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Anion-Selective Optical Sensors Based on a Coextraction of Anion-Proton Pairs into a Solvent-Polymeric Membrane**

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Abstract: The design of optical sensing devices for anions based on the coextraction of anions and protons into a PVC membrane is described. The extraction is controlled by the lipophilicity of the anions, while the coextracted protons in conjunction with a hydrogen ion selective chromoionophore provide the optical signal transduction. The absorbance of the membrane at a fixed pH of the aqueous sample solution and at constant anion concentrations is related to the hydration energy (Hofmeister series) of the anions. Calibration curves, response times, and reproducibilities of such sensor systems are presented. Membranes for optodes are at hand for an analytically relevant assay of lipophilic anions such as nitrate.

Because of the linear relationship between absorbance and concentration as well as the simplicity to measure light intensities accurately at different levels, UV/VIS spectrophotometry is a widely used quantitative analytical method. The assay of many ions, including biologically important cations, is thus routinely performed by forming coloured complexes between specific reagents and the analytes[1]. Typically a given amount of liquid reagent or a test device with an immobilized reagent is consumed, however, for each determination. Repeatedly usable optical measuring systems (optodes), that enjoy the advantages of UV/VIS spectroscopy, can be obtained if a reversible reagent phase can be immobilized on a support which is in contact with the test solution. Such a system would require little or no sample pretreatment, while the amount of consumable reagents is therefore limited. These methods are thus of considerable importance and indeed have become of great interest recently[2–10].

The signal generation in such chemical sensors usually relies on the detection of a change in absorbance or fluorescence and sometimes in other optical properties of the immobilized reagent phase. Special methods, such as Attenuated Total Reflectance (ATR)[10] and Surface Plasmon Resonance (SPR)[11] techniques have to be used when very thin reagent layers are to be probed. Optical waveguides in the form of fibres are often employed to efficiently transmit light to and from a small sensing area, but this is no prerequisite for the design of an optode. In most instances, chromophoric reagents have been immobilized on the sensor surface by covalent binding, adsorption, or entrapment (in hydrophilic or Langmuir-Blodgett[12] films), where the reagent is in direct contact with the aqueous test solution.

Chromoionophores which upon selective complexation with cations loose a proton are employed in some recently described sensors[13–16]. This deprotonation not only causes a colour change of the molecule, which is utilized for transduction, but also ensures charge neutrality[13–16]. It was found, however, that it is not necessary to have both the ion-sensing (ion recognition) and colour-change functions in the same molecule when solvent-polymeric membranes are used to contain the sensing species[17–21]. Such systems are based on a two-phase extraction where the lipophilic membrane acts as the organic phase. An ion-exchange principle has been used in the determination of cations by reflection on one-way test strips combining an electrically neutral ionophore with a lipophilic pH indicator in a polymeric matrix[17,18]. The indicator used is electrically neutral in its protonated form, therefore becomes negatively charged on deprotonation and thus the positive charge of the cation/neutral ionophore complex is balanced. The method allows existing highly selective ionophores, which are usually employed in ion-selective electrodes, to be used in optical detection. *Morf* et al. have recently suggested a series of ion exchange and coextraction devices and established theoretical principles for such systems[19–21]. The same authors have proven experimentally that reversible optical systems sensitive to cations can indeed be produced in such a manner[19–21]. They employed lipophilized pH indicators that are positively charged in the protonated form in conjunction with another neutral carrier and with non-selective negatively charged sites to ensure the electroneutrality condition[19–21].

In order to indirectly determine anions using the colour changes of a pH indicator, it is not possible to use the above described ion-exchange principle. To assure charge neutrality it is necessary that anions and protons are concurrently extracted into or released from the membrane phase. *Morf* et al. have also shown that it is possible to use such a coextraction system for an optical sensor by using two neutral carriers, one selective for a cation ($NH_4^{\oplus}$), the other selective for an anion ($CO_3^{2\ominus}$)[19]. Such a sensor responds to the activity product $a_{CO_3^{2\ominus}} \cdot a^2_{NH_4^{\oplus}}$. The distribution of anions between an aqueous and an organic phase, i.e. their lipophilicity, depends on the hydration energy of the anion[22] and follows the Hofmeister series[23]. An anion/proton coextraction sensor should therefore respond to the activity of anions at constant sample pH. Here we report on the design of an anion optode that exhibits a selectivity pattern which is derived from the lipophilicity of the anions.

Two schemes can potentially be employed for the design of an anion optode relying on the coextraction of anions and protons:

– Firstly, a lipophilized pH indicator (IND) that is electrically neutral in its non-protonated form is introduced into the membrane and used to coextract proton/anion pairs:

$$IND_{mem} + H^{\oplus}_{aq} + X^{\ominus}_{aq} \rightleftharpoons INDH^{\oplus}_{mem} + X^{\ominus}_{mem} \quad (1)$$

– Secondly, coextraction is facilitated by two charged species, a pH indicator ($IND^{\ominus}$) that is negatively charged in its non-protonated form and positively charged anion exchange sites ($R^{\oplus}$):

$$IND^{\ominus}_{mem} + R^{\oplus}_{mem} + H^{\oplus}_{aq} + X^{\ominus}_{aq} \rightleftharpoons INDH_{mem} + R^{\oplus}_{mem} + X^{\ominus}_{mem} \quad (2)$$

From the mass action law it follows that in such systems the absorbance of the pH indicator should ideally be proportional to the multiple of the activities of anions ($a_{X^{\ominus}_{aq}}$)

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and protons ($a_{H^{\oplus}_{aq}}$) in the sample solution. It should thus indeed be possible to determine the anion activity ($a_{X^{\ominus}_{aq}}$) in pH-buffered solutions. In addition, it can be expected that the equilibrium is controlled by the lipophilicity of the anion $X^{\ominus}_{aq}$. A differentiation of anions should therefore be feasible.

Experimental:

Synthesis of 1-octadecanoyloxy-4-(p-nitrophenylazo)-resorcinol (ETH 2412):

5 g (19.3 mmol) of 2,4-dihydroxy-4'-nitro-azobenzene (Fluka, p.A.) and 1.95 g (19.3 mmol) of triethylamine (Fluka, puriss. p.A.) were dissolved in 400 cm^3 tetrahydrofuran (Fluka, puriss. p.A.) and cooled with ice to about 5 °C. 5.84 g (19.3 mmol) of freshly distilled stearoyl chloride (Fluka, pract.) dissolved in 100 cm^3 of tetrahydrofuran were then added with a dropping funnel. The mixture was stirred for 2 h at room temperature and then filtrated. The solvent was evaporated and the crude product purified by double recrystallization from ethyl acetate. The pure product (4.13 g, 40.7% yield) had a melting point of 94–95 °C.

The constitution of ETH 2412 was confirmed by ^{1}H-NMR (300 MHz, $CDCl_3$, ^{13}C-NMR (75 MHz, $CDCl_3$), IR (KBr), and FAB-MS. The purity was tested by elemental analysis (calculated for $C_{30}H_{43}N_3O_5$: C 68.54%, H 8.24%, N 7.99%; found: C 68.67%, H 8.33%, N 7.89%).

Preparation of Membranes:

Membrane mixtures containing either lipophilized Nile Blue (ETH 5294[24], 6 mg) or lipophilized Azo Violet (ETH 2412, 6 mg) and tridodecylmethylammonium chloride (TDDMACl, Polysciences Inc. Warrington PA USA, 14.4 mg) as the active constituents and 160 mg of either bis(2-ethylhexyl)sebacate (DOS) or *ortho*-nitrophenyl octyl ether (*o*-NPOE) as plasticizer and 80 mg of poly(vinyl chloride) (PVC, high molecular, Fluka) or vinyl chloride-vinyl alcohol copolymer (OH-PVC)[25] were dissolved in 1.5 cm^3 of distilled tetrahydrofuran (Fluka). Nile Blue and Azo Violet have aqueous pH transition regions of pH 10.1 to 11.1 and pH 10.1 to 12.0, respectively, in their non-lipophilized form[26]. The actual pK's of the lipophilized forms in the membrane phase are not known. Membranes of approximately 4 μm thickness were cast from these solutions onto 35 mm diameter plates (Herasil quartz glass, W. Möller AG, Zürich) using a special spinning device.

Measurements:

The plates carrying the membranes were mounted in a cell and placed in a UV/VIS spectrometer (UVIKON Model 810, Kontron AG, Zürich). Standards were made up from analytical reagent grade KCl, KNO_3, KSCN, and $NaClO_4$. All solutions contained a universal buffer mixture at a 200fold dilution except those at pH 7.5 which contained TRIS (tris(hydroxymethyl)aminomethane) at a concentration of 10^{-2} M. The stock of the universal buffer mixture consisted of 10 mM NaH_2PO_4, 6.6 mM citric acid, and 21.5 mM $Na_2B_4O_7$[27]. Where indicated, solutions also contained 0.1 M of potassium acetate as an ionic strength adjustment buffer. All solutions were adjusted to the desired pH with either analytical grade sulfuric acid or sodium hydroxide solutions.

Results and Discussion:

The feasibility of a system based on a neutral pH indicator (Scheme (1)) was tested first. Lipophilized Nile Blue (ETH 5294)[24] was chosen for these experiments because it was expected to facilitate the extraction of anion/proton pairs due to its high basicity.

Initial experimental results indicated that ETH 5294 can indeed be protonated in PVC-based membranes, using either DOS or *o*-NPOE as plasticizer, but it was found to leach out of the membrane quite fast once protonated. This is due to the reduced lipophilicity of the molecule when charged. However, it was also found that the rate of leaching is strongly dependent on the counter-anion employed. For the four anions tested, the rate of leaching was found to decrease with increasing lipophilicity, i.e.: chloride < nitrate < thiocyanate < perchlorate[23]. In ion-exchange systems where the highly lipophilic tetraphenylborate is also present in the membrane phase, no rapid leaching of the protonated indicator was observed[24].

We found that the leaching problem of the Scheme (1) optode system can be overcome by employing a membrane phase with relatively high dielectric constant. This was achieved by using a vinyl chloride-vinyl alcohol copolymer (OH-PVC) together with *o*-NPOE. Leaching of the protonated form of the indicator ETH 5294 could not be detected when this membrane composition was used. This improvement is thought to be due to two reasons: (a) an increase in the dielectric constant will change the partitioning of the charged chromophore in favour of the membrane phase and (b) a probable stabilization of the chromoionophore in the membrane phase might result from a chemical interaction between the hydroxy groups of the OH-PVC and ETH 5294.

The extent of protonation of the lipophilized form of ETH 5294 in this membrane matrix at different pH and for a constant concentration (0.1 M) of four anions is shown in Fig. 1. Here the absorbance values at the maximum absorption of the protonated form (665 nm) are

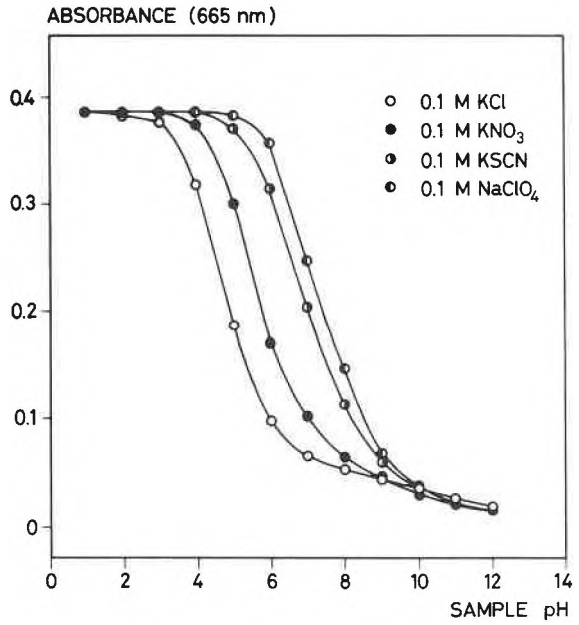

Fig. 1. Absorbances of ETH 5294 at $\lambda = 665$ nm at different pH for solutions containing a concentration of 0.1 M in either of four anions. Membrane composition: ETH 5294, o-NPOE, and OH-PVC.

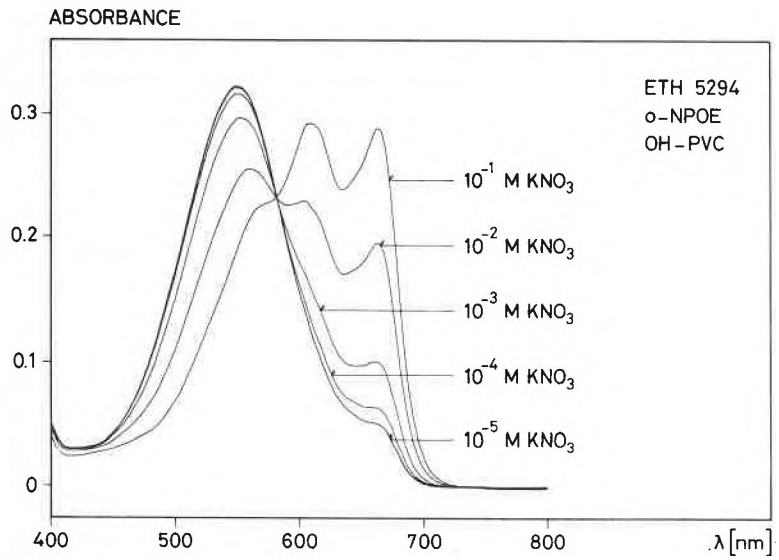

Fig. 2. Spectra for ETH 5294 for potassium nitrate solutions of different concentration at pH 5.0. Membrane composition as for Fig. 1.

plotted against the pH of the test solution. It can be seen that the pH range at which the pH indicator is in its protonated form depends on the anion used. The more lipophilic the anion is, the further this range extends into the alkaline region. Fig. 1 also indicates, that at pH 5.0 and a concentration of 0.1 M, all anions except chloride cause a nearly complete protonation of the indicator. This pH value was thus chosen as a suitable compromise for the determination of anion concentrations below 0.1 M.

In Fig. 2 the absorbance spectra of membranes containing ETH 5294 exposed to nitrate solutions between 10^{-6} and 10^{-1} M and at pH of 5.0 are shown. The absorbance band at about $\lambda = 665$ nm corresponds to the protonated form of the indicator and can be directly related to the nitrate concentrations. The larger the nitrate concentration, the larger is the protonated fraction of the indicator (Scheme (1)). The calibration curves for this anion as well as for chloride, thiocyanate, and perchlorate are given in Fig. 3. A relative selectivity of this system can be obtained from the plot. Test solutions buffered at pH lower than 5.0 might enable the determination of chloride at lower concentrations (lower than about 10^{-3} M).

Fig. 4 shows the response time and reproducibility of this system obtained when repeatedly changing between two different $NO_3^{\ominus}$ concentrations (10^{-3} and 10^{-2} M). The response time of the present assembly, as indicated by the chart recordings presented in Fig. 4, is in the order of a few minutes. The response of the membranes alone is expected to be in the order of seconds but the overall response is relatively slow because the present cell configuration does not allow stirring, and diffusion through the non-stirred layer on the optode membrane surface is expected to be rate determining[21]. The standard deviation of the absorbance values given in the graph and taken immediately before changing the solutions was 0.0005 for both concentrations. This corresponds to a precision of about ± 1% in concentration.

In practical applications it may be desirable to determine anion concentrations in solutions at a pH higher than 5.0. With the present system this is only possible for high concentrations of the more lipophilic anions such as thiocyanate and perchlorate. An even more basic neutral pH carrier might show a stronger tendency to extract anion/proton pairs and thus alleviate the problem. However, it is unlikely that such an indicator can be found, with the possible exception of guanidine derivatives. As an alternative route, the combination of charged carriers, corresponding to Scheme (2) as introduced above, was investigated.

In Fig. 5 the absorbances at $\lambda = 540$ nm of a membrane containing a lipophilized pH indicator (ETH 2412) and tridodecylmethylammonium chloride (TDDMACl), according to Scheme (2), are plotted. The

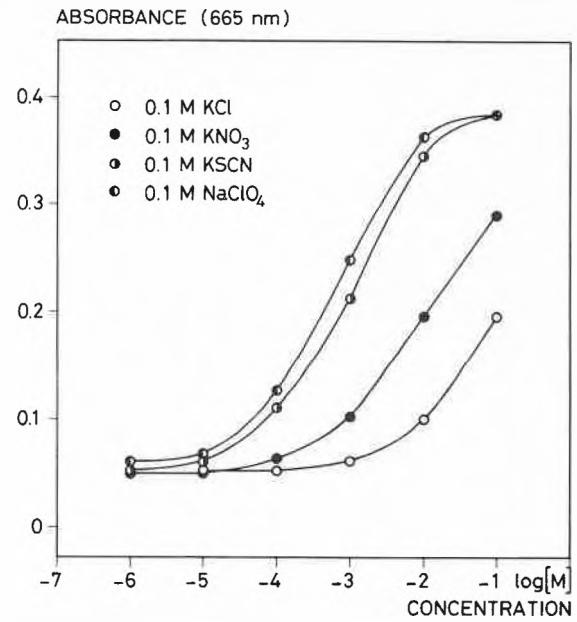

Fig. 3. Calibration curves for 4 anions at pH 5.0 obtained by measuring the absorbance at $\lambda = 665$ nm with membranes containing ETH 5294. Membrane composition as for Fig. 1.

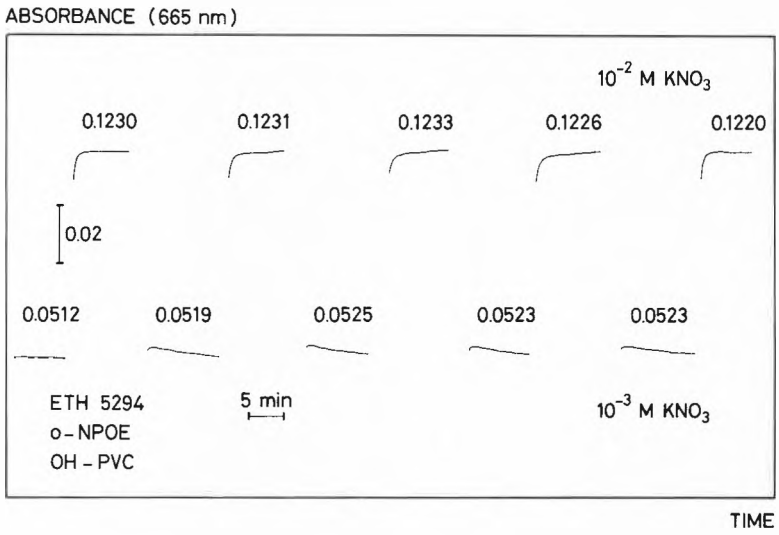

Fig. 4. Recorder traces for ETH 5294 obtained by changing solutions repeatedly between 10^{-2} M and 10^{-3} M KNO_3 at pH 5.0. Membrane composition as for Fig. 1.

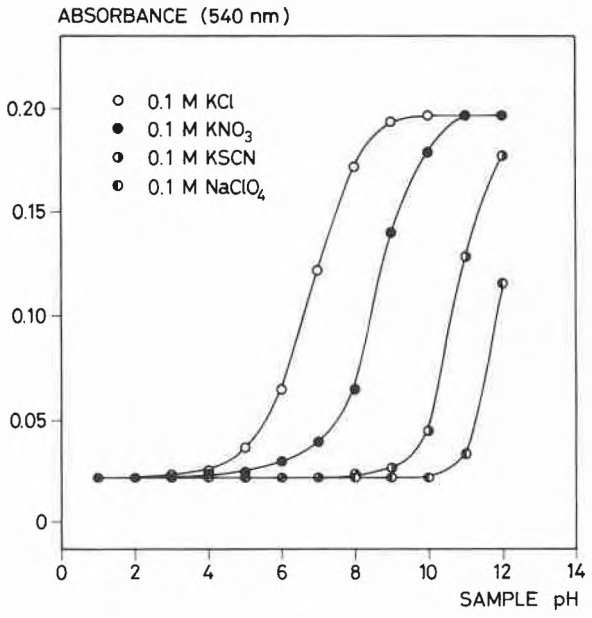

Fig. 5. Absorbances of ETH 2412 at $\lambda = 540$ nm at different pH for solutions containing a concentration of 0.1 M in either of four anions. Membrane composition: ETH 2412, TDDMACl, DOS, and PVC.

membrane was again exposed to solutions of constant anion concentration (0.1 M) and varying pH. High absorbance values are obtained at this wavelength for the deprotonated form of the indicator. Again, the pH range at which the indicator exists in its protonated form is dependent on the lipophilicity of the counter-anion. The plot is similar to the one obtained for the neutral indicator ETH 5294, but the transition regions are shifted towards the alkaline pH range. In contrast to ETH 5294, no leaching of the pH indicator in its charged form was encountered with ETH 2412 even in membranes employing PVC as matrix. This may be explained by the fact that in this case the problematic charged form of the pH carrier is countered by the strongly lipophilic quaternary ammonium ion.

In Fig. 6 the pH values at the middle of the transition regions are plotted against the hydration enthalpies of the anions used[28]. A good correlation is found for both systems and for all anions tested, except for thiocyanate. Here an irregularity is also encountered when the potentiometric selectivity coefficients of ion-selective electrodes based on quaternary ammonium ions are compared with the hydration energies[29].

Absorbance spectra for the system with the charged carrier ETH 2412 and TDDMACl at varying nitrate concentrations and a pH of 7.5 are given in Fig. 7. The dynamic range extends over about 2½ decades of nitrate concentration. Calibration curves for $Cl^{\ominus}$, $NO_3^{\ominus}$, $SCN^{\ominus}$, and $ClO_4^{\ominus}$ obtained by monitoring the absorbance at $\lambda = 540$ nm are given in Fig. 8. It should be noted that for concentrations lower than about 10^{-4} M it was necessary to repeatedly flush the measuring cell, if a response to that solution was found, in order to obtain equilibrium readings. This was due to the total amount of analyte in one cell volume being too little to satisfy the capacity of the membrane. The experimentally found lowest concentration that can satisfy the extraction capacity of the membrane of about 10^{-4} M corresponds to the theoretically expected value. The problem could be overcome by changing the design of the flow cell such that the ratio of cell volume to total membrane volume was more favourable and/or by using the cell in a continuous flow mode.

Response times and reproducibilities of the ETH 2412-based system are presented in Fig. 9. Potassium nitrate solutions 10^{-2} and 10^{-3} molar in concentration were measured repeatedly. The response time was found to be comparable to the membranes based on ETH 5294 and stable readings could be obtained within about 2 min. The standard deviations of the absorbance readings given in the plot were determined to be 0.0014 and 0.0002 absorbance units for the lower and higher concentration, respectively, which corresponds to a precision in concentration of about ± 4 and ± 0.5%, respectively.

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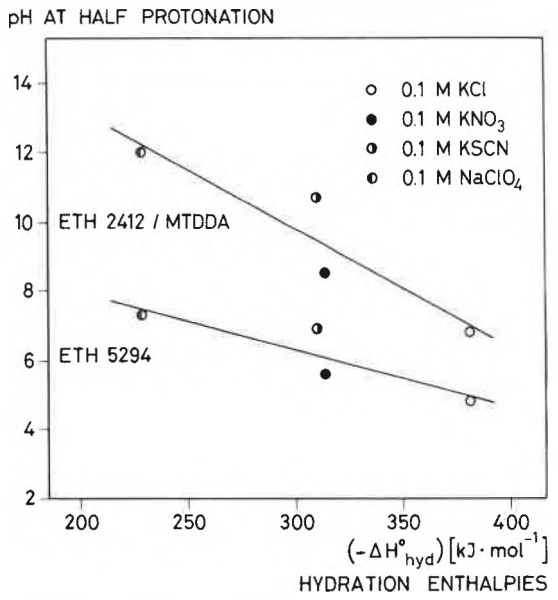

Fig. 6. Plot of hydration enthalpies ($-\Delta H^{0}_{hyd}$) of the four anions vs. the pH values at which half of the carrier is protonated with 0.1 M solutions of the respective anion.

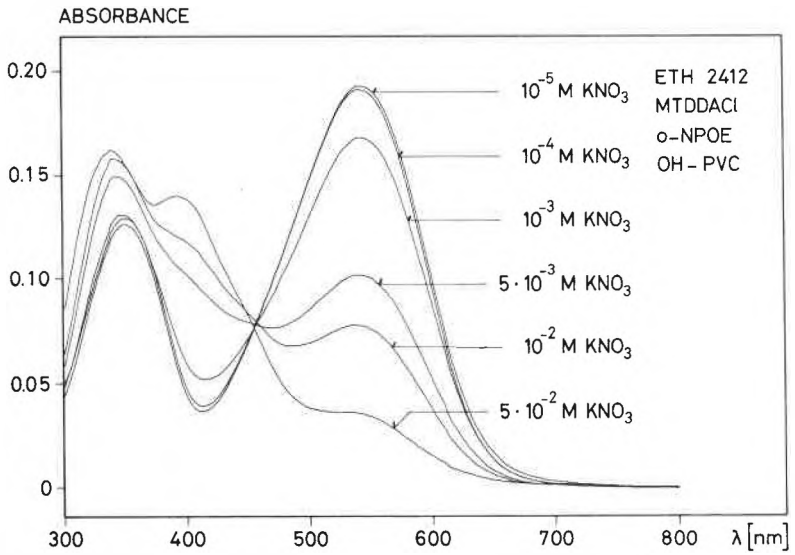

Fig. 7. Spectra for ETH 2412 for potassium nitrate solutions of different concentration at pH 7.5. Membrane composition: ETH 2412, TDDMACl, o-NPOE, and OH-PVC.

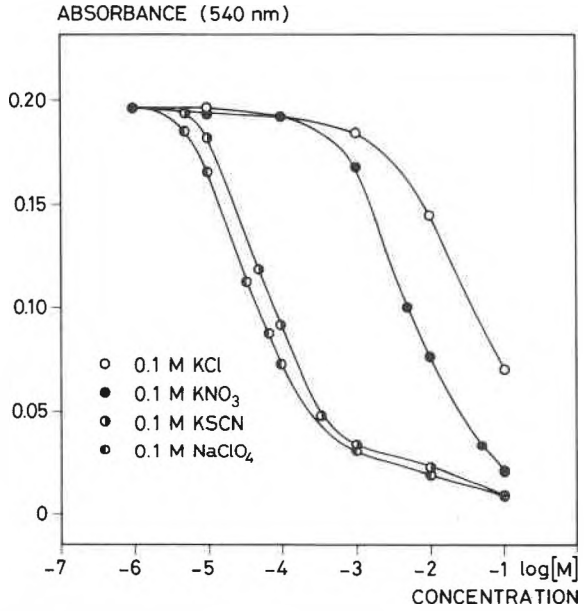

Fig. 8. Calibration curves for 4 anions at pH 7.5 obtained by measuring the absorbance at $\lambda = 540$ nm with membranes containing ETH 2412. Membrane composition as for Fig. 7.

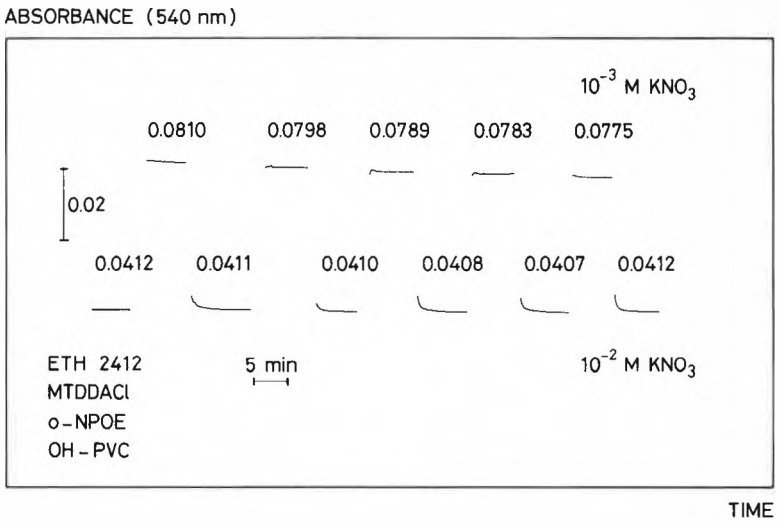

Fig. 9. Recorder traces for ETH 2412 obtained by changing solutions repeatedly between 10^{-2} M and 10^{-3} M KNO_3 at pH 7.5. Membrane composition as for Fig. 7. Solutions were buffered in ionic strength by adding 0.1 M potassium acetate.

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