two minutes delay, mainly due to the very high absorbance values. Spectrophotometer in such absorbance had to be saturated and error of measurement was growing (Graphs 4 and 5). The rate between dimer and monomer in the sensing phase seemed small. Transparency was minimal, because the volume of phthalocyanine’s dye looked huge. Also wettability was not optimal, perhaps due to the bulk quantity of PVC.



Graph 4.: Changes in absorption spectra with salicylate 10^{-3} M, during the time 2,6,12 and 14 minutes, in citrate buffer pH=3



Graph 5.: Absorbance at 386nm during 6 minutes, conditions: slit - 4nm, speed 60nm/min, every 10s taken absorbance

The noise associated with the high absorbance values associated with the two bands of interest leads one to consider in all the remaining studies, mean scans of five consecutive scans collected with a delay of four minutes. Another important procedure adopted was based on the use of the blank membrane. In the experiments where it was placed in the reference cell, the noise impaired all the expected repeatability, probably

due to the less intense light beam crossing the reference position. Hence, prior each experiment five scans of the blank membrane were collected and the mean spectrum calculated. This spectrum was subtracted to the membrane containing metalloporphyrin before reflecting on the data furnished.

4.1.2 Membrane containing 10 mg (0.5 w/w%) gallium(III)phthalocyanine chloride

Second membrane consisted of:

- 10 mg (0.5 w/w%) of Ga(PC)
- 1.44 g (73.8 w/w%) of PVC
- 0.5 g (25.6 w/w%) of o-NPOE.

This membrane was prepared in the same way described for the previous one, with added expectation for transparency improvement and hence better reproducibility of response. Although the amount of Ga(PC) was reduced six times, the colour of the 0.8 mm thick membrane appeared very intensive again and with an irregular distribution (see Fig. 28). The green colour was the complementary colour due to the Q bands of phthalocyanins, absorbing in the 610 to 730 nm range (Graph 6).

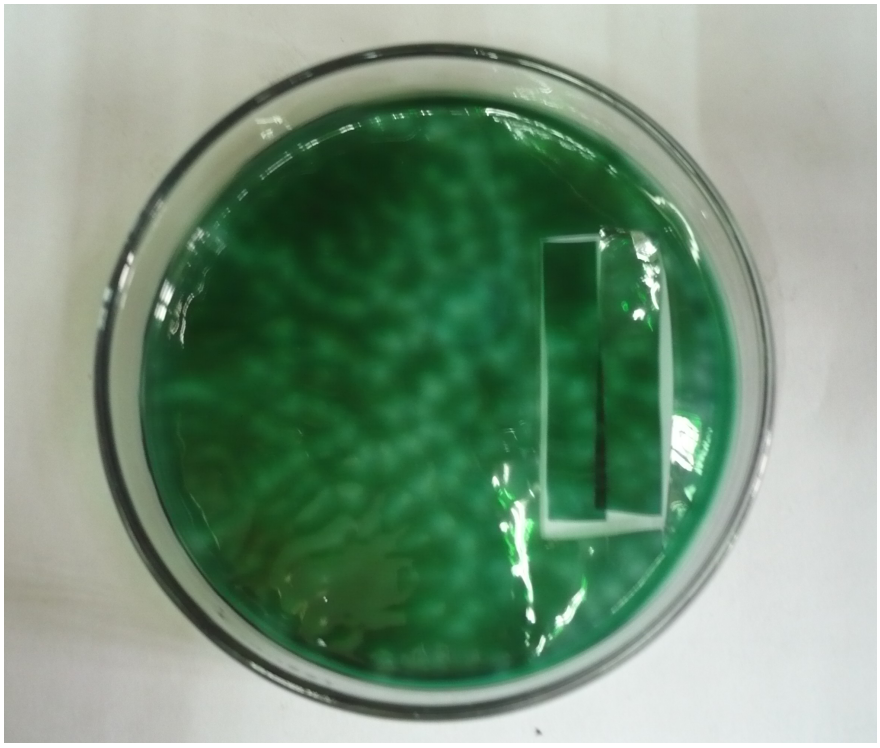
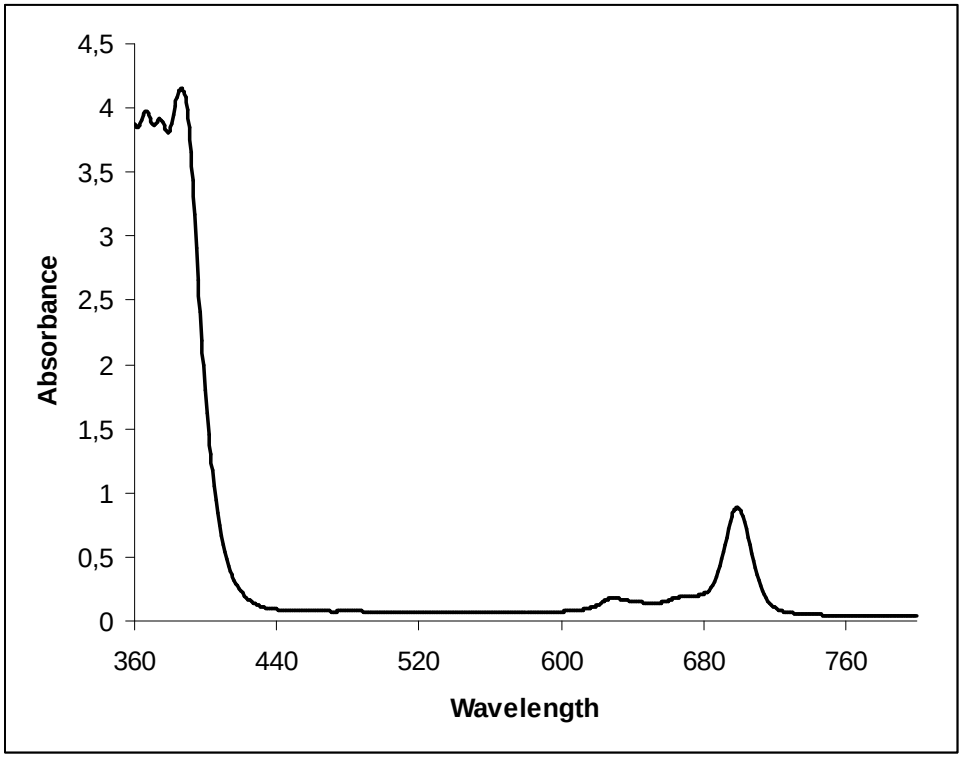
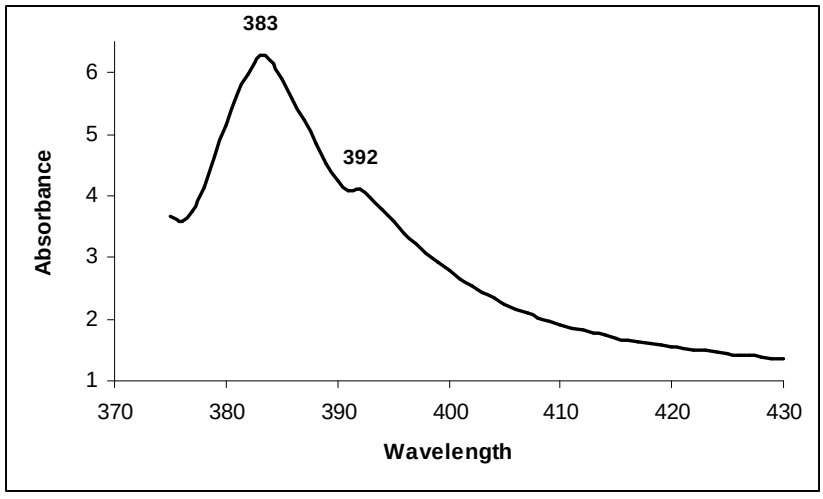


Fig. 28.: Illustration of 10 mg gallium(III)phthalocyanin membrane in Petri dish.

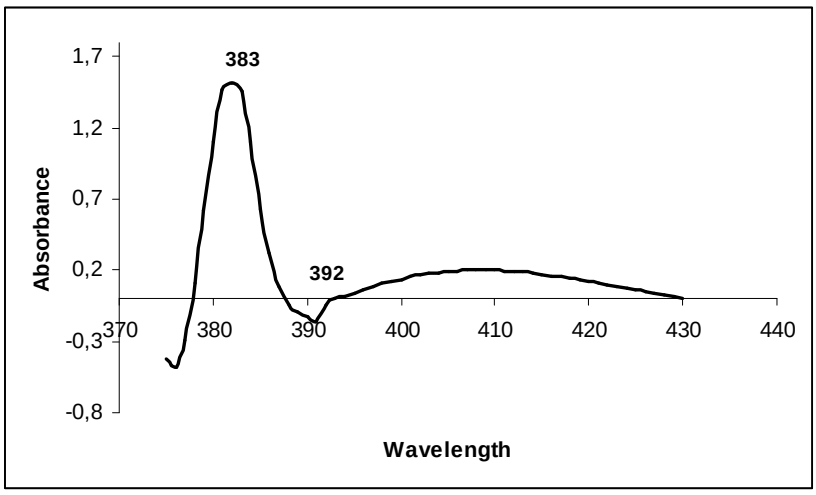


Graph 6.:Q bands in the range 610-730 nm, absorption spectrum of 2mg Ga(PC) membrane in MES buffer pH=4.5

Reducing to 0.5 w/w% from 3%, the amount of immobilized porphyrin, didn't bring the expected effect. The transparency kept poor too. Absorbance exhibited values around six was obtained as well for the dimeric band. Decreasing of Ga(PC) increased relatively percentages of o-NPOE and PVC. The raw spectrum before further testing was taken (Graph 7), and here it is now evident that by decreasing the amount of entrapped ionosphere allowed a significant decrease of the monomeric band.



Graph 7.: Raw absorption spectrum of 10 mg Ga(PC) membrane in water

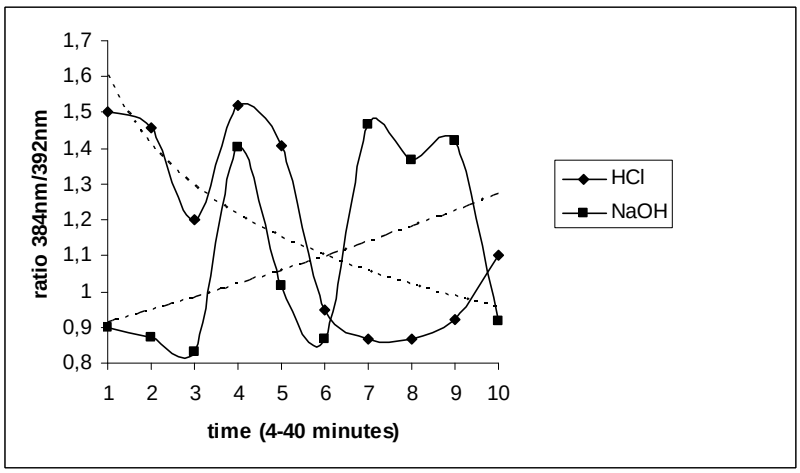


Graph 8.: Subtracted absorption spectrum of 10 mg Ga(PC) from blank membrane

As shown in the corrected spectrum, the absorbance band in the wavelengths of the monomer was clearly smaller than dimeric one. It could be caused by lower concentration of dye. Use of lower amounts of dye enabled too easiest dissolution by o-NPOE and then created dimer form.

4.1.2.1 Specification of dimer and monomer position and basic response towards chloride

The difficulties found to interpret obtained spectra were similar to the already observed with the first membrane. Absorbance achieved values around six. To trace up some behavior and regularity, we calculated ratios between maxima in 384nm and 392nm (between dimer and monomer wavelength). Experiments were implemented resorting to the overlay function of the spectrometer control software.Against the inner transparent surface of the sample cell, it was placed a piece of membrane immersed in about 3 mL of HCl solution (10^{-3} M) and every four minutes was taken a spectrum and repeated ten times. Afterwards the solution inside the cell was changed by a NaOH solution (10^{-3} M) and again ten spectra every four minutes were scanned. Ratios and behavior of formations towards HCl a NaOH are displaced on Graph 9.



Graph 9.: Ratios 384 nm/392 nm from spectra of membrane in HCl and NaOH solution, during the 40 minutes

In spite of the observed noise, steady lowering of absorbance at 384 nm is observed during the first three minutes followed by a constant decrease until reaching the value of 0.950 after ten minutes. Monomeric absorbancy increase is observed for the wavelength of 392 nm. Based on the results one could speculate both on the lengthy response of the membrane (ten minutes or more) still keeping low wet ability and on the constant increase of the band at higher wavelenghts perhaps meaning to obey Beer´s law regarding to monomer amount in the membrane phase.

4.1.2.2 Response towards salicylate

Salicylate anion was chosen as model molecule for observation the response of Ga(PC) membrane, although the high values of absorbance and large errors of measurements. Starting concentration for each membrane was 10^{-3} M of salicylate in MES buffer 10^{-1} M, pH 3.4. After discovery response to salicylate 10^{-3} M, namely in displacing the equilibrium in the membrane towards the monomeric form, lower and higher concentrations were tried (10^{-2} M and 10^{-4} M). It seemed that membrane gave response towards to the lower salicylate concentration. Response towards salicylate 10^{-2} M looked similar to 10^{-3} M. It meaned that detection limit is lower than concentration 10^{-3} M.

4.1.3 Membrane with 2 mg (0,13 w/w%) gallium(III)phthalocyanine chloride

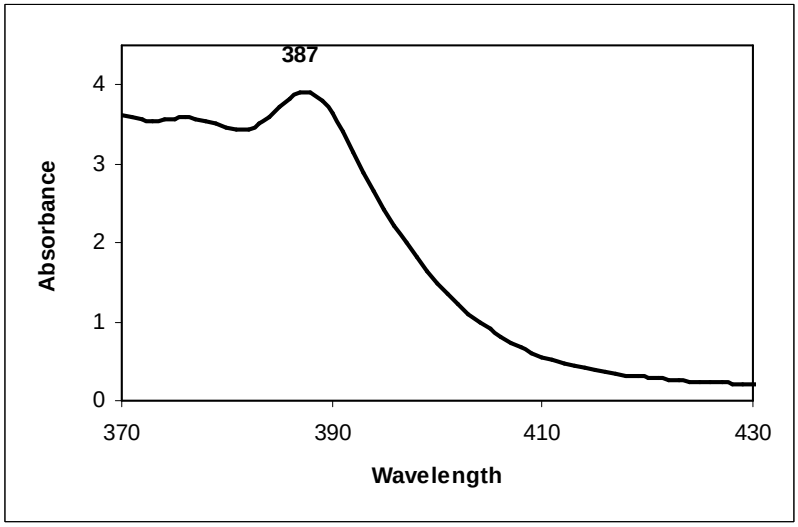
This membrane was very different from the two preceding. We changed not only amount of Ga(PC), but the amount of PVC as well. This last membrane consisted of:

- 2 mg (0.13 w/w%) of Ga(PC)
- 1 g (66.6 w/w%) of PVC
- 0.5 g (33.3 w/w%) of o-NPOE.

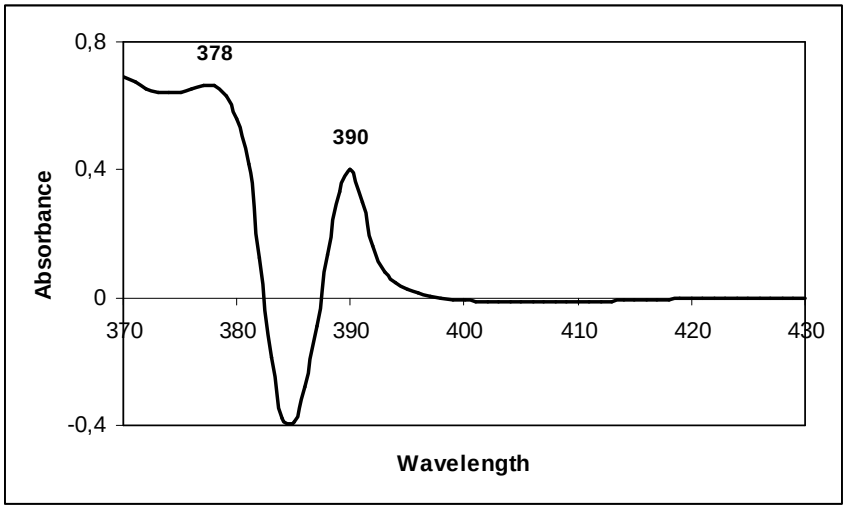
Mechanistic and targeted characteristics of membrane with 2 mg of Ga(PC) looked very well. Transparency of membrane was greater, colour was significantly lighter. Low concentration of Ga(PC) was dissolved very well (grown percentage of o-NPOE relatively). Concentration was decreased almost thirty times compared to the first (composed of 60 mg - 3 w/w% of Ga(PC)). Despite of this the membrane demonstrated clearer green colour. The percentage of PVC was diminished in 26% in comparison to the first membrane. The main goal of this decrease was to lower the membrane thickness and thus lower the optical path though it. At the same time to increase the percentage amount of mediator solvent thus providing reduced response time.

Membrane became less plastic and thinner, about 0.6 mm. Thickness of sensing phase is very important factor. Membrane has to be sufficiently thin to achieve response in acceptable time. Long time of answer is limiting for use as sensor [8]. Thickness of optode membrane should be <10 μm. Sample solution could get to contact with ionophore more easily because of the less plasticity of optode membrane. Less plasticity caused also that the membrane became with adhesive character.

We managed beginning by evaluation of the absorbance maximum of the optode membrane to value around 3.5 (see Graph 10). It was a noticeable difference over against two membranes above. Subtracted spectrum enabled to resolve overlapping bands and the monomer and dimeric forms clearly shown at 390nm and 378nm, respectively. Position of dimer and monomer forms changes in dependence on conditions in membrane and in ambient as well.



Graph 10.: Absorption spectrum of membrane with 2 mg of Ga(PC) in glycin-phosphate buffer pH 3



Graph 11.: Subtrated spectrum of Ga(PC) 2 mg membrane, absorption maxima at 378nm and 390 nm

4.2 Dimer-monomer equilibrium, mechanism of reaction

Development of this optical sensor is based on its dimer-monomer equilibrium. It was proved that the metalloporphyrins are capable to rearrange in dimer structures. Two planar molecules of Ga(PC) are connected via hydroxide ion bridge in axial position. Similar equilibriums for Ga(III)- , Sn(IV)- , Zr(IV)- , In(III)- were reported [10,12,13].

In the performed experiments we detected the salicylate anion. It was shown that salicylate is possible to shift this equilibrium to benefit for monomer formation. Supposed mechanism of Ga(PC) equilibrium is represented in this diagram:

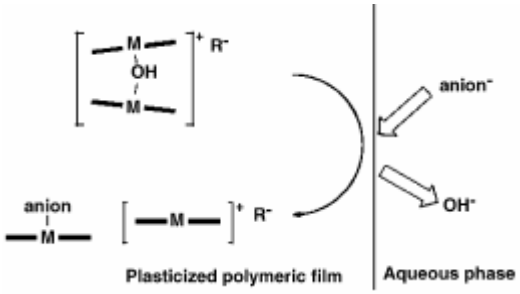
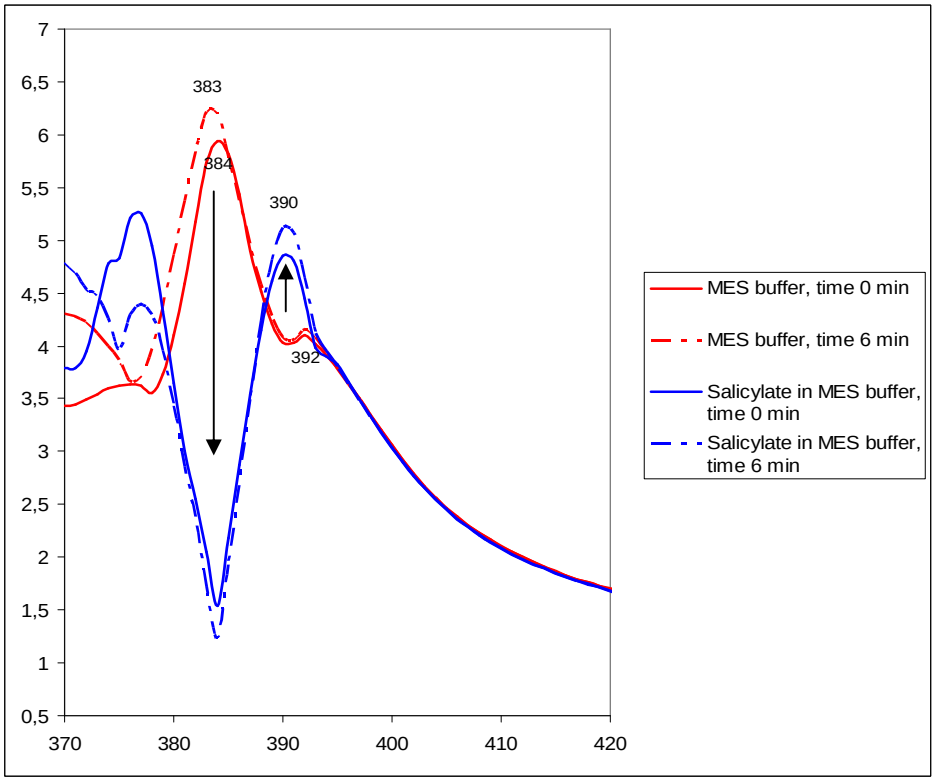


Fig. 29.: Mechanism of reaction salicylate (anion$^-$) with dimer form of gallium(III)phthalocyanine chloride, R$^-$ is chloride, M is gallium [13]

Equilibrium of our membrane was confirmed with obtaining spectrum on Graph 12:



Graph 12.: Red curves demonstrate absorption spectra 10 mg membrane with MES buffer in time 0 min and 6 min, Blue curves illustrate absorption spectra after reaction with salicylate in time 0 min and 6 min, arrows show decreasing of dimer at 384 nm and increasing of monomer at 392 nm

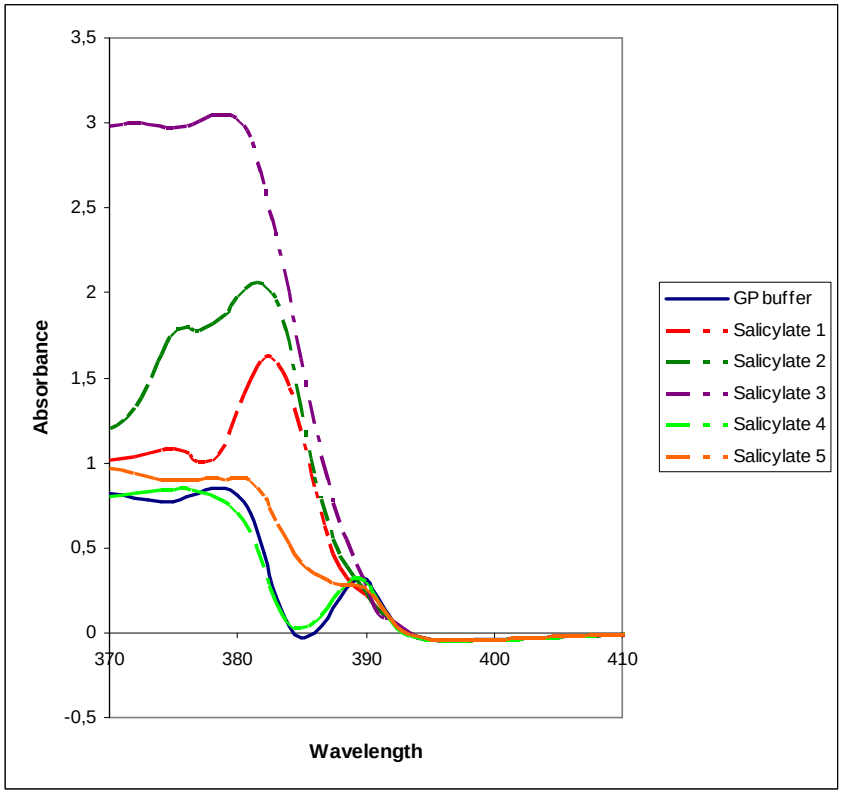
Shifting in wavelengths is clearly also to see. On the Graph 12 are two different shifting of maxima in wavelengths. First is between curves with only MES buffer in time 0 min and 6 min (384nm and 383nm), caused probably by increasing of dimer during the time in buffer. Second is in wavelengths of monomer form- 392nm and 390nm, caused by increasing of monomer. Increasing of the monomer amount in the membrane exhibits

overlapping dimer and monomer formation, which made difficult interpretation of other spectra.

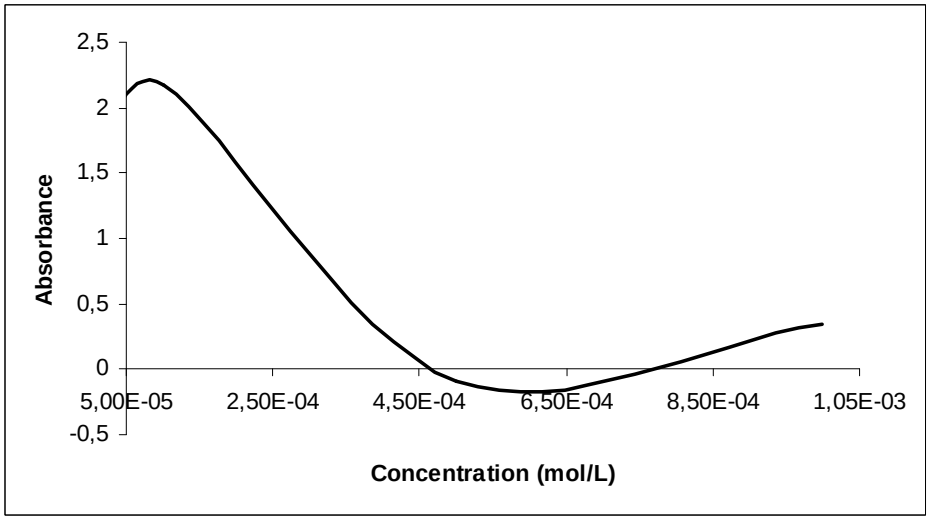
4.3 Sensitivity towards salicylate, valproate and chloride

4.3.1 Salicylate

From the Graph 14, obtained by considering the absorbance values obtained at 384 nm for solutions with increasing concentrations in salicylate, is possible to recognize a significante decrease in the concentration range laying between 10^{-4} M and 5.10^{-4} M. The absorbance in the start decreases with growing concentration of salicylate anion, but also increase at higher concentrations was noticed and caused by mutual overlapping of monomeric and dimeric band, so that we can see the growth of monomer as well (Graph 13). The same phenomenon can be seen in calibration curve of valproate (Graph 15).



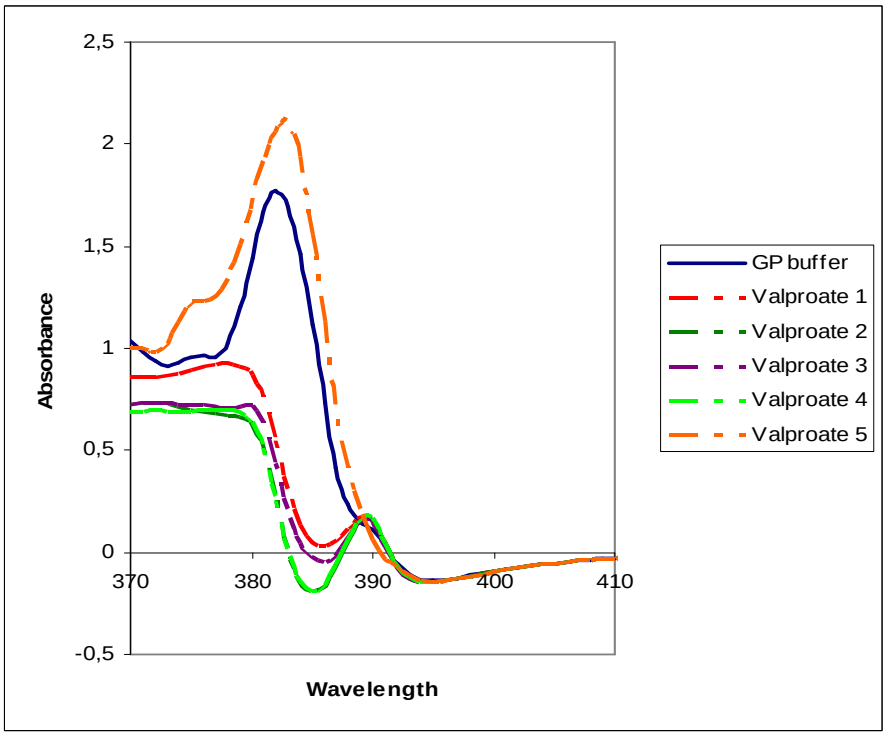
Graph 13.: Shifting of monomer is clearly see from 390nm to 379nm and dimer from 380nm to 372nm; Concentrations of salicylate solutions in GP buffer from 1 to 5 are 10^{-6} M, 10^{-5} M, 10^{-4} M, $5x10^{-4}$ M, 10^{-3}M



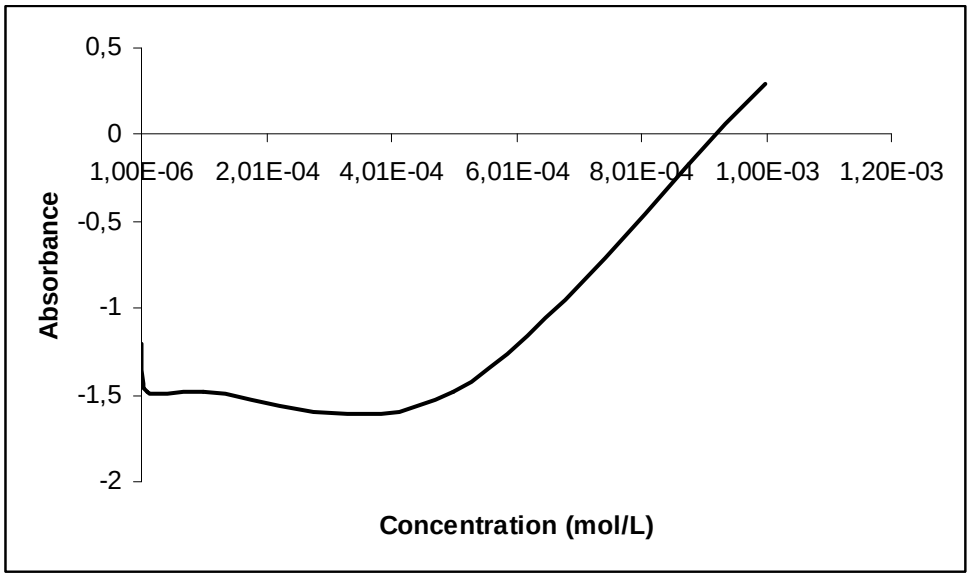
Graph 14.: Calibration curve towards salicylate

4.3.2 Valproate

In the calibration curve of valproate (Graph 16), we can observe that the absorbance decreased abruptly at concentrations of valproate in the micromolar range, but a linear increase due to monomeric band contribution leaded to a proportional increase in the interval between $5x10^{-4}$ to $1x10^{-3}$ M.



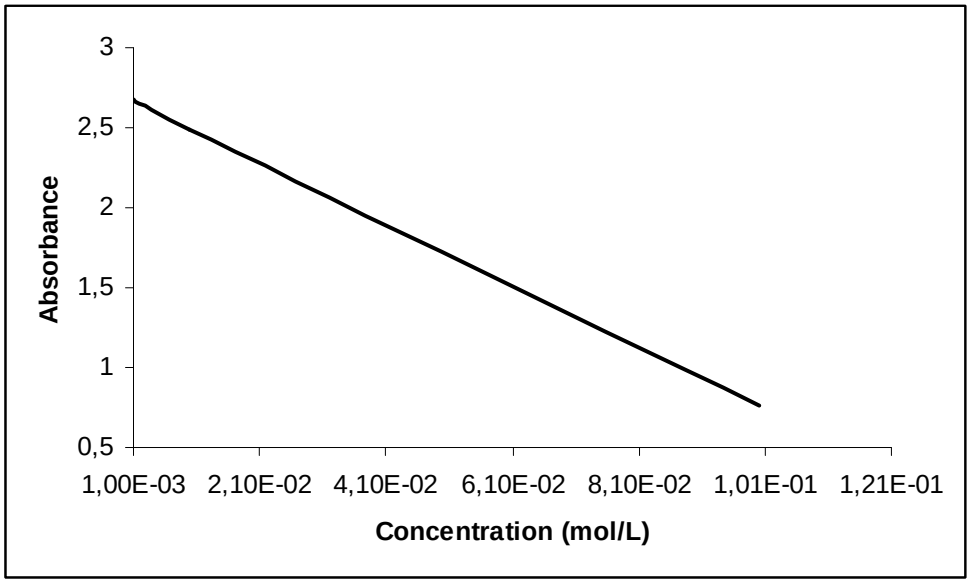
Graph 15.: Shifting in monomeric band between 390nm and 383nm and in dimeric band between 380nm and 375nm caused by solutions of valproate 1-5 (concetrations 1-5: 10^{-6} M, 10^{-5} M, 10^{-4} M, $5x10^{-4}$ M, 10^{-3}M)



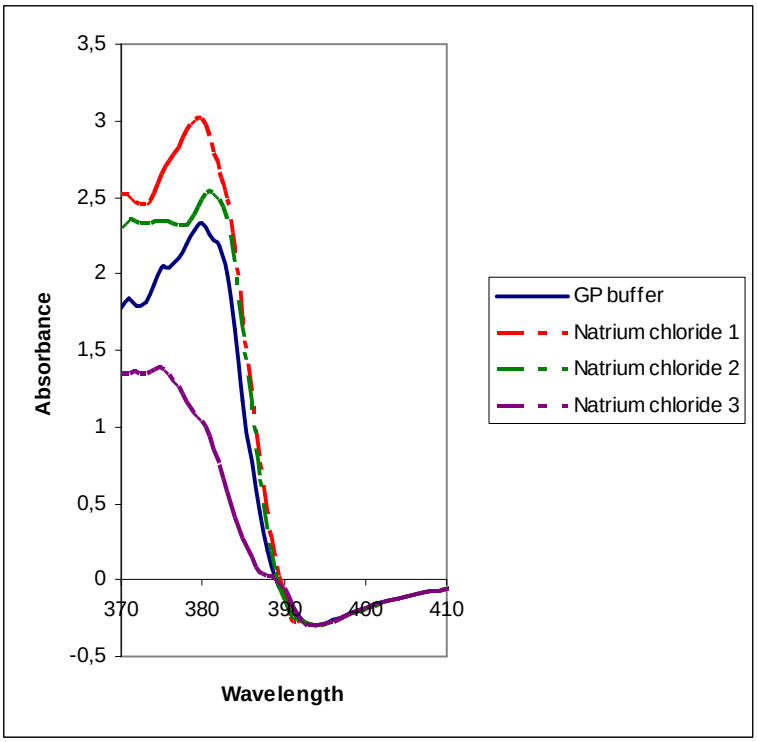
Graph 16.: Calibration curve towards valproate

4.3.3 Chloride

We also tried chloride in sample solution as potential interference. The absorbance of dimeric form started to decrease only for concentrations higher than those observed for salicylate or valproate (see Graph 18).



Graph 17.: Calibration curve towards chloride



Graph 18.: Shifting in dimeric band between 380nm and 375nm with natrium chloride is possible observed only in higher concetration 10^{-1} M (natrium chloride 3), concentrations of natrium chloride 1-3 in GP buffer were: 10^{-3} M, 10^{-2} M, 10^{-1} M

5 Conclusion

5.1 Thickness of sensing layer

From the previous studies it was possible to conclude that thickness of the membrane acts as one of the main operating features. Anion of interest has to transfer from aqueous phase to organic phase to react with its entrapped components. Further, thickness has influence on response time and absorbance. Bulky membranes increase absorbance, now known to be due to the translucent aspect of the final membrane, and damages reproducibility of absorption spectra. It is reported that desired thickness for optode membrane is <10 μm [8]. Optode membranes are usually prepared with spin-coating or other film-forming technique [12,13,14]. Our last membrane had thickness around 0.6 mm. However this membrane gave some response. Further decreasing of volume of PVC could lead to very low plasticity of film and easy leaching of ionophore from the film and worsen mechanistic resistance. Another possibility is spreading mixture before evaporating in Petri dishes with higher diameters and decrease thickness to half. Alternatively smaller amounts of sensing mixture could be poured in the Petri dish and carefully shaken to gently spread the sensor over the all surface. In our opinion this is the first searching direction because whatever the previous membrane considered they were very easy to cut in small pieces and handle in spectrophotometric measurements. Problems like membrane shrinkage were in this way absent and no chemical inert materials to support membranes are needed.

5.2 Nature of plasticizer

Dimer-monomer equilibrium can be shifted towards dimer with employing organic solvent with higher dielectric konstant [10]. Exploiting of o-NPOE ($\varepsilon = 24$) for answer characteristic should be preferable than DOS ($\varepsilon = 4,6$). But DOS demonstrated still good response concerning dimer-monomer equilibrium [10]. DOS shows better transparency and lower leaching profile than o-NPOE [14]. Perhaps, if we had enough time, we have implemented a membrane with DOS as plasticizer and compared with those based on o-NPOE.

5.3 Addition of ionic additive

It is well known that addition of lipophilic additives can improve selectivity of optode membrane or ISEs. In employing of ISEs is used 10 – 20 mol% relative to the ionophore. Optode membranes usually have this percentage higher. Literature reports up to 45 mol% relative to metalloporphyrin [10]. Except enhancing of selectivity, it is also possible to increase the dimeric band with employing of additives.

Membrane with Ga(PC) exhibits a charged carrier mechanism and for that reason could exploit the use of anionic additives to improve its qualities. Tetraphenylborate derivates were usually added [13,14]. We didn't exploit anionic lipophilic additives, because we stayed at determination of basic membrane characteristics and improving mechanistic features.

5.4 Immersing membrane before measurement

Storage of membrane has influence on its function, response and maximum of absorption spectrum.

Hydration helps membrane built more dimer band by doping it with hydroxide anions. If the membrane is left long time in immersing liquid, it could occur leaching of the ionophore, swell and turbidity of membrane. Leaching of ionophore diminishes lifetime and efficiency of sensor. Swell and turbidity lower reproducibility of obtained spectra. Hence, it is necessary to find optimum of time of immersing. Type of immersing liquid is very important as well. Water decreases lifetime of optode membrane and repeatability of measurements [8]. Buffer solution was proved as good immersing liquid. It ensures whole ionic stability and dopes membrane with hydroxide anions.

It depended on the type of membrane, which time of immersing we were used. First and second membranes, which were too much plastic and hydratation wasn't easy, since membrane needed a minimum of two hours to become hydrated. Perhaps, last membrane was enough up to one hour to stay in buffer solution.

5.5 Molecule structure

Molecule of porphyrin or phthalocynine should be carefully considered. Substituents link to porphyrin (or phthalocynine) core in meso-position can do steric hindrance to create desired dimer formation. Big substituents don't allow easy attack of hydroxide bridge.

Gallium(III)phthalocyanin chloride is probably suitable compound to create dimer formation. Ga(PC) haven’t got substituents. Or reversely it doesn’t have more stable dimer and monomer can be created very easy. If the producing of monomer is simple, distinction among concentrations of sample solutions can be impossible.

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7 Shrnutí

V této diplomové práci jsme se zabývali vývojem optického sensoru pro salicylát. Sensor je miniaturní zařízení, které přenáší informace v čase o určité látce, kterou sledujeme. Bez sensorů bychom nemohli existovat ani my. Například díky teplotním sensorům víme, že je venku zima a že se máme obléknout, abychom nezmrzli. Výhodou sensorů je jejich velikost a schopnost ihned informovat o změnách okolí. Každý sensor se skládá z rozpoznávající části a převodníku (Obrázek 13), který mění signál na měřitelnou veličinu. Ideální sensor by měl vždy reagovat stejně (neměl by podléhat stárnutí) a jen na látku, která nás zajímá a neměl by být ovlivňován okolím.
Existují tři základní kategorie sensorů:

1. *fyzikální:* pro měření fyzikálních veličin jako teplota, tlak, hmotnost
2. *chemické:* které měří chemické látky za pomoci chemických nebo fyzikálních signálů
3. *biosensory:* využívají biologické látky jako rozpoznávající části k identifikaci chemických látek

Převodníky jsou děleny do několika skupin podle veličiny, kterou zpracovávají a převádí na signál (Tabulka 1). V našem případě jsme sledovali a měřili optický signál. Měřenou veličinou byla absorbance.
Rozpoznávající část sensoru byla založena na metaloporfyrinu. Metaloporfyriny jsou barevné látky skládající se z tetrapyrolového kruhu a uvnitř mají navázán kovový ion (Obrázek 2,3). Metaloporfyriny jako barevné látky absorbují ve viditelné části spektra. Absorpční spektrum metaloporfyrinů se též využívá k jejich identifikaci, protože je pro každý metaloporfyrin charakteristické (Obrázek 9). Absorpční spektrum metaloporfyrinu se skláda ze dvou charakteristických absorpčních maxim. První, větší, v nižších vlnových délkách se nazývá Soretův pás, druhé se projevuje jako jedno nebo více malých maxim a nazývá se Q vazby (Obrázek 7,9).
Některé metaloporfyriny jsou schopné tvořit dimery (Obrázek 24) pomocí hydroxidových můstků. Již v minulosti bylo prokázáno, že dimerická forma metaloporfyrinu má vyšší absorbanci než monomerická. Na začátku měření se předpokládá, že veškerý metaloporfyrin je v dimerické podobě. Některé aniony mají schopnost rozbít hydroxidový můstek a snížit množství dimeru, které se projeví

snížením absorbance v oblasti Soretova pásu. Zároveň vzrůstá podíl monomeru. Tato vlastnost metaloporfyrinů se nazývá dimer-monomer rovnováha a bylo na ní založeno naše měření (Obrázek 25, 26, 29; Graf 12). Měří se tedy úbytek absorbance při určité vlnové délce maxima, který je úměrný koncentraci detekovaného anionu. V našem případě salicylátu.

Metaloporfyrin byl zabudován v polymerní membráně z PVC (Složení membrány viz kapitola 3.3). Jen při správném složení membrány a okolních podmínkách je možná reakce mezi detekovaným anionem a dimerem metaloporfyrinu. V této práci jsme se zabývali především optimalizací membrány a reakčních podmínek.

Prvním metaloporfyrinem použitým v membráně byl Cu(TPP) (Obrázek 27). Snížení absorbance membrány z tohoto metaloporfyrinu při reakci se salicylátem bylo téměř nepatrné. Podobná situace byla při testování membrány s Mn(TPP). Dalším vybraným metaloporfyrinem bylo Ga(PC). Membrána s Ga(PC) od prvních změřených absorpčních spekter vykazovala mnohem vyšší reaktivitu než předešlé metaloporfyriny s mědí a manganem. Pokračovali jsme tedy s vývojem membrány s Ga(PC).

Membrána obsahující 60 mg Ga(PC)

Jako první byla zkonstruována membrána, která obsahovala 60 mg metaloporfyrinu (Kapitola 4.1.1). Membrána byla velmi tmavě zelená a málo transparentní. Při měření dosahovala absorbance hodnot okolo šesti. Při takto vysoké absorbanci se předpokládá velká chyba měření. Díky velkému objemu PVC byla membrána velmi plastická a mechanicky odolná. Pravděpodobně kvůli vysoké hydrofobitě nedovolovala dostatečný kontakt analyzovaného roztoku s metaloporfyrinem. Reprodukovatelnost odpovědi membrány byla složitá, přesto bylo provedeno mnoho absorpčních spekter.

Základní spektrum membrány bylo sejmuto (Graf 1) a byla určena maxima v 384 nm a 393 nm a rameno v 402 nm. Protože se na začátku měření předpokládá, že veškerý metaloporfyrin je v dimerické formě, byl dimer určen při 393 nm a rameno v 402 nm jako monomer. K přesnému určení polohy dimeru a monomeru byly provedeny dva jednoduché pokusy. Díky těmto pokusům jsme zjistili, že původní určení poloh dimeru a monomeru nebylo správné. Nově byla poloha dimeru a monomeru určena v oblastech maxim v 384 nm a 393 nm. Z velikosti obou maxim v Grafu 1 je vidět, že podíl monomerické formy je příliš vysoký.

Schopnost membrány reagovat se salicylátem je zobrazena na Grafu 4. Velikost šumu při měření je zřejmá z Grafu 5. Membrána neměla vhodné vlastnosti pro další měření. Byla tedy připravena nová membrána již s nižším obsahem metaloporfyrinu s cílem zvýšit transparentnost a zlepšit přesnost spektrofotometrického měření.

Membrána obsahující 10 mg Ga(PC)

Ačkoliv tato membrána byla připravena ze šestkrát menšího množství Ga(PC) než předchozí, vykazovala stále velmi intenzivní zelenou barvu a transparentnost byla nízká, jak je patrno z Obrázku 28. Podařilo se zvýšit poměr dimeru k monomeru (Graf 7). To mohlo být způsobeno lepším rozpuštěním menšího množství Ga(PC) v o-NPOE a snazší tvorbou dimeru.

Membrána obsahující 2 mg Ga(PC)

Tato membrána s pouhými 2 mg Ga(PC) byla velmi odlišná od dvou předchozích. Kromě snížení objemu Ga(PC) bylo menší i množství PVC. Tím byla zajištěna vyšší transparentnost, menší plasticita a tloušťka membrány. Nižší plasticita a tloušťka membrány umožňují lepší kontakt analyzovaného roztoku anionu s ionoforem. Počáteční absorbance se snížila z původních hodnot kolem šesti na čtyři (Graf 10).

Kalibrační křivky a citlivost směrem k salicylátovému, valproátovému a chloridovému anionu

Membrána obsahující 2 mg Ga(PC) byla použita k vytvoření kalibračních křivek salicylátu a valproátu. Jako potenciálně interferující anion byl vybrán chlorid. Do kyvety s membránou byly postupně dávány analyzované roztoky v pufru v koncentracích 10^{-6} M, 10^{-5} M, 10^{-4} M, $5x10^{-4}$ M a 10^{-3} M.

Graf 14 znázorňuje kalibrační křivku týkající se salicylátu. Křivka byla sestrojena z hodnot absorbance při vlnové délce odpovídající dimeru (384 nm). Zprvu dochází k poklesu absorbance s rostoucí koncentrací salicylátu v koncentracích od $5x10^{-5}$ M do $4.5x10^{-4}$M. Kalibrační křivka poté začíná růst, protože dochází k překrývání a posunu vazeb. Růst v kalibrační křivce tedy odpovídá přírůstku monomeru. Na Grafu 13 jsou

vidět posuny vazeb monomeru mezi 390 nm a 379 nm a dimeru mezi 380 nm a 372 nm. Stejný jev se objevuje i u valproátu.

Z kalibrační křivky týkající se valproátu je především patrný lineární vzestup monomeru v intervalu od $5x10^{-4}$ M do $1x10^{-3}$ M (Graf 16). Překrývání vazeb znázorňují absorpční spectra membrány s valproátem na Grafu 15.

Chlorid byl vybrán jako potenciální interferující anion. K snížení absorbance rozložením dimeru metaloporfyrinu na monomery bylo potřeba vyšší koncentrace než u salicylátu a valproátu. Kalibrační křivka znázorňující detekční limit a odpověď směrem k chloridu je na Grafu 17.

8 Závěr

Při vývoji optického sensoru založeném na metaloporfyrinu, který je ukotven v PVC membráně jsme se zabývali především optimalizací membrány. Jako velmi slibný metaloporfyrin pro detekci salicylátu a valproátu se jevilo Ga(PC), narozdíl od Cu(TPP) a Mn(TPP). Postupně byly připraveny tři membrány s různým obsahem Ga(PC). K detekci anionů nebylo nutné použít velké množství chromoionoforu. Membrána s obsahem pouze 2 mg Ga(PC) se ukázala jako stále dostatečně sensitivní. Aplikací optody do průtokového systému jsme se bohužel nezabývali, protože optimalizace složení membrány (obsah metaloporfyrinu, PVC, obsah a typ plasticizéru), reakčních a skladovacích podmínek (typ pufru, jeho pH a koncentrace, hydratace membrány) se ukázaly velmi důležité a současně byly časově náročné.

Byla vyvinuta membrána, která při spektrofotometrickém měření byla schopna detekovat salicylát a valproát. Limit detekce salicylátu byl $5x10^{-5}$ M a valproátu dokonce v mikromolárních koncentracích. Chloridový anion, jako potenciální interferent, by neměl rušit měření, protože jeho limit detekce byl mnohem vyšší.

Ga(PC) a pravděpodobně i jiné metaloporfyriny obsahující gallium(III) mají dobrý potenciál ve využití detekce anionů jak při výrobě farmaceutik tak například při kontrole odpadních vod.

List of abbreviations

Cu(TPP)	5,10,15,20-Tetraphenyl-21*H*,23*H*-porphine copper(II)
Ga(PC)	Gallium(III)-phthalocyanine chloride
Mn(TPP)	5,10,15,20-Tetraphenyl-21*H*,23*H*-porphine manganese(III)chloride
DMSO	Dimethylsulfoxid
THF	Tetrahydrofuran
PVC	Polyvinylchloride
o-NPOE	ortho-nitrophenyloctylether
TPP	Tetraphenylporphyrine
CitBS	Citrate buffer solution
GPBS	Glycin-phosphate buffer solution
MES	2-(N-Morpholino)ethanesulfonic acid buffer solution
ISE	Ion-selective electrode
PU	Polyurethane
In(III)[OEP]Cl	Indium(III)-octaethylporphine chloride
DOS	Dioctyl sebacate
UV	Ultraviolet
VIS	Visible
NIR	Near infrared