Journal of Inclusion Phenomena and Macrocyclic Chemistry **35:** 111–122, 1999.

Novel Potentiometric Sensor for Determination of Cysteine Based on Substituted Poly(diphenylporphyrins and Metalloporphyrins)

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Abstract. This paper describes the application of an electropolymerized film of poly(bis(2-aminophenyl)-2,8,12,18-tetraethyl-3,7,13, 17-tetramethylporphyrin) and its metalloderivative for determination of amino acids. A remarkable selectivity for cysteine (log $K^{\text{pot}}_{\text{Cys/Amino acids}} = 10^{-2}$–$10^{-3}$) has been found. The potentiometric response to sulfur-containing amino acids is discussed and compared with selectivity data for PVC-membranes based on metallotetraphenylporphyrins.

Key words: porphyrin, metalloporphyrin, electropolymerization, ISEs, amino acids.

1. Introduction

The binding and transport of amino acids constitutes an important problem in supramolecular chemistry from both the biomimetic and analytical points of view [1]. Presently, some information is available about the dynamics and regulation of amino acid transport in nature, but a detailed mechanistic or structural understanding remains lacking [2]. This paucity of information is providing an incentive to develop synthetic systems that are capable of carrying out amino acid transport [3].

As a part of our ongoing research we are interested in *specific receptors* with potential application as novel *electrochemical sensor* elements. We recently described the application of porphyrins and expanded porphyrins for recognition of biologically important anions [4]. Also electropolymerized porphyrin and metalloporphyrin films are interesting as sensor material for the construction of potentiometric sensors – ion-selective electrodes (ISEs). Potentiometric methods offer the possibility to study factors governing the recognition process of the functionalized polymeric film. The response mechanism of ISEs results from either ion-exchange (as for conventional ISEs) or selective interactions between polymer and the targeted substrates. The former depends simply on the substrate's lipophilicity; the

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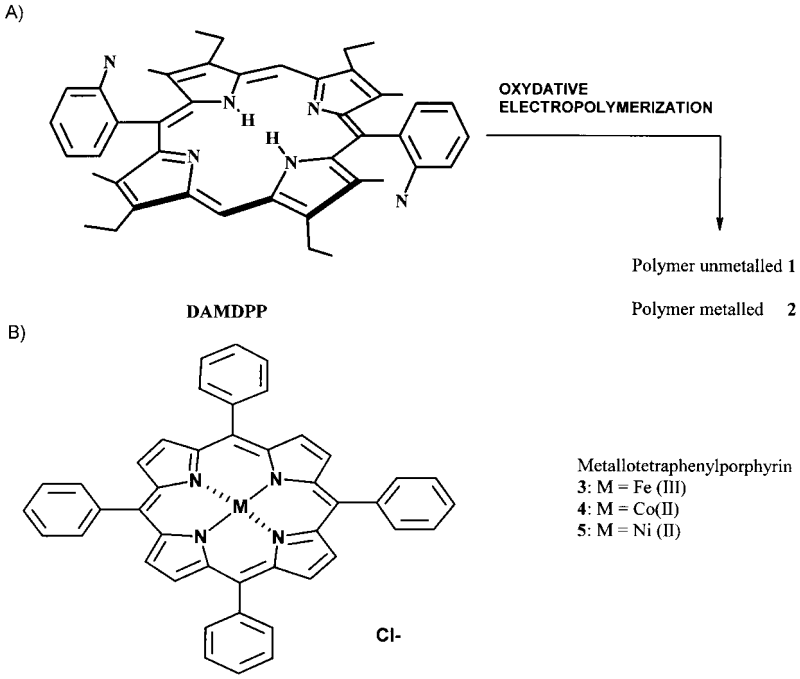


Chart 1. Structure of the compounds used in the present study for preparing polymerized films (A) and PVC-based membranes (B).

latter on chemical recognition principles: (i) complementary, (ii) specific interactions, (iii) proton uptake by ionophore and other particular binding modes. The structure of polymerized porphyrin film offers several binding modes including hydrogen bonding, π-π stacking, hydrophobic interaction, with the additional possibility to introduce a metal cation to the deposited layer and generate an axial ligand binding mode.

Recently, we have described the preparation of a novel poly(porphyrin) film based on the electropolymerization of 5,15-bis(2-aminophenyl) porphyrin from aqueous solutions with and without metal modification (Chart 1) [5]. The potentiometric selectivity patterns obtained for inorganic anions reflected the different response mechanism of the polymerized electrode: (1) ion-exchange for unmetalled film, and (2) selective axial ligation between metal center and certain anion for metalled film. Here we report the remarkable selective response of metalled poly(porphyrin) film toward cysteine ($\log K^{\mathrm{pot}}_{\mathrm{Cys/Amino\,acids}} = 10^{-2}$–$10^{-3}$) at neutral pH. This finding indicates that an electropolymerization technique of proper derivatized porphyrin macrocycle represents a new approach for the design of receptors for biologically important substrates, such as amino acids.

The recognition of amino acids has attracted considerable interest, but the change of ionization forms (cationic, zwitterionic, anionic) with pH makes design of specific receptors very challenging. In order to chelate an amino acid in its

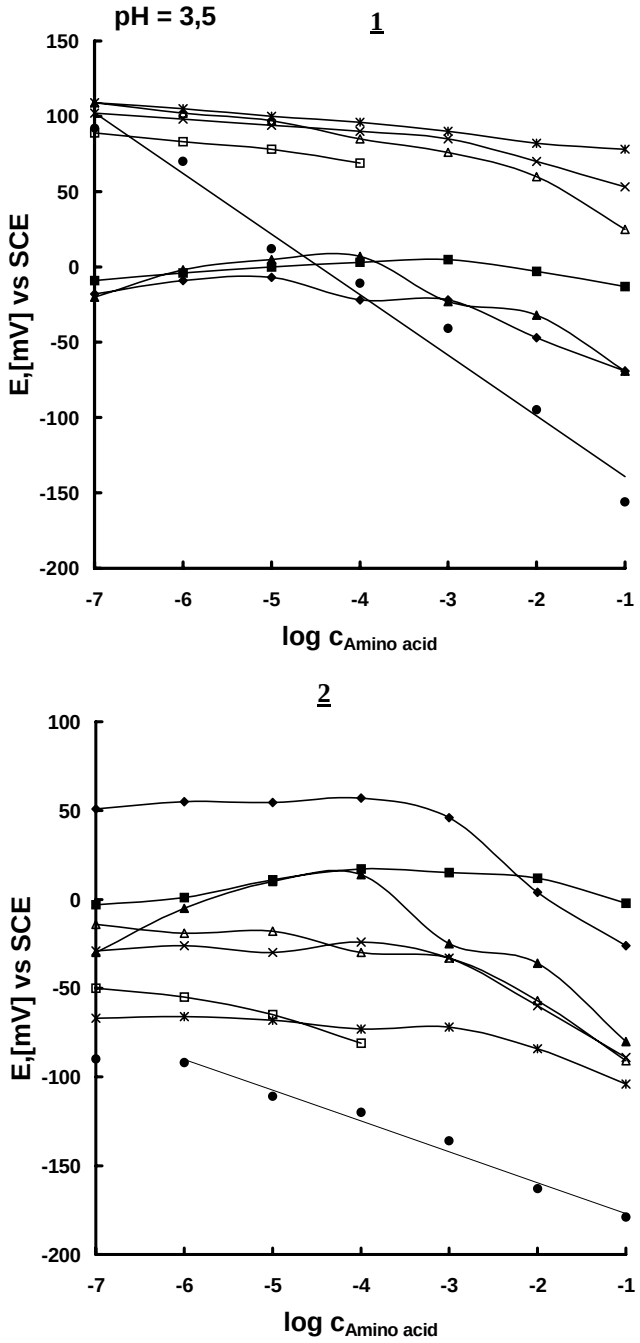
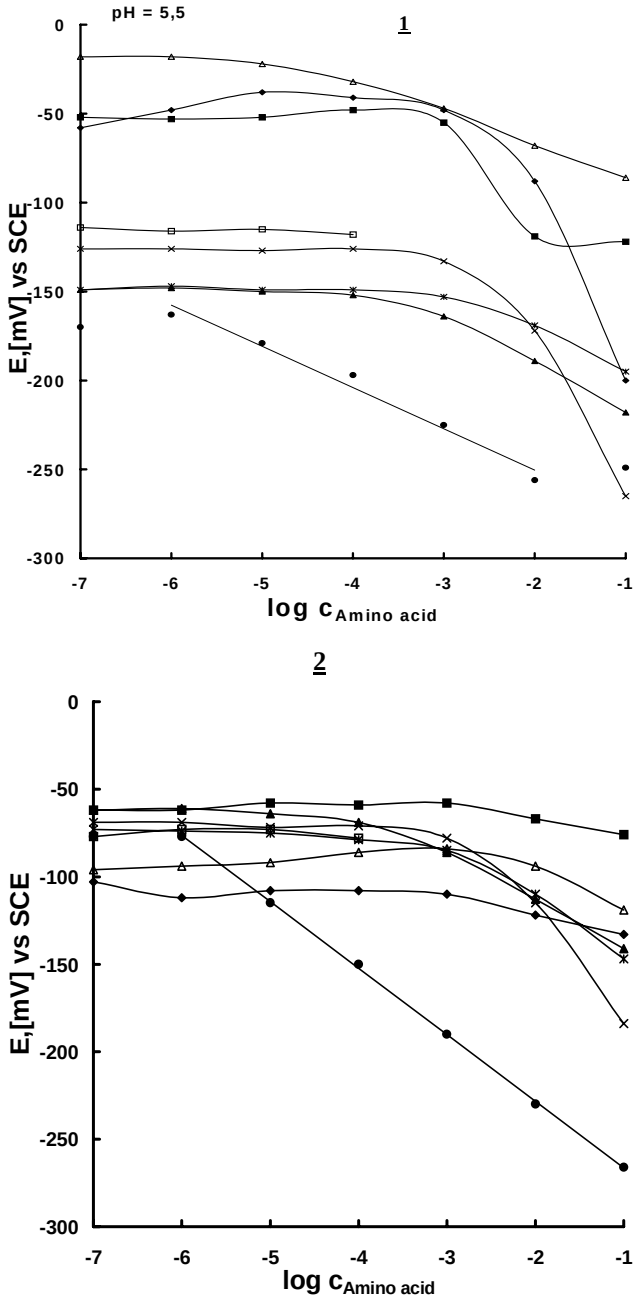


Figure 1. pH-effect on potentiometric response of unmetalled **1** and metalled **2** polymerized films to amino acids (♦, CH_3COO^-; ∗, Arg; ●, Cysteine; □, Cystine; ■, Gly; ×, Lys; ▲, His; △, Met).



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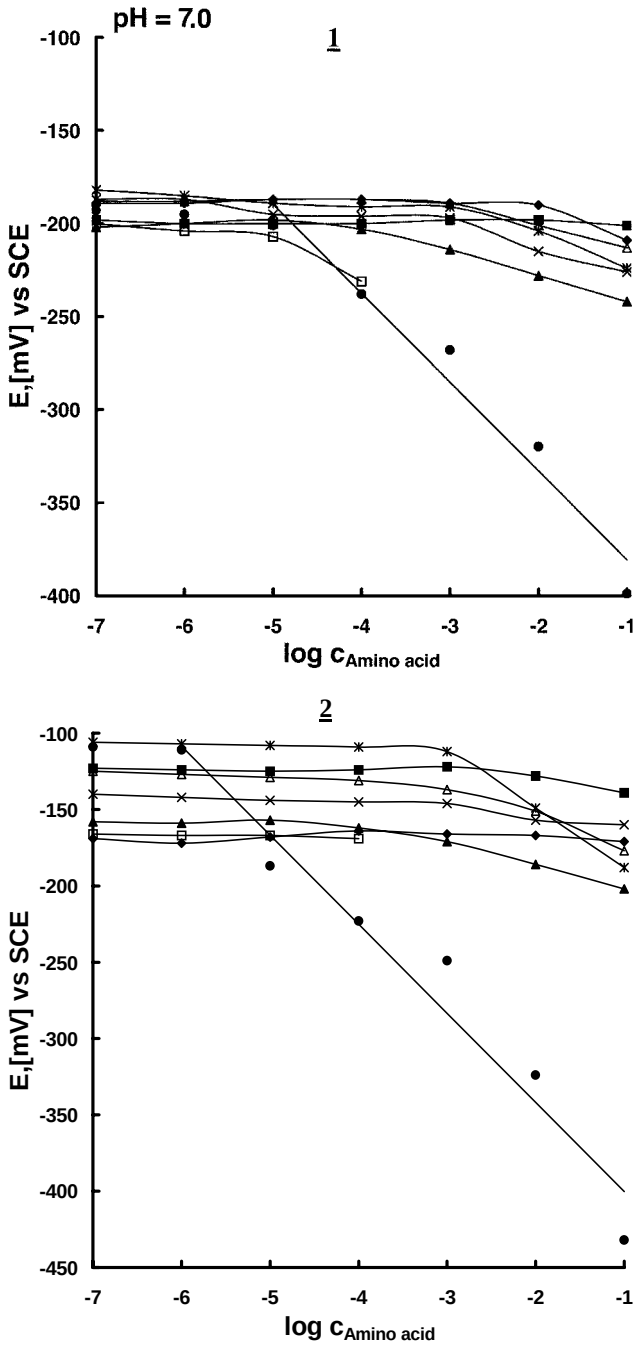

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zwiterrionic form (at neutral pH), bidentate receptors, containing recognition sites for both carboxylate anion and protonated amino group have to be employed.

From a mechanistic point of view, we have been interested to gain insight into the mechanism of recognition by which the unmetalled and metalled poly(porphyrins) recognize the amino acids, in particular, cysteine. Herein we represent the pH dependence and transition metal influence on potentiometric discrimination of amino acids by polymerized porphyrin electrodes. Our data indicate that the potentiometric response is based on the coordination interaction between the substrate group —*S*—*H* and the metallo-center of polymerized porphyrin.

2. Experimental

2.1. REAGENTS

The syntheses of 5,15-bis(2-aminophenyl)-2, 8, 12, 18-tetraethyl-3, 7, 13, 17-tetramethylporphyrin (DAMDPP), the metalloporphyrins containing Fe (III), Co (II) and Ni (II) have been reported [6–8]. The structure of used DAMDPP and metalloporphyrins are represent in Chart 1. The following amino acids and related compounds were of the highest grade commercially available, and used without further purification: alanine (Ala) from Merck, Germany; cystine from Loba Feinchemie, Germany BRD; glycine (Gly) from Lachema, Czech Republic; histidine (His), serine (Ser) from Sigma-Aldrich, Germany; arginine (Arg), lysine (Lys), threonine (Thr), tryptophane (Trp) from Sigma-Aldrich Chemie GmbH, Germany; aspartic acid (Asp), cysteine, leucine (Leu), glutamic acid (Glu), methionine, phenylalanine (Phe), proline (Pro), tyrosine (Tyr) from Fluka Biochemie, Switzerland. Cysteine and methionine as their methyl ester hydrochlorides, cystine dimethyl ester dihydrochloride were bought from from Sigma-Aldrich Chemie GmbH, Germany.

Nitrogen was from Linde-Technoplyn (Prague, Czech Republic).

Poly(vinyl chloride) high molecular weight (PVC), 2-nitrophenyl octyl ether (*o*-NPOE), tetrahydrofuran (THF; stored over 3 Å molecular sieves) used for preparation ion-selective membranes were purchased from Fluka Chemika, Switzerland.

Sodium hydroxide and 2-[*N*-Morpholino]ethanesulfonic acid hydrate (MES) were purchased from Onex (Roznov, Czech Republic) and Sigma-Aldrich Chemie (Germany), respectively. All the other chemicals used were of analytical-reagent grade and were obtained from Lachema (Brno, Czech Republic). Distilled water was used to prepare buffer and standard solutions.

2.2. APPARATUS AND ELECTRODES

The instrumentation used for electropolymerization was a polarographic analyzer PA 2 (Laboratory Devices Prague) with a cyclic voltammetric adapter (Department of Analytical Chemistry, Institute of Chemical Technology, Prague, Czech

Republic) connected to an XY-recorder BAK 5T (Aritma Prague). The polymerization was performed in a three-electrode electrochemical cell. The Ag/AgCl (3 M KCl) and platinum wire inserted in a glass tube (diameter 5 mm; 3 M H_2SO_4) with porous diaphragm were employed as a reference electrode and an auxiliary electrode, respectively. The working electrode was a platinum wire (Pt; diameter 0.4 mm, length 7 mm), fixed by means of Wood alloy within a glass tube (diameter 5 mm). Prior to polymerization, the working electrode was polished with Al_2O_3 and $CaCO_3$ powder, then kept in a mixture of $K_2Cr_2O_7$ with H_2SO_4 for 1 h.

The potentiometric measurements were made with a digital voltammeter, Model M1T330 (Metra Blansko, Czech Republic). The reference electrode was Hg/Hg_2Cl_2, KCl (sat.) electrode (Crytur, Monokrystaly s.p., Turnov, Czech Republic). The cell assemblies for potentiometric measurements with polymerized and PVC-based electrodes were as follows:

> Hg/Hg_2Cl_2, KCl (sat.) | 3 M KCl | 0.05 M MES buffer test solution | polymer film/Pt
>
> Hg/Hg_2Cl_2, KCl (sat.) | 3 M KCl | 0.05 M MES buffer test solution | membrane | 0.01 M KCl, 0.05 M MES | Ag / AgCl.

All of the potentiometric measurements with prepared electrodes were carried out at ambient temperature. The pH was monitored using glass electrode Type 01-29 B (Labio Prague, Czech Republic) and a pH-Meter Type OP – 205/1 (Budapest, Hungary).

2.3. ELECTRODE PREPARATION AND EMF MEASUREMENTS

2.3.1. *Electropolymerization*

The electrolytic solution (0.005 M DAMDPP, 3 M H_2SO_4) was deoxygenated by bubbling nitrogen through the solution for approximately 10 min. Electropolymerization was carried out from solutions of DAMDPP by repeated cyclic scanning of the platinum electrode potential from -0.05 to +1.2 V at 50 mV/s [5]. The thickness of the film was controlled by the polymerization time. Part of the prepared electrodes was modified by Co (II) as described by Ikeda *et al*. [9]. The formed electrodes were thoroughly washed with distilled water and with 0.1 M ammonium hydroxide in the case of metalled electrodes.

2.3.2. *Preparation of polymer membranes*

The ion-selective membranes based on **3–5** were prepared by dissolving in 0.7 ml THF approximately 100 mg of mixture composed of 33 wt % PVC, 66 wt % *o*-NPOE, 1 wt % receptor. A membrane solution was poured into a metallic tube (stainless steel, face ground on both sides; height: 20 mm; thickness: 2 mm; inner diameter: 16 mm), and solvent was allowed to evaporate for 24 h. A circle (diameter: 12 mm, thickness: 0.15 mm) was cut out from the membrane thus prepared,

fixed on a polymer ring (inner diameter: 8 mm) and mounted on a liquid membrane type ISE body from Crytur, Czech Republic.

2.4. EVALUATING POTENTIOMETRIC ANION RESPONSE AND SELECTIVITY

Working solutions were prepared by dilution of stock solutions with MES adjusted to experimental pH with NaOH. Calibration curves were constructed by plotting the potential *vs.* logarithm of concentration of the substrate present in the buffer solution. Before each set of measurements, the electrodes were soaked overnight in 0.05 M MES under experimental pH solution without guest (5–10 min). The response time t (Δt, ΔE) was defined as the time at which the differential quotient ($\Delta E/\Delta t$) of the potential-time curve becomes smaller than a prechosen value ($\Delta E < 1.0$ mV within $\Delta t = 1.0$ min in the present study). Potentiometric selectivity coefficients ($K_{i/j}^{\text{pot}}$) were evaluated by the matched potential method [10, 11].

3. Results and Discussion

3.1. INFLUENCE OF pH ON POTENTIOMETRIC RESPONSE OF ELECTROPOLYMERIZED FILMS TO AMINO ACIDS.

The potentiometric responses for a series of amino acids as a function of pH were measured with following results (Figure 1).

(1) It was found that pH 3.5 and 5.5 are the optimal conditions for the sensitivity of metalled and unmetalled films to CH_3COO^-: -36 mV/decade, 10^{-3}–10^{-1}M; -76 mV/decade, 10^{-3}–10^{-1} M, respectively (Figure 1). The potentiometric response for the simplest amino acid Gly was observed only with unmetalled film under pH 5.5.

(2) The ability of polymerized films to differentiate the aromatic- (Phe, Tyr and Trp) and imidazolyl-containing (His) amino acids was examined.

In the presence of His both **1** and **2** electrodes demonstrated a strong anionic response near pH 3.5: -37 mV/decade, 10^{-2}–10^{-1} M and -29.3 mV/decade, 10^{-4}–10^{-1} M, respectively, which was reduced with increasing pH. The remarkable difference in response for the measured concentration range by unmetalled and metalled poly(porphyrin) film attains two orders of magnitude. It is well known that the measuring range of ISEs is defined as the activity ratio of the upper and lower detection limit [12]. In addition, the kind and concentration of the interfering electrolyte influence the maximum measuring range. Our experimental data indicated uptake of proton by the electropolymerized poly(porphyrin) surface, of course this effect was weaker in the case of metalled film [5]. Thus, the lower detection limit of unmetalled film is limited probably because of protons interfering.

Interestingly, at the same conditions the polymerized films are more selective to His than to other aromatic amino acids (Tyr, Trp), with the exception of Phe. In the case of Phe, the near-Nernstian potentiometric response was observed under pH

Table I. pH-Effect on potentiometric sensitivity of unmetalled **1** and metalled **2** polymerized films to Cysteine

pH	**1**			**2**		
	S, mV/decade	Linear range, M	C_{min}, M	S, mV/decade	Linear range, M	C_{min}, M
3.5	−40	10^{-7}–10^{-1}	2.5×10^{-8}	−16	10^{-6}–10^{-1}	–
5.5	−30	10^{-4}–10^{-2}	4.0×10^{-5}	−38	10^{-6}–10^{-1}	4.0×10^{-7}
7.0	−48	10^{-5}–10^{-1}	4.0×10^{-6}	−58	10^{-6}–10^{-1}	4.0×10^{-7}

3.5 at narrow concentration range 10^{-6}–10^{-5} M with electrode **2**. It is known that the imidazole ring acts as one of the strongest σ electron donating ligands [13]. The potentiometric discrimination of His is feasible due to imidazolyl ring-metal interaction within the metallated polymerized film.

Thus, both kinetic (*kinetics of the phase transfer equilibria*) and steric limitations (*morphology of the polymerized film and the structure of the target substrate*) may influence the selective ISE response.

(3) The basic acids (Lys and Arg) represent another class of tested amino acids. Note the preference of both unmetalled and metalled films to Lys at pH 5.5: −66 mV/decade and −53 mV/decade beginning from 10^{-3} M, respectively. The electrode **2** displayed the selectivity to Arg: −38 mV/decade from 10^{-2} and 10^{-3} M, respectively for pH 5.5 and 7.0 (Figure 1).

(4) From the sulfur-containing amino acids: Cysteine (—*S*—*H*), Cystine (—*S*—*S*—) and Methionine (—*S*—CH_3) were investigated. We found that poly(porphyrin) films are selective for Cysteine only. Results are presented in Table I.

The ISE's characteristics indicate that the polymerized porphyrin electrode displays high sensitivity to Cysteine at acidic medium (Figure 1). Actually, the coulombic interaction between the sulfur atoms in the amino acid and porphyrin ring increases with decreasing pH (Figure 2), since the porphyrin ring is effectively protonated at the given conditions (for porphyrin pK_{a1} = 3.5, pK_{a2} = 5.5; spectroscopically determined [14, 15])

In contrast, the potentiometric activity of the metal-containing film toward Cysteine increases at pH range 5.5–7.0. As seen from Figure 2, Cysteine includes two acid groups (—NH_3^+ and —*S*—*H*) of comparable strength, the thiol group is slightly stronger [17]. Therefore, the number of Cysteine molecules with —S^- groups would rise with increasing pH and, as a consequence, the metal-sulfur bond strength would enhance (Table 1). In support of the possibility of —S^- binding at the metal center, we noted an absence of the pH-effect with Methionine substrate, which includes a —*S*—CH_3 group. Also, the sensitivity of electrode **2** to Methionine remained without marked changes ($-29 \div -26$ mV/decade) at wide pH range 3.5–7.0.

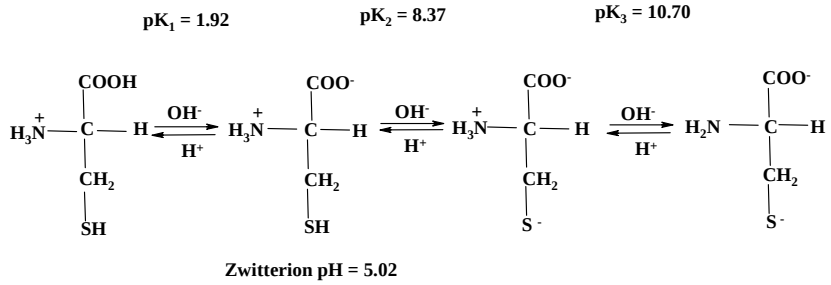

Figure 2. Ionization of Cysteine in aqueous solutions of different pH. The pK values quoted are at a temperature of 35 °C [16].

Table II. Potentiometric response of metalloporphyrin-based electrodes for cysteine methyl ester hydrochloride

Ligand	S, mV/decade	Linear range, M	E^0, mV	R^2
DAMDPP	28	10^{-2}–10^{-1}	−45	0.9566
3	33	10^{-4}–10^{-1}	−26	0.9588
4	34	10^{-3}–10^{-1}	−174	0.9981
5	19	10^{-2}–10^{-1}	+15	0.9570

However, a weak response was observed in the case of Cystine containing the disulphide group (—*S*—*S*—). The total response toward a given substrate is likely to depend not on the charge or the axial ligand binding, but rather on the planar structure of the substrate and the polymer morphology.

Thus, the pH-effect on potentiometric activity reflects the role of both axial ligand binding and coulombic attraction which are the most important interactions for Cysteine discrimination.

3.2. TRANSITION METAL AND POTENTIOMETRIC SELECTIVITY OF METALLOPORPHYRIN-BASED PVC-MEMBRANES TO SULFUR-CONTAINING AMINO ACIDS

Further, an attempt was made to examine how the metal-sulfur bond strength (for different transition metals) is influenced by the other possible binding groups of the amino acid: bond formation *via* carboxylate and/or amino-group. In this connection, the metalloporphyrins **3–5** having Fe (III), Co (II) and Ni (II) as central metal cations (Chart 1) were incorporated into PVC-membranes and characterized as receptors for sulfur-containing amino acids and their methyl ester hydrochlorides.

The cationic responses obtained for the investigated PVC-membranes are closely linked to the ability of the central metal ion to bind the sulfur atom (Table II). The slope of 33 mV/decade from 10^{-4} and 10^{-3} M, respectively for

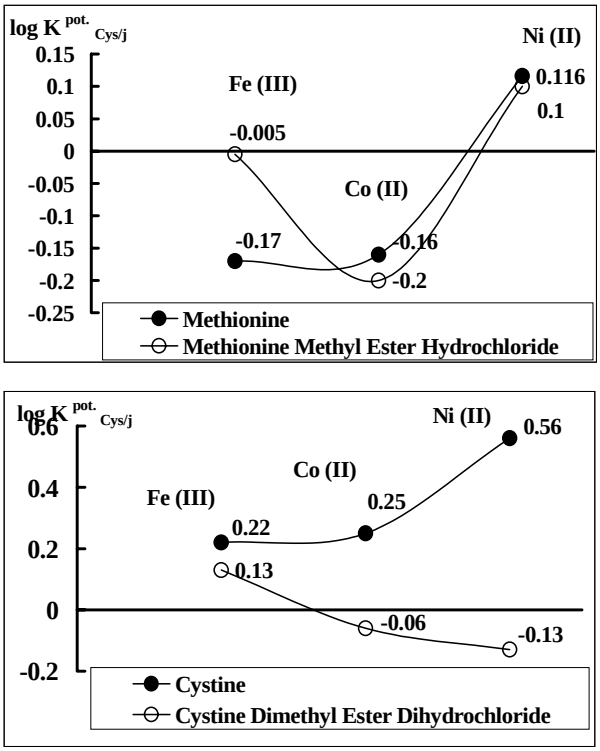

Figure 3. Influence of the metal on the selectivity coefficients, $\log K^{pot}_{Cys/j}$, for PVC-membranes based on metalloporphyrins **3–5**.

3 and **4**, indicated that Fe (III) and Co (II) are much more selective to cysteine methyl ester hydrochloride than Ni (II). No such effect was observed, however, with DAMDPP (a metal free monomer porphyrin).

The patterns of the selectivity of the prepared PVC-membranes to other sulfur-containing amino acids (Methionine and Cystine), relative to Cysteine are represent in Figure 3. The interfering influence of Methionine and Cystine changes for the transition metals in the following order: Fe(III) $\approx$ Co(II) $<$ Ni(II). The values of $\log K^{pot}_{Cys/j}$ at the presence of Cystine was found to markedly increase. Complexation of Cystine *via* both sulfur atoms could be an explanation of the observed effect.

The inversion of the selectivity order for transition metals was observed for the cystine methyl ester hydrochloride used as interfering substrate: Ni(II) $<$ Co(II) $<$ Fe(III) (Figure 3). Compared to Methionine, the selectivity of the Fe (III)-containing porphyrin ($\log K^{pot}_{Cys/j}$) in the presence of methionine methyl ester hydrochloride is reduced.

4. Conclusions

The potentiometric response of nonmetalled polymerized porphyrin films **1** is selective for Cysteine at acidic pH (3.5) with coulombic attraction as the main binding mode. Metalled poly(porphyrin) **2** is highly selective for Cysteine at neutral pH; this selectivity is governed by the strong sulfur-metal center binding (axial ligand). This mechanism of binding was also confirmed by use of PVC-membranes with metallotetraphenyl-porphyrins. The novel poly(porphyrin) films represent a novel electrochemical sensor element for Cysteine. The potentiometric sensitivity of the protonated poly(porphyrin) reached a value (-40 mV/decade) with $C_{\min}$ 2.5×10^{-8} and 4.0×10^{-6} M at acidic and basic medium, respectively.

The comparison of polymeric films with monomeric porphyrin units showed the higher binding efficiency of the polymeric films.

Acknowledgements

Financial support from Czech Grant Agency (grants No. 203/96/0740 and No. 203/97/1099 to V.K.), the Ministry of Education of Czech republic (grant VS 97135 to V.K.), the Howard Hughes Medical Institute (grant No. 74195-541101 to V.K.) and Project No. CEZ: J19/98: 223400008 is gratefully acknowledged.

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