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Application of a Novel Lipophilized Derivative of Rhodamine 19 in an Optical Sensor Suitable for Measurements of pH in Alkaline Regions

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pH determination

An optical pH sensor based on the fluorescence of a new lipophilic rhodamine dye immobilized in a thin poly(vinyl chloride) membrane was developed. The response to hydrogen ions was a result of reversible changes in the molecular structure of rhodamine. Excited at 528 nm, the membrane fluoresced at 547 nm. The sensor system showed a usable sensitivity over the 9.5-11.9 pH range and very good reproducibility.

INTRODUCTION

A request for rapid and continuous monitoring of pH is present in almost every branch of science, including chemistry, biochemistry, medicine and environmental analysis. Owing to its reliability, precision and short response time, the glass pH electrode is the most widely used. The interferences caused by the local electrical potential, field or current can be overcome by optical pH sensing. The very small size and flexibility of optical sensors are advantageous for measurements *in vivo*.

An optical sensor (optode) is a small analytical device, which can generate a measurable signal on the basis of a certain optical characteristic of the analyte. The active component of the pH optode is a pH indicator, which should, in an ideal case, possess the following properties: high selectivity towards protons, ability to generate a pH sensitive signal, sufficient lipophilicity to prevent washing out of the polymer surface, or the molecular structure suitable for covalent immobilization, good solubility in the membrane phase, a short response time, photostability and reversible proton binding. It is almost impossible to find a species that would fulfil all these requirements. Currently, many investigations have been focused on the synthesis and use of adequate chromoionophores.

The pH optodes are mostly based on the absorbance of indicators or on the changes in their fluorescence. For instance, the absorption properties of immobilized azo dyes depend on the pH value, providing reproducible and relatively fast pH determination in the whole pH range.[1] Merocyanine dyes serve as indicators in multidimensional optical sensors, which could be applied to simultaneous measurement of the water content and its pH value in organic solvents.[2] Furthermore, sensors for measurements in the physiological pH range use, among others, aminofluorescein chemically doped in tetramethoxysilane based sol-gel,[3] a mixture of 5 (and 6)-carboxynaphthofluorescein entrapped in polyacrylamide gel matrix[4] or co-

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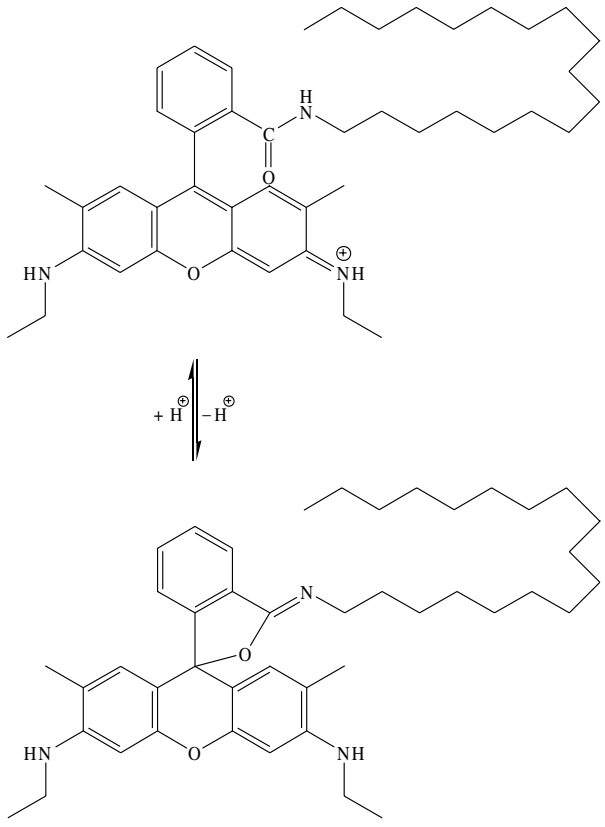
valently bound 4-(4'-hydroxyethylsulpho-2'-nitrophenylazo)-1-naphthol.[5] The pH optodes containing ruthenium(II) complexes as active components are suitable for measuring the hydrogen ion activity in mildly acidic and neutral pH regions.[6] Although numerous modes of immobilization and various types of chromophores have been used to overcome membrane swelling, dye leaching, photobleaching and small relative signal changes, most optodes still suffer from these problems.

Owing to their high fluorescence quantum yield and photostability, rhodamine dyes are promising chromoionophores for the use in optical sensors. So far, they have been applied as active components of optical sensors for the determination of metal ions,[7] humidity,[8] NH_4^+ ions [9, 10] and various anions,[11, 12] but they have not been used for pH sensing.

Recently, we synthesized an octadecylamide derivative of rhodamine 19. During a detailed study of its characteristics in solution, we noted that depending on time, type of solvent, pH and concentration, either conversion of the cationic form of the dye into the neutral form or aggregation took place.[13] Both equilibria observed were followed by a change in absorption and emission properties of the dye and can be used as sensing mechanism in optical sensors. A long alkyl chain introduced in the rhodamine structure provided sufficient lipophilicity of the dye in polymer support.



Scheme 1. Chemical structures of the cationic and the electrically neutral forms of rhodamine 19 octadecylamide.

In this paper, we report on a pH sensing system consisting of rhodamine 19 octadecylamide dissolved in a poly(vinyl chloride) membrane. The working principle is based on the pH dependent and reversible equilibrium between the cationic fluorescent form of the dye and its electrically neutral nonfluorescent form (Scheme 1). The effects of anionic additive, plasticizer, fluorophore concentration and membrane thickness were investigated. The optimized sensor membrane was characterized in terms of the response to pH, response time, dynamic range, leaching, photostability and reproducibility.

EXPERIMENTAL

Chemicals and Solutions

Poly(vinyl chloride) (PVC, high molecular weight), tris(2-ethylhexyl) phosphate (TOP), 2-nitrophenyl octylether (*o*-NPOE), potassium tetrakis(4-chlorophenyl)borate (KTCPB), and tetrahydrofuran (THF) were purchased from Fluka AG (Buchs, Switzerland). Poly(propylene glycol) sebacate (PPGS) was obtained from Supelco (Deisenhofen, Germany). Rhodamine 19 octadecylamide (R19OA) was synthesized according to the procedure described elsewhere.[13]

All chemicals used for adjusting pH were of analytical purity grade. Hydrochloric acid and sodium hydroxide of defined pH value were prepared by dilution of 0.10 mol dm^{-3} stock solutions. Ionic strength was adjusted by adding 0.15 mol dm^{-3} sodium chloride. Glycine/NaOH buffer ($8.6 < pH < 12.8$) was prepared according to reference 14. Doubly deionized water was used throughout.

Membrane Preparation

Exact compositions of the membranes are given in Table I. All membranes contained (mass fractions) 33 % PVC and 66 % plasticizer (TOP, *o*-NPOE, PPGS). Membrane components were dissolved in an appropriate volume of THF and 0.100 cm^3 of this solution was injected onto a rotating glass plate of the spinning device (constructed at the ETH, Zürich, rotating frequency 200-1000 rpm).[15] After 30 s of rotation in the THF saturated atmosphere, a glass plate with a 2-4 mm thick sensing membrane was removed from the device.

Apparatus

The glass plate with a PVC membrane was mounted in a specially designed flow-through cell constructed at the ETH, Zürich,[15] and placed into a Perkin Elmer LS 50 spectrofluorimeter. The flow rate of the continuous flow-through system was kept at 2 cm^3 min^{-1} by means of a Varioperpex peristaltic pump (type LKB Bromma 2120). The cell was thermostated to 25 ± 1 ºC by means of a Medingen Dresden thermostat (model U3). The pH was measured with a PHM 64 Radiometer pH/mV meter using a Radiometer combined glass-calomel electrode GK 2401 C.

TABLE I. Composition of membranes M0 to M7

Membrane	Plasticizer	Rhodamine 19 octadecylamide			Potassium tetrakis(4-chlorophenyl)borate			THF
		$\frac{m}{\text{mg}}$	$\frac{c^{(a)}}{\text{mol dm}^{-3}}$	$\frac{b^{(b)}}{\text{mmol kg}^{-1}}$	$\frac{m}{\text{mg}}$	$\frac{c^{(a)}}{\text{mol dm}^{-3}}$	$\frac{b^{(b)}}{\text{mmol kg}^{-1}}$	$\frac{V}{\text{cm}^3}$
M0	DOS	0.49	2.79×10^{-3}	30	–	–	–	0.25
M1	*o*-NPOE	0.49	2.79×10^{-3}	30	0.35	2.82×10^{-3}	30	0.25
M2	*o*-NPOE	0.24	1.37×10^{-3}	15	0.17	1.37×10^{-3}	15	0.25
M3	*o*-NPOE	0.16	9.1×10^{-4}	10	0.12	9.7×10^{-4}	10	0.25
M4	*o*-NPOE	0.16	4.6×10^{-4}	10	0.12	4.8×10^{-4}	10	0.50
M5	TOP	0.16	9.1×10^{-4}	10	0.12	9.7×10^{-4}	10	0.25
M6	PPGS	0.16	9.1×10^{-4}	10	0.12	9.7×10^{-4}	10	0.25
M7	PPGS	0.16	4.6×10^{-4}	10	0.12	4.8×10^{-4}	10	0.50

(a) In THF solution.
(b) In membrane.

RESULTS AND DISCUSSION

Sensing Mechanism

The sensing scheme used in this work is based on the pH dependent fluorescence of rhodamine 19 octadecylamide immobilized in a PVC membrane. The sensor membrane is purple at a low pH and colorless at a high pH. Spectral changes of the dye are due to the change in molecular structure. By decreasing hydrogen ion activity, a bond between the oxygen of the amide group and the C–9 atom of the xanthene part of the molecule is formed (Scheme 1), thereby leading to a restriction of the electron flow of the xanthene π-system and causing a change in absorption properties and loss of fluorescence. The equilibrium between the fluorescent cationic form of the chromoionophore and its nonfluorescent electrically neutral form is reversible and therefore a suitable sensing mechanism for pH determination.

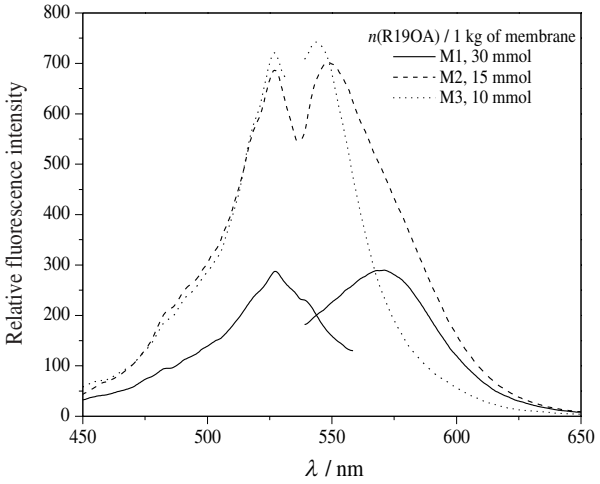


Figure 1. Excitation and emission spectra of membranes M1, M2 and M3 with different amounts of R19OA.

Membrane Optimization

The membrane without anionic additive (M0, Table I) did not fluoresce and was not sensitive to pH changes. It was assumed that in this membrane the neutral form of the dye was preferred. The formation of ion pairs between the lipophilic anion, like KTCPB, and the positively charged R19OA seemed to stabilize the cationic form in the membrane phase. Furthermore, lipophilic counterions improved the mobility of protons between the membrane and the aqueous phase. Consequently, KTCPB in equal amounts with respect to the dye was added into the membranes (M1–M7).

The optimal concentration of the fluoroionophore in the sensing membrane was 10 mmol kg^{-1}. Increase in the dye concentration above this value (membranes M1 and M2) caused reabsorption and selfquenching of cationic molecules, resulting in a shift of the emission maximum to higher wavelengths and in a decrease in emission intensity (Figure 1).

Membranes containing different kinds of plasticizer showed different response times (Table II). The response time of the membrane containing TOP (M5) was 3 minutes in 0.10 mol dm^{-3} NaOH and several hours in 0.10 mol dm^{-3} HCl. The response time of the membrane plasticized with *o*-NPOE (M3) was 40–50 minutes in both solutions. The fastest response time was recorded for the membrane plasticized with PPGS (M6): 4–5 minutes upon exposure to 0.10 mol dm^{-3} NaOH and 20 minutes upon exposure to 0.10 mol dm^{-3} HCl. This can be explained by the improved transport of protons in the polymer phase through the hydroxyl groups of PPGS.

To shorten response times, thinner membranes were prepared using diluted THF solutions of membrane components (M4 and M7). Achievement of equilibrium was faster in *o*-NPOE membranes when they were thinner,

TABLE II. Response time of membranes on exposure to 0.01 M HCl and 0.01 M NaOH

Membrane	Plasticizer	c / mol dm^{-3}	λ_{ex} / nm	λ_{em} / nm	t_{95}[(a)] / min	
					HCl	NaOH
M3	*o*-NPOE	9.1×10^{-4}	528	544	50	40
M4	*o*-NPOE	4.6×10^{-4}	528	544	20–25	5–10
M5	TOP	9.1×10^{-4}	528	549	>330	3
M6	PPGS	9.1×10^{-4}	528	545	20	4–5
M7	PPGS	4.6×10^{-4}	528	547	15	4–5

[(a)] Time for 95 % of the total signal change to occur.

but it was still not satisfactorily fast. No significant differences in response time were observed for the PPGS membranes of various thicknesses.

Excitation and emission spectra of the membrane with the best characteristics, M7, before measurement and after exposure to 0.10 mol dm^{-3} HCl and 0.10 mol dm^{-3} NaOH for 20 min are shown in Figure 2.

Response Time

Figure 3 shows the response of membrane M7, exposed alternately to solution of hydrochloric acid (pH = 1.0) and sodium hydroxide (pH = 11.7 and 12.8, respectively) for 20 minutes. In general, the response time was rather long and depended on hydrogen ion activity in solution. The flow of HCl, pH = 1.0, increased the membrane emission. The corresponding response took as long as 15 minutes. When NaOH, pH = 12.6, was passed over the membrane, the fluorescence signal decreased. The equilibrium was reached within five minutes. However, when the membrane was exposed to NaOH, pH = 11.7, the time necessary to achieve equilibrium was much longer, amounting to more than 20 minutes. The slow membrane kinetics can be explained by the slow conversion of one form of R19OA into another due to changes in molecular structure, *e.g.*, rehybridization of C-9 atom of the xanthene part of the dye. Furthermore, exposure of the membrane to NaOH solutions of various concentrations showed that not only the ratio of both forms in the equilibrium but also the rate of conversion of one form into another depended on the hydrogen ion activity. Therefore, the rate of equilibrium achievement in the membrane can be used as a parameter for pH determination, instead of the value of fluorescence signal after the steady state has been reached.

The rate of fluorescence intensity changes in the first three minutes of the membrane response was taken to be the measure of the rate of equilibrium achievement. The slopes of the corresponding segments of the curves fluorescence *vs.* time were then plotted against pH to obtain the pH response curve of the membrane.

The fluorescence intensity values recorded during the achievement of equilibrium showed very good reproducibility on repeated exposures to the same solution. Table III illustrates relative standard deviations for each minute of the three repeated exposures of membrane M7 to NaOH, pH = 11.7. The obtained values are very low,

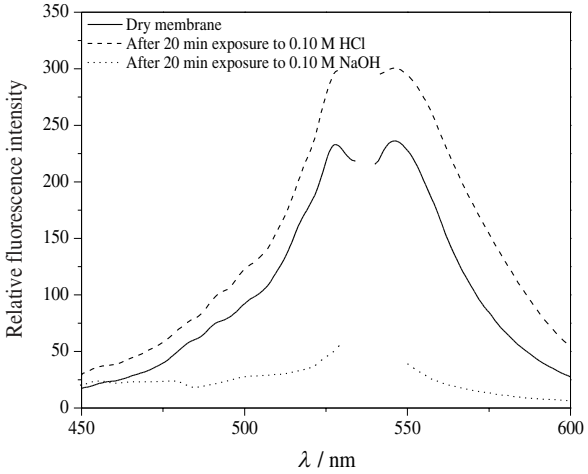


Figure 2. Excitation and emission spectra of membrane M7 before measurement and after 20 minutes of passing over of 0.10 M HCl and 0.1 M NaOH.

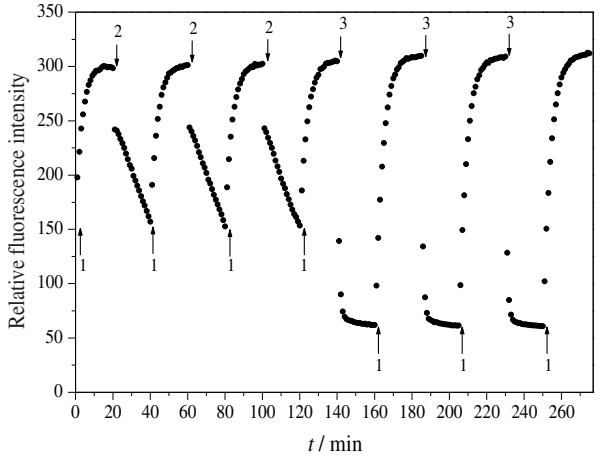


Figure 3. Response of sensing membrane M7 to cycling between HCl, pH = 1.0 (1) and NaOH, pH = 11.7 (2) or pH = 12.6 (3); λ_{ex} = 528 nm, λ_{em} = 547 nm.

TABLE III. Reproducibility of the fluorescence signal of membrane M7 on exposure to NaOH, pH = 11.7, calculated on the basis of 3 measurements[a]

t / min	Average value	Standard deviation	Relative standard deviation / %
1	245.8	3.3	1.3
2	245.2	2.0	0.8
3	243.3	0.6	0.3
4	240.3	0.7	0.3
5	236.0	1.7	0.7
6	231.7	1.8	0.8
7	227.1	2.2	1.0
8	221.9	2.7	1.2
9	216.6	3.2	1.5
10	211.3	3.3	1.6
11	206.5	3.2	1.6
12	201.7	4.6	2.3
13	195.8	3.6	1.9
14	191.7	3.6	1.9
15	186.7	3.9	2.1
16	181.9	4.0	2.2
17	177.0	3.9	2.2
18	172.4	3.8	2.2
19	168.2	3.9	2.3
20	164.2	2.8	1.7

[a] See Figure 3.

0.3–2.3 %. Due to this characteristic of the membrane, it was possible to construct a pH response curve by using relative fluorescence intensity values recorded in the defined time interval before equilibrium achievement. The third minute of the membrane response was considered to be optimal because of the best reproducibility of the corresponding signals (RSD = 0.3) and the possibility of fast measurement.

Membrane M7 showed the same constant value of the maximal fluorescence signal in 0.1 mol dm^{-3} HCl during the operational lifetime (two weeks). This was an indication that exposure to UV/Vis radiation and flow of solutions caused neither photobleaching nor leaching of the dye out of the membrane. However, the membrane had to be recovered with acid after (before) exposure to an alkaline solution.

Dynamic Range

To determine the dynamic pH range, membrane M7 was exposed to hydrochloric acid and sodium hydroxide solutions of various concentrations. A 0.1 mol dm^{-3} HCl solution was used to obtain a reverse response. As can be seen in Figure 4, illustrating the dependence of relative fluorescence intensity recorded in the third minute of measurement on the pH value, the membrane responded to changes of the hydrogen ion activity in the strong alkaline region. Adjustment of ionic strength of solutions with 0.15 mol dm^{-3} NaCl was followed by a faster response and shift of the response curve to a lower pH, resulting in a dynamic range 9.5 < pH < 12.0.

Figure 5 illustrates the response of the membrane exposed to a series of glycine/NaOH buffer solutions of 8.6 < pH < 12.8. The glycine/HCl buffer solution of pH = 1.2 was used to obtain a reverse response. A plot of relative fluorescence intensity of the fluorophore *versus* pH provided a sygmoidal curve (Figure 6a). A similar curve was observed using slope values instead of emission intensity (Figure 6b). Unfortunately, in that case the points at pH > 12.0 should be neglected, because of a very fast

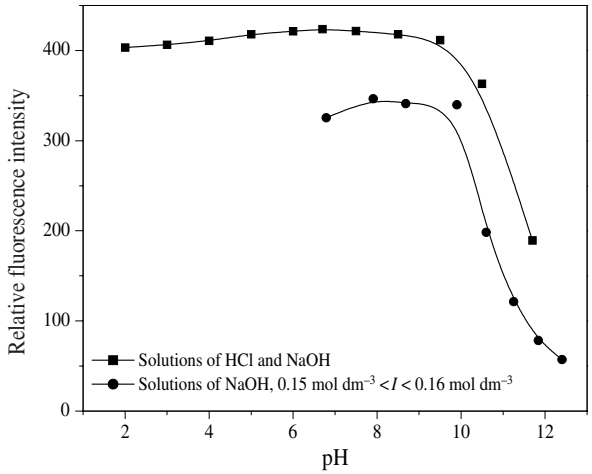


Figure 4. Response curves of membrane M7 on the pH of HCl and NaCl solutions in the third minute of measurement.

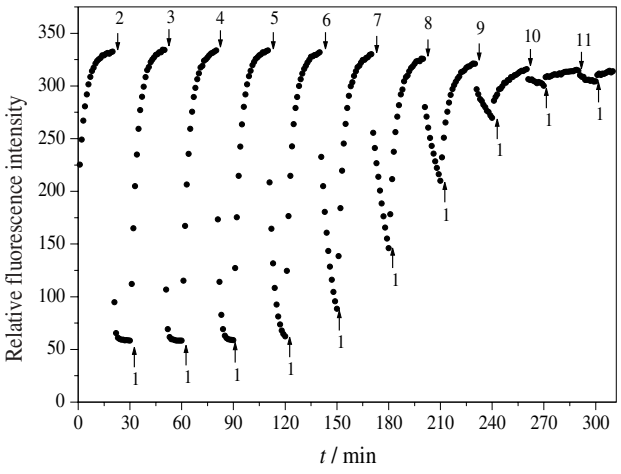


Figure 5. Response of sensing membrane M7 to glycine/HCl, pH = 1.2 (1) and glycine/NaOH buffer solutions, pH = 12.8 (2); 12.6 (3); 12.0 (4); 11.5 (5); 11.0 (6); 10.5 (7); 10.1 (8); 9.6 (9); 9.0 (10); 8.6 (11); λ_{ex} = 528 nm, λ_{em} = 547 nm.

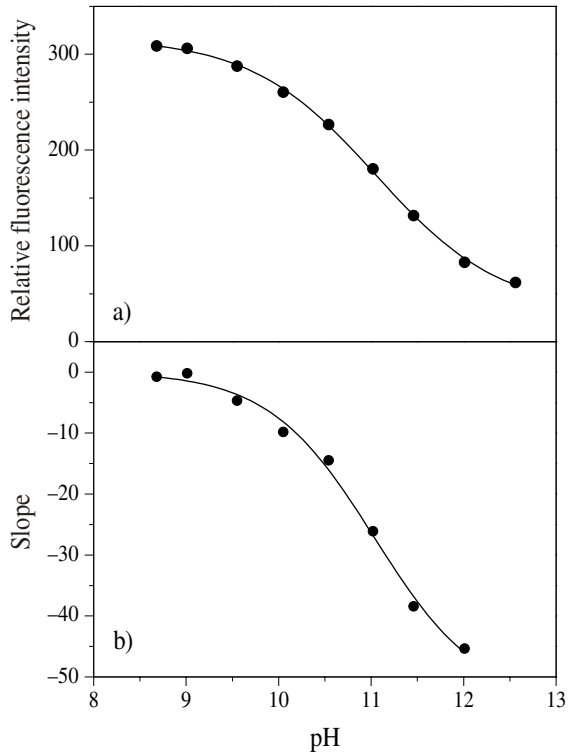


Figure 6. Response curves and dynamic range of M7 during the flow of glycine/NaOH buffer solutions over the membrane constructed using: a) the fluorescence intensity of the dye in the membrane in the third minute; b) the rate of fluorescence signal changes within the first three minutes of measurement.

equilibrium and the steady state achievement within the time interval chosen for calculation of the slope. The dynamic range covered the values 9.5 < pH < 11.9 while the inflection point of the curves was noted at pH = 10.7.

TABLE IV. Comparison of the pH induced fluorescence signal of three membranes M7[a]

t / min	pH			
	10.0	10.5	11.0	11.5
1	277 ± 7 (± 17)	255 ± 5 (± 13)	233 ± 4 (± 10)	209 ± 7 (± 18)
2	269 ± 6 (± 15)	241 ± 3 (± 7)	205 ± 2 (± 4)	166 ± 3 (± 6)
3	261 ± 4 (± 11)	226 ± 1 (± 3)	180 ± 6 (± 16)	134 ± 2 (± 5)
4	252 ± 4 (± 9)	213 ± 3 (± 7)	159 ± 9 (± 23)	111 ± 2 (± 6)
5	245 ± 2 (± 5)	201 ± 5 (± 12)	141 ± 11 (± 26)	94 ± 1 (± 4)

[a] Average values ± SD. The 95 % confidence limits are given in parentheses.

Reproducibility

The reproducibility of the pH induced signal change of M7 was evaluated by exposing three freshly prepared membranes to the flow of a series of glycine/NaOH buffer solutions covering the dynamic pH range of the proposed sensing system. The fluorescence intensity of the membranes was recorded every minute during a five-minute interval. The average values of signals for the three tested membranes, the corresponding standard deviations and the 95 % confidence limits are listed in Table IV. The obtained deviations are low with respect to pH induced signal changes, confirming that the new dye can be successfully used for reproducible pH measurements.

CONCLUSION

PVC membranes with an immobilized new lipophilic derivative of rhodamine 19 can be employed for optical pH sensing. The sensing mechanism is based on the pH dependent and reversible equilibrium between the fluorescent cationic form of rhodamine 19 octadecylamide and its nonfluorescent, electrically neutral form. The dynamic range of the membrane covers pH of 9.5-11.9. Although the membrane displays rather long response times, dependent on the hydrogen ion activity, the relative signal changes measured before equilibrium achievement are satisfactorily large and reproducible. Introduction of a long alkyl chain into the structure of rhodamine 19 provides sufficient lipophilicity and solubility of the dye in plasticized polymer. No leaching or photobleaching were observed. Strong fluorescent properties and good photostability of this new lipophilic dye are an advantage compared to the other fluorescent dyes used in optical sensors.

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SAŽETAK

Primjena novoga lipofilnoga derivata rodamina 19 u optičkome senzoru pogodnome za mjerenje pH u bazičnome području

Snežana Miljanić i Zvjezdana Cimerman

Razvijen je pH optički senzor koji se temelji na fluorescenciji nove lipofilne rodaminske boje imobilizirane u tankoj membrani od poli(vinil klorida). Odziv na vodikove ione posljedica je reverzibilnih promjena u molekularnoj strukturi rodamina. Pobudom pri 528 nm, membrana je fluorescirala kod 547 nm. Senzorski sustav pokazao je upotrebljivu osjetljivost u području pH = 9,5–11,9 te vrlo dobru reproducibilnost.