Full Paper

PVC Supported Liquid Membrane and Carbon Paste Potentiometric Sensors Incorporating a Mn(III)-Porphyrin for the Direct Determination of Undissociated Paracetamol

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Received: April 2, 2008
Accepted: June 5, 2008

Abstract
PVC supported liquid membrane and carbon paste potentiometric sensors incorporating an Mn(III)-porphyrin complex as a neutral host molecule were developed for the determination of paracetamol. The measurements were carried out in solution at pH 5.5. Under such conditions paracetamol exists as a neutral molecule. The mechanism of molecular recognition between the Mn(III)-porphyrin and paracetamol, leading to potentiometric signal generation, is discussed.
The sensitivity and selectivity toward paracetamol of carbon paste and polymeric liquid membrane electrodes incorporating an Mn(III)-porphyrin host were compared. The applicability of these sensors to the direct determination of paracetamol was checked by performing a recovery test in human plasma.

Keywords: Mn(III)-porphyrin, ISE, Carbon paste, Neutral guest, Paracetamol

DOI: 10.1002/elan.200804280

1. Introduction

The study of the potentiometric sensing of neutral compounds is an ongoing challenge for scientists. In previous work, we have already applied diverse nitrogen-containing macrocyclic compounds as sensitive elements in potentiometric sensors destined for the detection of undissociated phenol derivatives [1 – 5]. The crucial step of potentiometric signal generation involved the formation of hydrogen bonds between electron pair of oxygen atom from OH group of phenolic guest and hydrogen atom from NH or NH_2 group from host macrocycles. These hydrogen bonds increased the acidity of OH group, and as the consequence, H^+ ions dissociated to the aqueous layer adjacent to the organic phase [1]. It has been recently discovered, that the potentiometric signals could be also generated when the guest molecules contained NH_2 groups, and OH groups were attached to the host molecules [3]. These phenomena indicated that formation at the interfaces of supramolecular complexes based on hydrogen bonding lead to the changing of acidity/basicity of guests molecules, which facilitated their dissociation.

Herein, we present a Mn(III)Cl-tetrakis[3,5-bis(*t*-butyl)-phenyl]porphyrin (Mn(III)-porphyrin 1) as host molecule, which is able to generate potentiometric signals in the presence of undissociated guest molecules, such as paracetamol (PCT, **2**) (Fig. 1). Paracetamol (acetaminophen) is a common analgesic antipyretic drug. It is widely used for the relief of mild to moderate pain associated with headache, backache and arthritis [6]. Paracetamol is also very effective to bring down high temperature in fevers, including colds and flu, and for relieving the aches and pains associated with these common illnesses [7]. The drug is well tolerated by people with peptic ulcers and in general those who suffer from asthma. However, its primary metabolic pathways involve oxidation by the liver and excessively high concentrations of PCT in plasma may cause renal and hepatic toxicity [8, 9]. Thus it is necessary to develop a rapid, simple and highly selective method to determine PCT in body fluids by understanding its clinical distribution and toxicokinetic parameters. Several methods including titrimetry [10], spectrophotometry [11] and chromatography [12] have been used to determine PCT. However, most of these methods are either time consuming or expensive.

Electroanalytical methods, which are simple in handling and relatively cheap, seem to be good candidates for application in routine analysis in a diagnostic laboratory.

Paracetamol is an electroactive compound, which can be readily oxidized at carbon paste or glassy carbon electrodes. However, these amperometric procedures are not selective, since the potential involved in this process ranges from 0.6 to 0.8 V and various substances are electroactive in this potential interval [7].

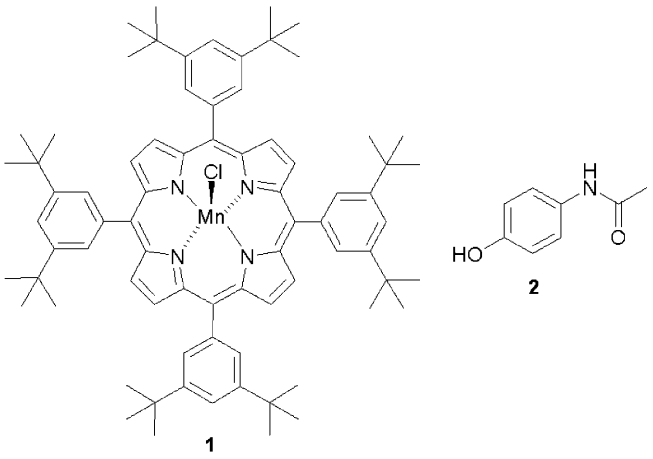


Fig. 1. Chemical structure of Mn(III)-porphyrin 1 and paracetamol (PTC).

Many amperometric methods have been used to determine PCT directly in physiological samples such as blood [13–15] and urine [16]. Most popular electrodes used for this purpose are screen-printed carbon electrodes [16], chemically modified glassy carbon electrodes [17–20], boron doped diamond film electrodes [21] or electrodes modified with gold nanoparticles [22].

It might be concluded, that amperometric methods for the determination of PCT can not be fully recommended, because of interferences caused by the presence of many electroactive substances in physiological samples. The more suitable for this purpose are potentiometric sensors, ion-selective electrodes (ISEs) or carbon paste electrodes (CPEs).

Therefore, the developing of new potentiometric sensors for determination of paracetamol is highly desirable. Ion-selective liquid membrane electrodes have been already widely used for medical and pharmaceutical analysis [23–26]. Specialized reviews concerning this subject have been published [23, 27]. The issue of selectivity and detection limits of ISEs has been reviewed by [23, 28–30].

Another type of electrodes used in the potentiometry is based on carbon paste. Potentiometric sensors based on carbon paste have been also applied for determination of compounds crucial for pharmaceutical formulations such as: ascorbic acid [31], sulfate [32], chlorpheniramine maleate [33], just to name a few. The applications of carbon paste electrodes were review by Kalcher et al. [34].

The selectivity of ISEs relies on the phase boundary equilibrium [23, 30, 35], and represents the free energy of complete ion transfer across the interface of an aqueous (sample) phase and water-immiscible organic phase. This free energy is particularly high, in the case of very hydrophilic anions [23, 30, 35]. It has been proved the free energy connected with transfer of target molecule form the aqueous phase to the one containing host molecule depends on the structure of the interface. The same receptor, hydrogen bon – forming bis(thiourea), used for the modification of ISE and highly oriented pyrolytic graphite (HOPG) showed different selectivity [36].

In both type of electrodes, ISEs and CPEs, the host molecules are dissolved in organic solvent. The main difference between them concerns the matrix material, polymer and carbon paste, which create the different interfaces [28, 34]. As the consequence, the accessibility of target compounds to the host molecules immobilized in polymeric or carbon paste matrix is different. Also, in most cases, carbon paste electrodes could be incorporated with higher concentration of host molecules, than ISEs, in which concentration of ionophores is limited by their solubility in organic solvent applied.

Therefore, the aim of the present work was to investigate the possible application of a Mn(III)-porphyrin **1** (Fig. 1) as the analytically active element of a potentiometric sensors (ISE and CPE) destined for the direct detection of the undissociated form of paracetamol, occurring at the border of the aqueous/lipophilic liquid (solid) phase. As a sensing element of the above sensor Mn(III)-porphyrin **1** was selected. This ligand has been already applied in ISE for salicylic acid determination [24].

The sensors presented rely on the supramolecular host-guest recognition occurring at the border of the aqueous/lipophilic liquid (solid) phase. The influence of the type of immobilization of the host molecule (interface type) on the sensitivity and selectivity of the PCT determination will be discussed.

2. Experimental

2.1. Reagents

Free base tetrakis[3,5-bis(*t*-butyl)phenyl]porphyrin was synthesized according to a previously published procedure [37]. Mn-insertion could be performed quantitatively using $MnCl_2 \cdot 4H_2O$ in refluxing DMF (overnight) [38]. The metalation progress was monitored by UV-vis spectroscopy. Tridodecylammonium chloride (TDDMACl), potassium tetrakis(*p*-chlorophenyl)borate (K-TPClPB), and bis(2-ethylhexyl)phthalate (DOP) were used as received from Fluka-Aldrich (Poland). High molecular weight PVC was purchased from Wako (Japan). Paracetamol (PCT) was obtained from Sigma-Aldrich. Paraffin was obtained from Riedel-deHaën. Graphite powder was obtained from Fluka. Sodium dihydrogen orthophosphate, sodium phosphate, sodium chloride and THF were obtained from POCh (Poland). THF was distilled over solid KOH prior to use.

All solutions were prepared with deionized water (with resistivity 18.2 MΩ cm) purified with a Milli-Q reagent grade water system (Millipore, Bedford, MA).

2.2. Preparation of PVC Supported Liquid Membrane Electrodes

The membranes were prepared by a standard procedure. The composition of the membranes studied was as follows: 1 wt% ionophore (10 mM *vs.* DOP volume), 66 wt% DOP,

33 wt% PVC. Some membranes were additionally incorporated with 50 mol% (vs. ionophore concentration) of lipophilic salts TDDMACl or K-TPClPB. All membrane components were dissolved in 2 mL of freshly distilled THF. The resulting mixture was placed into a glass ring of 30 mm inner diameter and kept for 24 h to allow complete evaporation of THF. The standard thickness of the resulting membranes was approximately 100 μm. Circles of 6.0 mm diameter were punched from the membranes and directly mounted on a liquid membrane type Philips ISE body (Glasbläserei Moler, Zürich, Switzerland).

2.3. Preparation of Carbon Paste Electrodes

The carbon powder was made by mixing 0.1 g graphite powder and 60 μL paraffin in a mortar to obtain a homogeneous paste. Then the paste was housed in a 3.0 mm diameter electrode Teflon body (BAS, Lafayete, USA). The electrode surface was smoothed with paper. A small amount of paste (1 mm depth) was removed from the Teflon body and replaced by carbon paste containing the Mn(III)-porphyrin ionophore (0.1 g graphite powder, 60 μL paraffin and 25 mg Mn(III)-porphyrin 1). The concentration of Mn(III)-porphyrin 1 vs. paraffin volume was 3.4×10^{-1} M. Finally, the electrode surface was smoothed again with paper.

2.4. EMF Measurements

The *EMF* measurements were performed at room temperature (approximately 20 °C) by the use of a multi-channel pH meter supplied by Lawson Labs, Inc. (USA). An Ag/AgCl double junction electrode was used as the reference electrode. The cell assembly was as follows.

For the PVC supported liquid membrane electrodes:

Ag/AgCl | 3 M KCl | 1 M CH_3COOLi || sample solution | membrane | 0.1 M KCl | Ag/AgCl

For the carbon paste electrodes:

Ag/AgCl | 3 M KCl | 1 M CH_3COOLi || sample solution | modified carbon paste | carbon paste

All electrodes were conditioned in a 0.01 M (pH 5.5) phosphate buffer for 24 h prior to use.

2.5. Determination of Paracetamol in the Presence of an Artificial Matrix Mimicking Physiological Samples

The potentiometric responses of a CPE incorporating Mn(III)-porphyrin **1** toward PCT were checked in the presence of an artificial matrix with following composition: 0.01 M phosphate buffer, 0.09% NaCl, 0.5% BSA; pH 5.5.

In order to perform the recovery test, 25 mL of the artificial matrix was spiked using a stock solution of PCT (1 mM and 10 mM). The potentials generated by a known amount of PCT were recorded and PCT was determined based on the calibration curve obtained in the presence of the artificial matrix.

2.6. Optical Thin-Film Preparation and Spectroscopic Measurements

Thin films of polymeric membranes, having the same composition as for the potentiometric measurements, were deposited on clean glass slides (50 mm × 8 mm × 1 mm). Glass slides covered with thin films of the membranes were placed into quartz cuvettes containing 2 mL 0.01 M phosphate buffer (pH 5.5). The concentration of paracetamol was changed by adding a stock solution of PCT (1 mM or 10 mM).

The UV-vis absorption spectra of the thin films containing Mn(III)-porphyrin **1** were recorded in the presence of different concentrations of PCT in the wavelength range of 200 – 1000 nm. The measurements were carried out every 10 minutes until the UV-vis spectra did not show any further change.

3. Results and Discussion

3.1. Potentiometric Responses of Mn(III)-Porphyrin-Containing Sensors Toward Paracetamol

The potentiometric responses of these sensors toward paracetamol were measured in 0.01 M phosphate buffer at pH 5.5. Under these conditions, paracetamol ($pK_a = 9.5$) exists as the undissociated compound in solution. Figure 2 shows the responses of a polymeric liquid membrane and carbon paste based sensor toward paracetamol. Both of the sensors contain Mn(III)-porphyrin 1 (Fig. 1) as the host molecule.

The sensor based on carbon paste modified with the investigated host porphyrin displayed a better response toward paracetamol in comparison to the PVC liquid membrane sensor, with a lower detection limit (3.9×10^{-5} M) and a larger slope (-45.7 mV log C^{-1}) (Table 1). The detection limit was estimated by the linearization method recommended by IUPAC [39].

The response properties of ISEs based on ion carriers are strongly influenced by membrane composition, in particular lipophilic ionic sites [40 – 42]. The type of sites, cationic or anionic, depends on the charge of host and guest molecules. In the case of ISEs based on the neutral host, ionic sites with the charge opposite to that of primary ions are necessary to obtain a Nerstian response, to decrease the membrane resistance, to reduce the co-ion interference, and to improve the detection limit and selectivity. On the other hand, in ISEs based on electrically charged hosts, the ionic sites with the same charge sign as

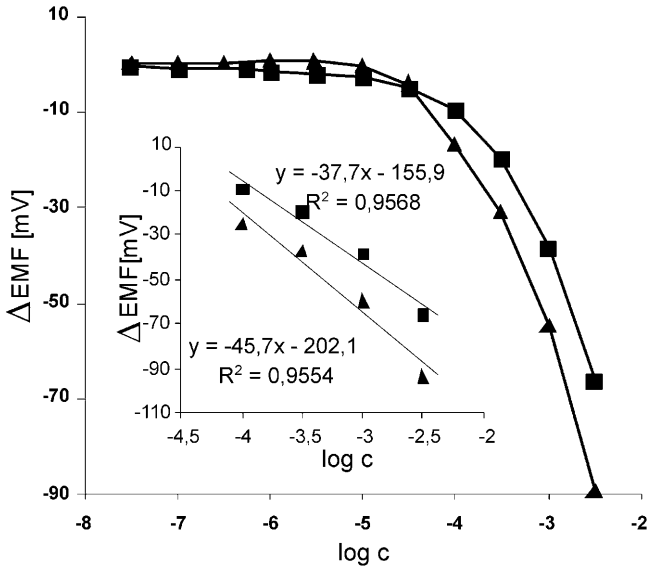


Fig. 2. Potentiometric response toward paracetamol of Mn(III)-porphyrin ISE (■) and Mn(III)-porphyrin CPE (▲). The measurements were performed in the presence of 1.0×10^{-2} M phosphate buffer, pH 5.5 (■, $n = 8$; $0.24 < SD < 9.35$), (▲, $n = 6$; $0.25 < SD < 11.92$).

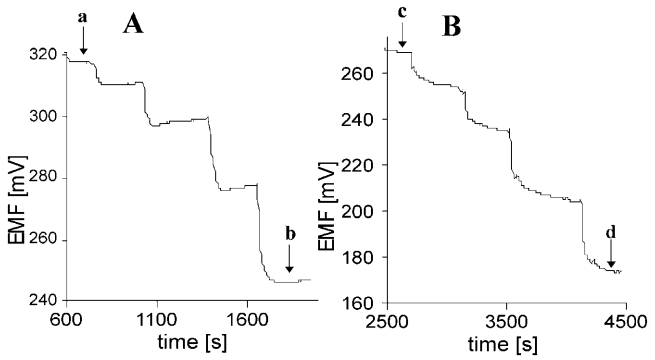


Fig. 3. Dynamic responses of Mn(III)-porphyrin ISE (A) Mn(III)-porphyrin-CPE (B). The analyte concentration was changed from a) 3.2×10^{-5} M to b) 3.2×10^{-3} M (A) and from c) 3.2×10^{-5} M to d) 2.0×10^{-5} M (B).

primary ions are recommended. To our knowledge, there is no recommendation concerning the type of ionic sites in the case of ISEs destined for the determination of neutral target compounds. Therefore, the influence of both types of lipophilic additives on the response behavior of the PVC membrane sensor was studied using 50 mol% (vs. ionophore) of potassium tetrakis(*p*-chlorophenyl)borate (KTPCIPB) and tridodecylammonium chloride (TDDMACl). The obtained results show that the incorporation of both types of lipophilic additives into the membrane slightly decreases the sensor responses toward PCT (results are not shown). Therefore, for further experiments, sensors incorporated with the Mn(III)-porphyrin **1** host only were applied.

The dynamic response time is an important factor for any potentiometric sensor. The response time of the investigated sensors was recorded by changing the PCT concentration from 1.0×10^{-8} to 1.0×10^{-1} M and was determined as 60 s for ISE and 40 s for CP sensors (Fig. 3, Table 1).

Table 1. Potentiometric response characteristics of PCT-selective electrodes based on Mn(III)-porphyrin **1** ($n = 5$). Detection limits and response times were estimated by methods recommended by the IUPAC [39].

Type of electrode	Slope ($\pm SD$) (mV/decade)	Detection limit (M)	Response time (s)
PVC Mn(III)-porphyrin	-37.7 ± 6.4	8.9×10^{-5}	60
Carbon paste Mn(III)-porphyrin	-45.7 ± 5.9	3.9×10^{-5}	40

Both types of sensors, when stored in 0.01 M phosphate buffer (pH 5.5), could be used for at least 2 weeks without any detectable divergence in PCT sensitivity.

3.2. Selectivity of Polymeric Liquid Membrane and Carbon Paste Electrodes Incorporating Mn(III)-Porphyrin Toward Paracetamol

The influence of interfering ions on the response behavior of a sensor is usually described in terms of selectivity coefficients K_{ij}. Potentiometric selectivity coefficients of the membrane sensors were evaluated by the matched potential method [42, 43]. This method is the only one suitable for the determination of selectivity coefficients when the target is a neutral molecule. This method does not depend on the Nicolsky – Eisenmann equation because the potentiometric selectivity coefficient is defined as the activity ratio of primary and interfering compounds that give the same potential change under identical conditions. The selectivity coefficients were calculated from the equation:

$$K_{ij} = C_i / C_j$$

Where C_i is the concentration of paracetamol and C_j is the concentration of the interfering compound. All measurements were performed in a background solution of 1.0×10^{-4} M paracetamol in an appropriate buffer. The experimental conditions employed and the resulting values obtained are summarized in Table 2. The data show that the proposed sensors are very selective to paracetamol over the interfering compounds studied, which are present in physiological samples. Comparison of the selectivity coefficients indicated that the carbon paste based sensor is more selective than the PVC based one.

3.3. Determination of Paracetamol in the Presence of an Artificial Matrix Mimicking Physiological Samples

In order to show the applicability of the sensors based on Mn(III)-porphyrin **1**, the potentiometric determination of PCT was performed in the presence of an artificial matrix,

Table 2. Potentiometric selectivity coefficients ($K_{i,j}^{MPM}$) for PCT sensors based on Mn(III)-porphyrin **1** ($n=5$). The log $K_{i,j}^{MPM}$ value was calculated for $\Delta EMF = -3.0$ mV. The concentration of paracetamol in a background solution was 1.0×10^{-4} M. The measurements were performed in 0.01 M phosphate buffer solution, pH 5.5.

Type of electrode	$K_{i,j}^{MPM}$			
	Interferents			
	SO_4^{2-}	Cl^-	PO_4^{2-}	Lactic acid
PVC Mn(III)-porphyrin	-2.28 ± 0.45	0.59 ± 0.09	– [a]	0.00 ± 0.02
Carbon paste Mn(III)-porphyrin	– [a]	-0.74 ± 0.18	– [a]	-1.87 ± 0.12

[a] The potential was constant until 9.9×10^{-2} M of interfering salt, before a cationic potentiometric response was observed.

which mimics the 10 times diluted human plasma (see Sec. 2).

The Mn(III)-porphyrin-containing carbon paste electrode, being a more sensitive sensor, was selected for this experiment. The results obtained are presented in Figure 4. The potentiometric signals generated in the presence of PCT were lower in comparison to these observed in pure buffer solution (Fig. 2). Nevertheless, linear potentiometric responses toward PCT were observed within the concentration range from 4.6×10^{-4} M to 4.9×10^{-3} M. The detection limit, estimated by the linearization method, was 3.5×10^{-4} M. Thus, this sensor might be used for the determination of a toxicological concentration of PCT in human plasma which is in the range of 2 mmol/L [9].

The PCT calibration curve obtained in the presence of an artificial physiological matrix (Fig. 4) was used for performing a recovery test. 25 mL of artificial physiological matrix was spiked with a known amount of PCT and the generated potential changes were recorded. Next, PCT concentrations were determined based on the calibration curve (Fig. 4). The recovery data collected in Table 3 suggest that the sensor presented might be applied for PCT determination in the concentration range from 0.6 to 2.0 mM with good precision.

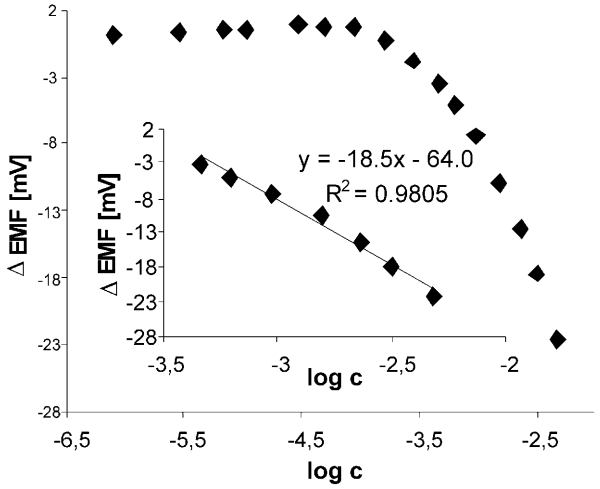


Fig. 4. Potentiometric response of the Mn(III)-porphyrin-CPE toward paracetamol. The measurements were performed in the presence of 0.01 M phosphate buffer, 0.09% NaCl, 0.5% BSA; pH 5.5 ($n=8$; $0.23 < SD < 4.2$).

Table 3. Recovery test in an artificial physiological matrix using PCT determination with the Mn(III)-porphyrin **1** carbon paste electrode (measuring conditions see Fig. 4; $n=7$).

Paracetamol added (mM)	Paracetamol determined (mM)	Recovery (%)
0.60	0.66 ± 0.08	103.7 ± 3.1
2.00	2.02 ± 0.09	108.8 ± 4.6

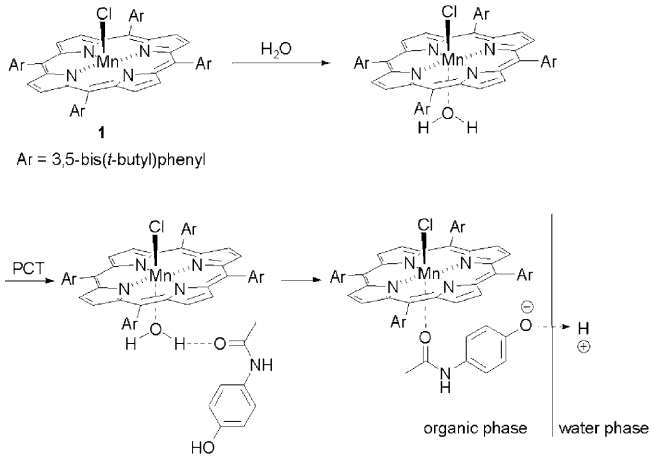
3.4. Mechanism of Potentiometric Signal Generation by Mn(III)-Porphyrin-Containing Sensors after Stimulation with Undissociated Paracetamol

The generation of membrane potential changes after stimulation with undissociated paracetamol molecules could be explained as follows. In the first step, chloride ligated Mn(III)-porphyrin 1 creates an aqua-complex via simple binding of water as a sixth ligand (Scheme 1). The creation of such a complex was described by Meyerhoff et al. [44]:

$$Mn(TPP)Cl + H_2O \rightleftharpoons Mn(TPP)ClH_2O$$

In the next step, a second-sphere supramolecular complex of paracetamol molecules with $Mn(TPP)ClH_2O$ [44] is created (Scheme 1). The existence of such a complex at the surface of a polymeric liquid membrane modified with metalloporphyrins was postulated by Kibbey et al. [45]. This second-sphere interaction of paracetamol molecules occurs probably at a low sample concentration. When the concentration of paracetamol increases, an exchange of second-sphere coordinated paracetamol for inner-sphere water ligands occurs, and, as a consequence, a complex between the Mn(III) centers and paracetamol, via the oxygen atom from the amide group, is created (Scheme 1).

In the measuring condition (pH 5.5), PCT moelcules exist in undissociated form ($pK_a = 9.5$). The formation of the above mentioned complex, according to a combination of mesomeric and inductive effects, causes an increase of the acidity of the phenolic OH function from PCT molecule.



Scheme 1. The illustration of the mechanism of potentiometric signal generation by potentiometric sensors incorporating Mn(III)-porphyrin in the presence of undissociated paracetamol.

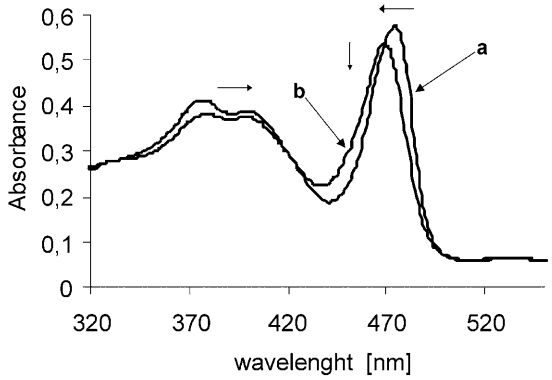


Fig. 5. UV-vis spectra of a thin PVC film doped with Mn(III)-porphyrin **1** upon exposure to paracetamol at the following concentrations: a) 0 M; b) 1.0×10^{-3} M. The measurements were performed in the presence of 0.01 M phosphate buffer, pH 5.5.

This decreases the pK_a of the PCT at the surface of PVC or carbon paste supported organic phase. As a consequence, this leads to a more facile dissociation of the OH group and finally to H^+ ejection from the interface to the aqueous layer adjacent to the organic phase (Scheme 1).

The energy gained from the proton solvation process is probably the driving force allowing the dissociation of phenol derivatives at the aqueous/organic membrane interface. This event is responsible for the generation of an anionic response of ISE and CPE modified with Mn(III)-porphyrin 1 after their stimulation with undissociated paracetamol.

The reaction of Mn(III)-porphyrin 1 with paracetamol was confirmed by spectroscopic measurements at the border between water and the polymeric membrane (Fig. 5). The UV-vis absorption spectrum of a thin membrane film containing Mn(III)-porphyrin **1** deposited onto glass slides conditioned in 0.01 M phosphate buffer solution (pH 5.5) exhibited one main band at 470 nm and two weaker bands at 376 and 400 nm (Fig. 5). After conditioning in phosphate buffer with an increasing concentration of paracetamol, the absorbance maximum decreased and shifted to shorter wavelength. This blue shift was expected due to the increase in electron density around the Mn(III) centers by the coordinated amide. These data confirm the creation of a complex between the Mn(III)-porphyrin 1 and paracetamol.

The differences between the selectivity and sensitivity of the sensors investigated are the result of the structure of the solid phases. The polymeric liquid membrane surface is very hydrophobic and less developed in comparison to the carbon paste surface. Probably, these properties have an influence on the accessibility of the doped Mn(III)-porphyrin **1** host for the PCT guest, and it could also influence the process of H^+ ejection from the interface to the aqueous layer adjacent to the organic/solid phase.

In addition, CPEs compared to ISEs are simpler to prepare and regenerate. They give stable responses with very low Ohmic resistance [33, 34].

4. Conclusions

It was proved that Mn(III)-porphyrin 1 was sensitive and selective ionophore in polymeric liquid membrane and carbon paste electrodes destined for the direct determination of paracetamol. The potentiometric signals were generated by Mn(III)-porphyrin 1 incorporating sensors in the presence of undissociated form of paracetamol. The carbon paste electrode incorporating Mn(III)-porphyrin 1 showed better linear range, lower detection limit and faster response time then polymeric liquid membranes. The CPEs properties are mainly connected with their lower Ohmic resistance and more developed interface structures in comparison to ISEs. In addition, CPEs are easier to prepare and regenerate than ISEs.

The recovery test done with Mn(III)-porphyrin 1 – carbon paste electrode proved that this type of sensor might be recommended for the direct determination of toxic level of paracetamol in physiological samples.

5. Acknowledgements

The work was realized within the Indo-Polish joint Science & Technology project No 5/06, with the aid of a statutory fund of the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland and Department of Science and Technology, Government of India, New Delhi, India. The authors thank The Regional Blood Donation Centre in Olsztyn, Poland for their supply of human plasma. W. D. thanks the University of Leuven, the Ministerie voor Wetenschapsbeleid and the FWO for financial support.

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