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Protonation of porphyrins in liquid PVC membranes: Effects of anionic additives and application to pH-sensing

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Abstract

We report on a study on the protonation of representative prophyrins, metalloporphyrins, porphyrin-ketones and a Pd-porphyrin Schiff base in aqueous solution and when dissolved in plasticized PVC membranes. The respective protolytic forms were characterized by absorption and emission spectra as well as apparent pK_a values. It is shown that the protonation of porphyrins dissolved in PVC membranes is much more difficult than in solution, resulting in an extraordinarily large decrease in the apparent pK_a values which can be as large as 5 units when compared to the corresponding data for the porphyrins in solution. In certain cases, no protonation at all occurred within the pH 2–12 range. At the same time, N-MeEP which is much more basic (its intrinsic pK being 11.2) can be protonated in such membranes into the monocation at weakly acidic pH, but the apparent pK_a also is lowered by about 6 units.

On addition of a tetraphenylborate anion to the PVC membranes, protonation was found to proceed much easier. Both the monocation and dication was identified in case for the porphyrins, while for the prophyrin–ketones direct protonation into dication took place, and N-methyletioporphyrin was present as the monocation over the whole pH 2–12 range.

Membranes composed of solutions of porphyrin–ketones or the Pd-porphyrin Schiff base in plasticized PVC were identified as being useful for purposes of continuously sensing pH in the physiological range. Their response time, calibration curves, signal stability and effects of buffer ionic strength and temperature were investigated in more detail. © Elsevier Science S.A.

Keywords: Prophyrins; Protonation; pK_a values; Plasticized PVC membranes; Optical pH sensors

1. Introduction

Porphyrins and related compounds display very characteristic and longwave absorption and luminescence and hence appear to be promising candidates for use in optical chemical sensors [1,2]. Indeed, certain porphyrins have been found to be useful probes for oxygen [3] and anions [4]. Porphyrins are also known to display pH-dependent spectra [5], and protonation has been investigated in more detail [5,6]. Our interest in novel probes for optical sensing of pH has caused us to perform a more systematic study on the protonation of porphyrins both in water and in liquid polymer membrane solution. The results show that the protonation of porphyrins not only strongly depends on the respective chemical structures, but also that protonation in plasticized PVC strongly depends on the presence of anionic additives. Membranes made from porphyrin solutions in plasticized PVC are shown to be useful for optical sensing of pH in the physiological range.

2. Experimental

2.1. Materials

High molecular weight poly(vinyl chloride) (PVC), 2-nitrophenyl octyl ether (NPOE), bis(2-ethylhexyl) sebacate (DOS), tris(2-ethylhexyl)phosphate (TOP) and potassium salt of tetra(o-chlorophenyl)borate (PTCPB), were purchased from Fluka. Poly(vinyl chloride-co-vinyl acetate-co-2-hydroxypropyl acrylate), M.W.15 000 (PVCp) was from Aldrich. Octyl and dodecyl ethers of 2-cyanophenol (CPOE and CPDDE, respectively) were synthesized according to procedure described elsewhere [7]. All salts and solvents were of analytical grade.

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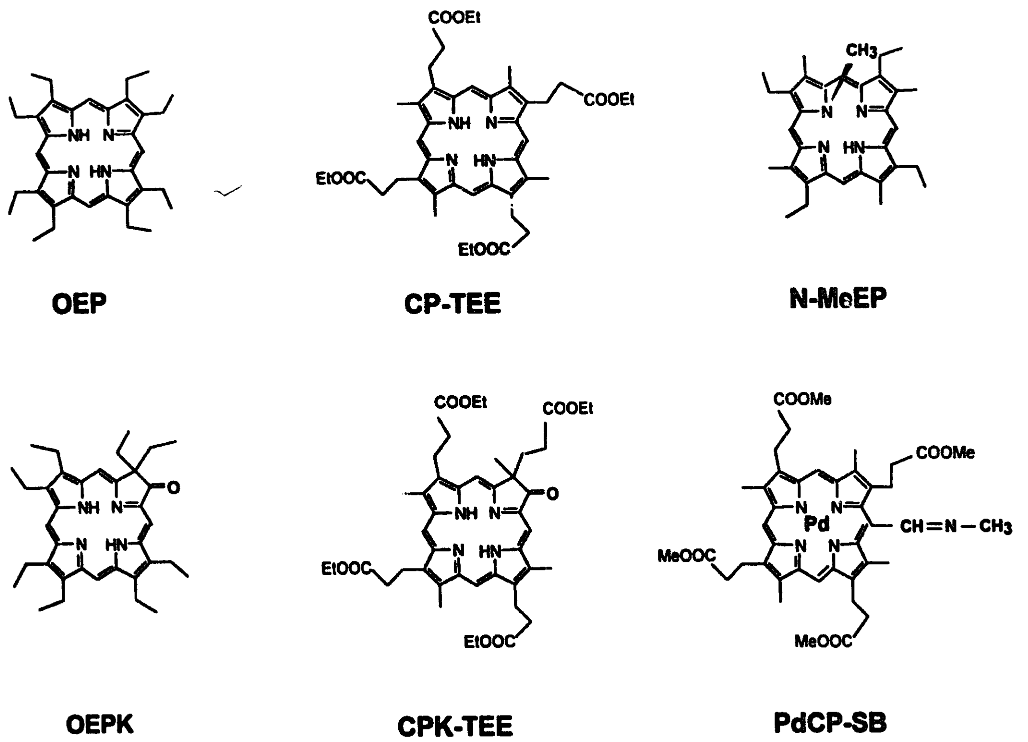

Fig. 1. Chemical structures of the dyes under investigation.

The porphyrin dyes of purity >97% were synthesized at the Institute of Biomedical Chemistry, Moscow. Octaethylporphyrine (OEP), coproporphyrin-I tetraethyl ester (CP-TEE) and N-methylethioporphyrin (N-MeEP) were prepared according to standard procedures [8,9]. Octaethylporphine-ketone (OEPK) and coproporphyrin-I-ketone tetraethyl ester (CPK-TEE) were synthesized according to Ref. [10], and the meso-substituted palladium(II) complex of coproporphyrin I tetramethyl ester Schiff-base (PdCP-SB) according to Ref. [11]. Their chemical structures are given in Fig. 1.

2.2. *Preparation of the polymer membranes*

A polymer solution was prepared containing (a) 120 mg of polymer and (b) 240 mg of plasticizer in 3 g of solvent (see Table 1). This solution (200 µl) was mixed with 100 ;gml of a solution of the respective porphyrin in chloroform (5 mg/ml) to give the so-called "cocktail". This cocktail was used to make the sensor films. Where necessary, 100 µl of a solution of PTCPB in THF (5 mg/ml) were added to this cocktail which then was spread onto a transparent polyester foil (Mylar, Du Pont) as a 10-µm thick layer using a home-made coating device. After solvent evaporation the coatings were approximately 2 µm thick. Disks ("sensor membranes") of 30-mm diameter (or slides of 12×30 mm) were cut and used for optical measurements. The membranes were stored dry at room temperature in the dark and used when necessary without special conditioning, except for washing them with aqueous buffer for 10–20 min prior to use. The composition of the cocktails and corresponding sensor membranes is given in Table 1.

2.3. *Apparatus*

Absorption spectra were acquired on a Shimadzu UV-2101PC dual-beam spectrophotometer in 1 × 1-cm cuvettes. PVC membranes were integrated into a flow-through cell to form one of its walls. Buffer solutions were pumped—at a flow rate of 2 ml/min—through both this cell and a reference cell (containing an undyed membrane) using a peristaltic pump (Minipuls-3, Gilson). Absorbance was monitored continuously at peak wavelengths or repetitively scanned. pH measurements were performed with a microprocessor pH-meter (pH 196, WTW) calibrated with standards of pH 7.00 and 4.00, respectively.

3. Results and discussion

3.1. *Structures of the dyes and protonation mechanisms*

OEP and CP-TEE (structures I and II) are classical lipophilic porphyrins. Their pyrrolic nitrogens (in the free base form) can be stepwise protonated into the monocation and dication [5,6]. The pK_1 and pK_2 values are 7.2 and 4.2, respectively [6]. In most solvent systems only the free base and the dication can be observed [5]. Fluorescence pH-titrations of porphyrins in aqueous solution gave pK_a values of about 5–6 [12]. The protonation equilibria are shown in Fig. 2 (Scheme A).

Table 1
Composition of cocktails used for preparation of sensor membranes. The a-type membranes contain the borate anion TCBP

Membrane	Polymer [a]	Plasticizer [a]	Solvent	Additive [b]	Porphyrin [c]
M1	PVC	CPDDE	$CHCl_3$	–	OEP
M1a	PVC	CPDDE	$CHCl_3$-THF	TCPB	OEP
M2	PVC	NPOE	THF-$CHCl_3$	–	CP-TEE
M2a	PVC	NPOE	THF-$CHCl_3$	TCPB	CP-TEE
M3	PVCp	CPDDE	$CHCl_3$	–	CP-TEE
M3a	PVCp	CPDDE	$CHCl_3$-THF	TCPB	CP-TEE
M4	PVC	NPOE	THF-$CHCl_3$	–	N-MeEP
M4a	PVC	NPOE	THF-$CHCl_3$	TCPB	N-MeEP
M5	PVCp	CPDDE	$CHCl_3$	–	OEPK
M5a	PVCp	CPDDE	$CHCl_3$-THF	TCPB	OEPK
M6	PVC	NPOE	THF-$CHCl_3$	–	CPK-TEE
M6a	PVC	NPOE	THF-$CHCl_3$	TCPB	CPK-TEE
M7	PVCp	NPOE	$CHCl_3$	–	CPK-TEE
M7a	PVCp	NPOE	$CHCl_3$-THF	TCPB	CPK-TEE
M8	PVC	CPDDE	THF-$CHCl_3$	–	CPK-TEE
M8a	PVC	CPDDE	THF-$CHCl_3$	TCPB	CPK-TEE
M9	PVCp	CPDDE	$CHCl_3$	–	CPK-TEE
M9a	PVCp	CPDDE	$CHCl_3$-THF	TCPB	CPK-TEE
M10	PVC	DOS	THF-$CHCl_3$	–	CPK-TEE
M10a	PVC	DOS	THF-$CHCl_3$	TCPB	CPK-TEE
M11	PVC	TOP	THF-$CHCl_3$	–	CPK-TEE
M11a	PVC	TOP	THF-$CHCl_3$	TCPB	CPK-TEE
M12	PVC	NPOE	THF-$CHCl_3$	–	PdCP-SB
M12a	PVC	NPOE	THF-$CHCl_3$	TCPB	PdCP-SB
M13	PVCp	NPOE	$CHCl_3$	–	PdCP-SB
M13a	PVCp	NPOE	$CHCl_3$-THF	TCPB	PdCP-SB
M14	PVC	CPDDE	THF-$CHCl_3$	–	PdCP-SB
M14a	PVC	CPDDE	THF-$CHCl_3$	TCPB	PdCP-SB
M15	PVCp	CPDDE	$CHCl_3$	–	PdCP-SB
M15a	PVCp	CPDDE	$CHCl_3$-THF	TCPB	PdCP-SB
M16	PVCp	CPOE	$CHCl_3$	–	PdCP-SB
M16a	PVCp	CPOE	$CHCl_3$-THF	TCPB	PdCP-SB
M17	PVC	DOS	$CHCl_3$	–	PdCP-SB
M17a	PVC	DOS	$CHCl_3$-THF	TCPB	PdCP-SB
M18	PVCp	TOP	$CHCl_3$	–	PdCP-SB
M18a	PVCp	TOP	$CHCl_3$-THF	TCPB	PdCP-SB

[a] 120 mg of polymer and 240 mg of plasticizer first dissolved in 3 g of solvent, throughout; 2 parts.
[b] 5 mg/ml solution in THF; 1 part.
[c] 3 mg/ml in $CHCl_3$, 1 part.

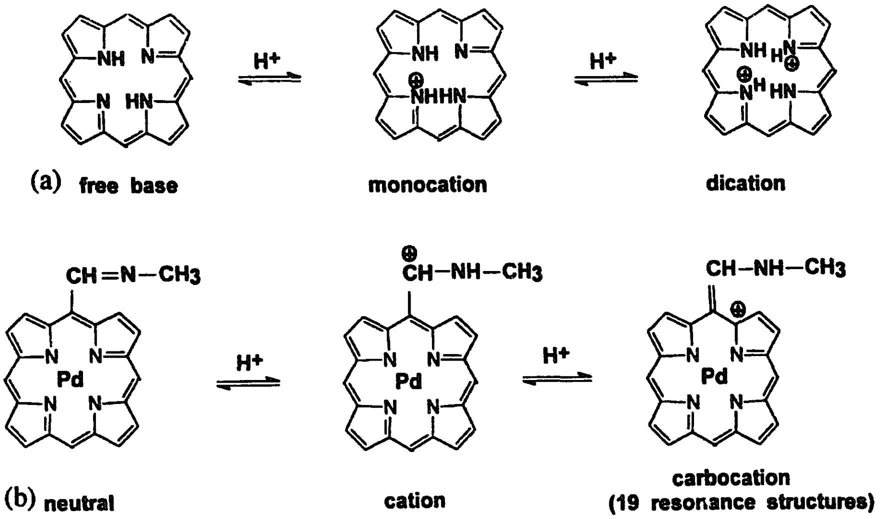

Fig. 2. Protonation equilibria of porphyrins (A) and PdCP-SB (B).

N-MeEP (structure III) is a typical representative of the N-alkylated porphyrins, whose optical properties and protolytic equilibria are similar to those of the conventional porphyrins. Since N-methylporphyrins are more basic, their monocations are easily obtained. For N-methylcoproporphyrin the pK_1 and pK_2 values were reported to be 11.3 and 0.7, respectively [9]. OEPK and CPK-TEE (structures IV and V), though derived from the corresponding porphyrins

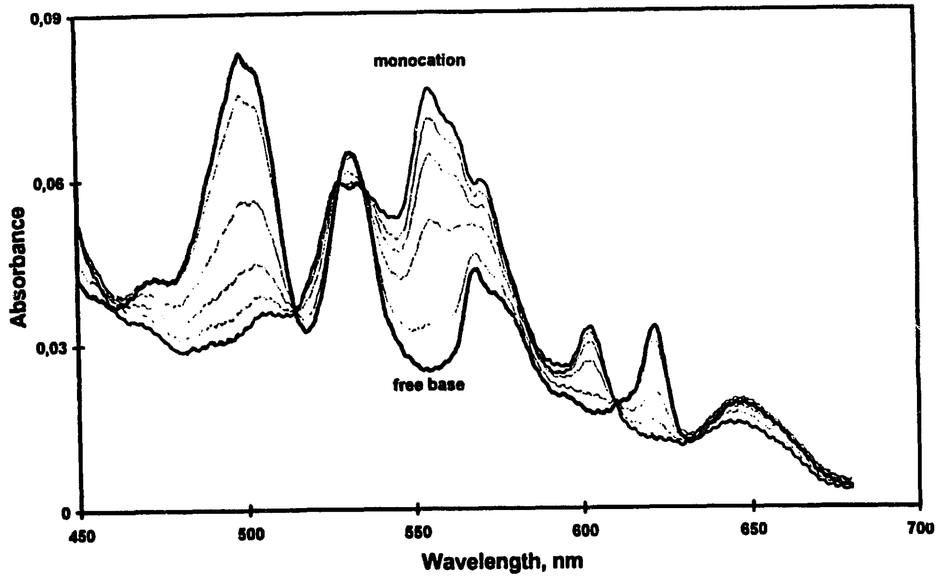

Fig. 3. Visible absorption spectra of membrane M1a with OEP as the indicator and showing the free base (bold line, pH 8.9), and the monocation (solid line, pH 3.73). Intermediate spectra at pH 7.5; 6.79; 6.16 and 5.51 (dashed lines).

Table 2
pK_a values and protolytic species of the porphyrin pH probes in polymer solution

pH probe(pK_a in water)	Membrane no.	Apparent pK_a in membrane	Protolytic forms detected in membrane
OEP	M1	<2	free base only
(7.2 and 4.2)	M1a	6.70	free base and monocation
		ca. 2.5	monocation and dication
CP-TEE	M2	<2	free base only
(7.2 and 7.4)	M2a	8.1	free base and monocation
		ca. 2.5	monocation and dication
	M3	<2	free base only
	M3a	6.45	free base and monocation
		ca. 2.5	monocation and dication
N-MeEP	M4	5.29	free base and monocation
(11.3)	M4a	>12	monocation only
OEPK	M5	<2	free base only
	M5a	5.42	free base and dication
CPK-TEE	M6	<2	free base only
(5.1)	M6a	6.35	free base and dication
	M7	<2	free base only
	M7a	5.76	free base and dication
	M8	<2	free base only
	M8a	5.13	free base and dication
	M9	<2	free base only
	M9a	4.5	free base and dication
	M10	<2	free base only
	M10a	5.25	free base and dication
	M11	<1	free base only
	M11a	<2	free base only

[13], display a markedly enhanced and longwave-shifted $Q_{(0,0)}$ band, along with a more intense fluorescence and improved photochemical stability. Their electronic spectra resemble the chlorins. Porphyrin-ketones and their complexes are considered as promising longwave luminescent probes [14]. At the same time, protonation of the porphyrin-ketones was shown to proceed differently and to be more complicated than in case of simple porphyrins [15]. The effective pK_a was determined to be 5.1 for CPK-TEE in aqueous solution [14].

For PdCP-SB (structure VI), protonation of the pyrrole ring is prevented by the metal ion, but possible at the external C = N double bond (the "Schiff base"). Such complexes have electronic spectra which are similar to those of unsubstituted porphyrin complexes [11,16,17]. Protonation of the Schiff-base dramatically changes the absorption spectrum in that the

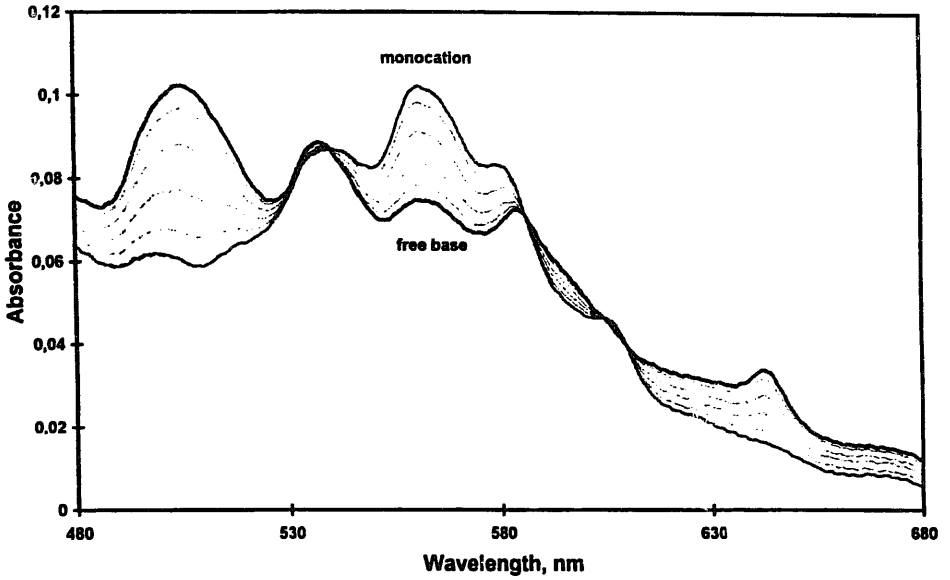

Fig. 4. Visible absorption spectra of membrane M3 (without borate additive) containing N-MeEP, showing the free base at pH 8.9 (bold line) and the monocation at pH 3.19 (solid line). Intermediate spectra at pH 7.31, 6.16, 5.55, 4.87 and 4.2 (dashed lines). Conditions: 10 mM phosphate buffer containing 150 mM NaCl, 20°C.

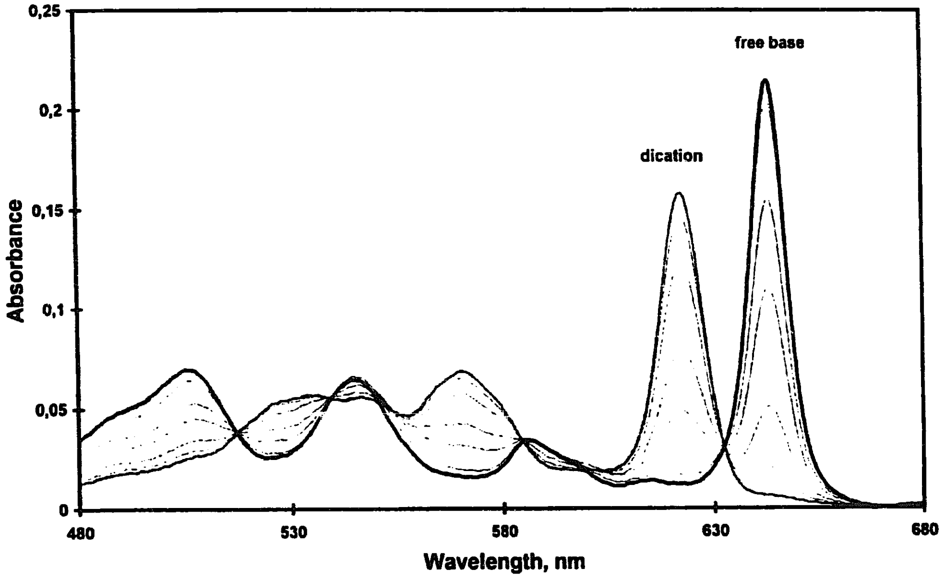

Fig. 5. Absorption spectra of membrane M10a containing CPK-TEE, showing the free base at pH 8.9 (bold line) and the dication at pH 2.78 (solid line). Intermediate spectra at pH 6.82, 5.80, 5.33, 4.61 and 3.94 (dashed lines). Same conditions as in Fig. 4.

α- and β-bands almost disappear, while the Soret is red-shifted by about 50 nm and a broad new band appears between 600 and 800 nm [16]. This was explained by partial charge transfer from the nitrogen atom to the porphyrin π-electron system under formation of a stabilized cation which can be represented in 19 different resonance structures (Fig. 2).

3.2. Protonation in liquid polymer membranes

3.2.1. OEP and CP-TEE

These dyes were found to incorporate into liquid polymer membranes as free bases. However, without an anionic additive (membranes M1 to M3) no spectral changes were observed on contact with aqueous buffer of any pH between 2 and 12. Protonation obviously is completely suppressed in such membranes. The apparent pK_a's must be smaller by at least 4 units compared to the values obtained with aqueous solutions [5].

If, however, an anionic additive such as tetrakis(p-chlorophenyl)borate (TCPB; referred to as "the borate") is added to the PVC membranes to give membranes M1a to M3a (where the suffix a indicates the presence of the borate additive in the membrane), distinct spectral response to pH is observed. The spectra indicate the formation of the porphyrin monocation, which is the predominating species within the

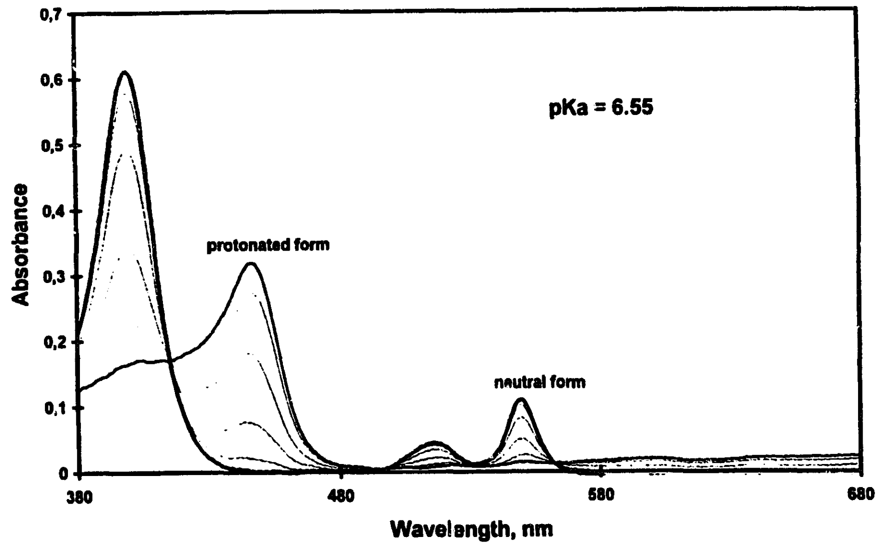

Fig. 6. Absorption spectra of membrane M16a with PdCP-SB as the indicator, showing the neutral form at pH 8.9 (bold line) and the protonated form at pH 2.78 (solid line). Intermediate spectra at pH 6.82, 5.80, 5.33, 4.61 and 3.94 (dashed lines). Same conditions as in Fig. 4.

pH 3–5 range (Fig. 3). At even lower pH, further protonation and formation of the porphyrin dication was observed in these membranes, the apparent pK_a being approximately 2.5 (see Table 2). The two apparent pK_a's obtained for the porphyrins in polymer membranes M1a–M3a are rather close to the respective pK's in water [5,6].

3.2.2. N-MeEP

In membranes without borate additive (M4), N-MeEP incorporates as the free base, and unlike OEP it is easily protonated to form the monocation at weakly acidic pH. The effect of pH on the absorption spectra is shown in Fig. 4. The apparent pK_a this process is estimated to be 5.3 (Table 2), which is about 6 units lower than the intrinsic pK_1 of N-alkylporphyrins [9]. On addition of borate to the PVC membranes (M4a), N-MeEP is present as the monocation over the pH 2–12 range. The N-MeEP monocation is spontaneously formed during preparation of the M4a membrane from the cocktail.

3.2.3. OEPK and CPK-TEE

Their behavior resembled that of OEP and CP-TEE. Thus, the presence of PTCPB additive was critical to enable protonation, which then readily occurred even in weak acid (M6 to M10 and M6a to M10a in Table 2). In contrast to the porphyrins, the OEPK and CPK-TEE free bases are double-protonated to give the dications (with no monocationic intermediate) as evidenced by the apparence of distinct isobestic points (Fig. 5). Again, the apparent pK_a values for the porphyrin-ketones in liquid polymer membranes are close to the respective data for aqueous solution [14].

3.2.4. PdCP-SB

This dye was also found to be protonable only in membranes with anionic additive (M12a to M17a). Protonation was accompanied by the transformation of the porphyrin type spectrum into a new one with an intense band at about 450 nm, along with a wide band at above 500 nm. The process was reversible and the porphyrin spectrum was restored on going back to neutral and alkaline pH. Typical absorbance changes are shown in Fig. 6, and apparent pK_a values are given in Table 3. The spectra of the porphyrins in a solution of plasticized PVC are summarized in Table 4.

Table 3

Apparent pK_a values of PdCP-SB in polymer solution as determined by exposure to 10 mM phosphate buffer of varying pH and containing 150 mM NaCl at 20°C (unless otherwise stated)

Membrane	pK_a
M12	<2
M12a	7.37
M13	<2
M13a	6.85
	7.36 [a]
M14	<2
M14a	7.31
M15	<2
M15a	6.65
M16	<2
M16a	6.55 [b]
	6.44
	6.36 [c]
	6.29 [d]
M17	<2
M17a	5.6
M18	<0
M18a	<1

[a] 37°C.
[b] 100 mM NaCl.
[c] 200 mM NaCl.
[d] 37°C.

Table 4
Spectral characteristics of the various protolytic forms of the porphyrin dyes dissolved in liquid polymer membranes

pH probe(s)	Species	Absorption maxima (nm)	Emission maxima (nm)	Wavelength for monitoring pH
OEP, CP-TEE	free base	401,499,531,568,622	626	499
	monocation	396,528,555,603	655	555
	dication	406,552,591	595	
N-MeEP	free base	400,504,537,562,585,642	644	504
	monocation	399,498,540,561,580	583	561
OEPK, CPK-TEE	free base	405,505,545,587,643	646	643
	dication	402,417,534,570,623	626	623
PdCP-SB	neutral	401,519,551	677 [a]	551
	protonated	447	- [b]	447

[a] Very weak in the presence of oxygen.
[b] Not detectable.

3.3. Dynamic response of the sensor films to pH

It is obvious from the above findings that membranes based on porphyrin-ketone (CPK-TEE) or the Pd-porphyrin Schiff base (PdCP-SB) are viable sensors for physiological pHs. They undergo distinct spectral changes on protonation, and the various protolytic forms are well-resolved and can be monitored using inexpensive LED light sources. The pH-induced optical changes of the porphyrins and N-alkyl-porphyrins, in contrast, are less expressed, and the spectra sometimes complex and overlapping.

Various membranes with dyes CPK-TEE or PdCP-SB (all differing in the composition of the cocktail) were prepared (Table 1) and investigated. Depending on the polymer and plasticizer added, the pK_a's of sensors based on the use of CPK-TEE (M6a–M11a) between 4.5 and 6.4 (Table 2; except for M11a), while sensors based on the use of PdCP-SB (M12a to M16a) had values of around 7.0±0.5 (see Table 3). In PVCp, which is a more hydrophilic polymer, pK_a values are lower by 0.5–0.6 units than in PVC, both for CPK-TEE and for PdCP-SB.

The pK values decrease by about 0.1 unit steps on increasing the ionic strength of the buffer from 0.10 to 0.15 to 0.20 mM. They also decrease by about 0.15 pK units on increasing the buffer temperature from 20 to 37 °C. The plasticizers have a small but distinct effect: On changing from NPOE (a notorious quencher of luminescence) to CPOE [7], pK_a values remain similar, while in case of DOS were lowered by about 1.5 units in case of M15a and M17a, but virtually unchanged for M8a and M10a. For TOP, the least lipophilic plasticizer, no protonation was observed both in the presence and absence of borate (M11, M11a in Table 2; M18, M18a in Table 3).

The sensor membranes were exposed to a stream of buffer of varying pH, and changes in absorbance recorded. A typical response to pH is given in Fig. 7 (for M13a). The response is fully reversible and occurs within 100 s. The stability of the signal at high and low pH over several hours is shown as well. The sensor membrane obviously is suitable for continuous optical recording of pH in the physiological range, par-

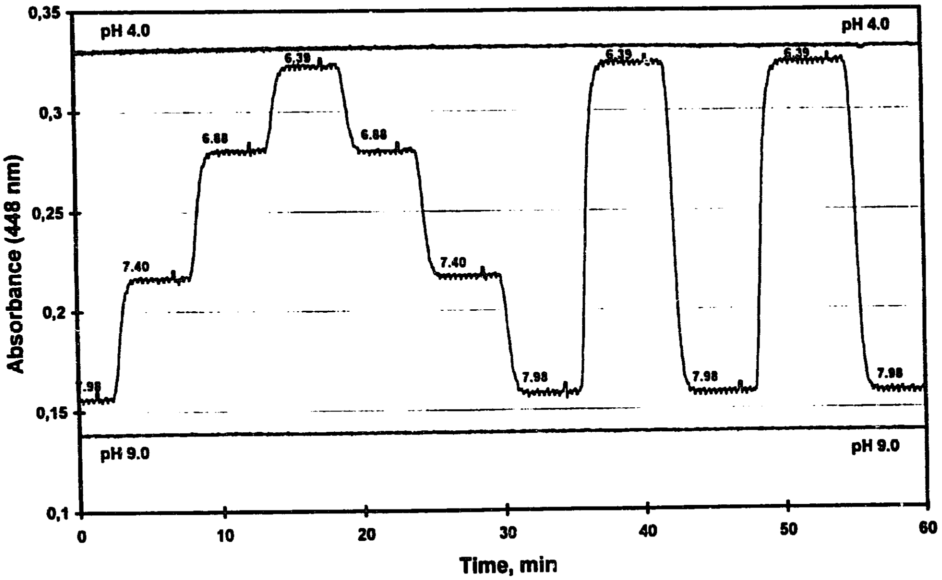

Fig. 7. Typical optical response to pH (middle curve) and signal stability at pH 9.0 (lower line) and pH 4.0 (upper line) of sensor membrane M13a. Same conditions as in Fig. 4.

ticularly of blood whose pH is 7.38 and can vary from 6.8 to 7.8. Both absorbance and fluorescence can be measured.

4. Conclusions

We show that lipophilic porphyrins can be protonated if dissolved in plasticized PVC. However, in the absence of the anionic borate additive, the apparent pK_a values are lowered by at least 5 units, except for the N-methyl etioporphyrin. In the presence of the anionic additive PTCPB, porphyrins are protonated already in weak acid. A two-step protonation (under formation of both the monocation and the dication) was observed for the polymer-dissolved porphyrins, whilst the corresponding porphyrin-ketones form dications but no monocation. Based on the finding that the pK_a's of certain porphyrins can be shifted into the near-neutral pH range by addition of anionic additives, sensor membranes have been prepared with apparent pK_a's between 4.5 to 8.0. Sensors based on PdCP-SB cover the pH 5.5 to 8.5 range and are promising candidates for measurement of blood pH, while sensors based on porphyrin-ketones cover the weakly acidic pH range (3.5–7.5). The actual pK_a of such membranes can be fine-tuned by proper choice of either dye, polymer, plasticizer or additive.

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References

[1] O.S. Wolfbeis (ed.), *Fiber Optic Chemical Sensors and Biosensors*, CRC Press, Boca Raton, 1991.
[2] M.J.P. Leiner, *Sensors Actuat.* B., B29 (1995) 169.
[3] D.B. Papkovsky, *Sensors Actuat.* B., B11 (1993) 293.
[4] E. Wang and M.E. Meyerhoff, *Anal. Chim. Acta*, 283 (1993) 673.
[5] J.E. Falk, *Porphyrins and Metalloporphyrins*, Elsevier, Amsterdam, 1964.
[6] A. Neuberger and J.J. Scott, *Proc. R. Soc. London, Ser. A.*, 213 (1952) 307.
[7] D.B. Papkovsky, G.J. Mohr and O.S. Wolfbeis, *Anal. Chim. Acta*, 337 (1997) 201.
[8] D. Dolphin (ed.), *The Porphyrins*, Vols. 1 and 2, Academic Press, New York, 1978.
[9] A.H. Jackson, in D. Dolphin (ed.), *The Porphyrins*, Vol. 3, Academic Press, New York, 1978, p. 341.
[10] C.K. Chang and C. Sotiriou, *J. Heterocycl. Chem.*, 2 (1985) 1739.
[11] G.V. Ponomarev and G.B. Maravin, Khim. Geterotsikl. Soedin., 85 (1977) (in Russian).
[12] D.B. Papkovsky, A.P. Savitsky and G.V. Ponomarev, J. Appl. Spectrosc., 51 (1989) 786.
[13] A.M. Stolzenberg, P.A. Glazer and B.M. Foxman, *Inorg. Chem.*, 25 (1986) 983.
[14] D.B. Papkovsky, G.V. Ponomarev and O.S. Wolfbeis, *Spectrochim. Acta, A.*, 52 (1996) 1629.
[15] D.B. Papkovsky, G.V. Ponomarev, *Spectrochim. Acta, A.*, 1997 (in press).
[16] G.V. Ponomarev, B.V. Rozinov and G.B. Maravin, *Khim. Geterotsikl. Soedin.*, 139 (1975) (in Russian).
[17] G.V. Ponomarev, *Khim. Geterotsikl. Soedin.*, 90 (1977) (in Russian).