Spectroscopic Method for the Determination of the Ionic Site Concentration in Solvent Polymeric Membranes and Membrane Plasticizers

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The built-in site density of either fixed sites or mobile hydrophobic ion sites determines whether a membrane is permselective for cations or anions of the sample. The molar ratio of the ionophore to the intrinsic or added ionic sites in an ion-selective membrane significantly influences the potentiometric response of ionophore-based electrodes. Consequently, full knowledge of the "site inventory" in an ion-selective membrane may be essential when new, uncharacterized polymers or plasticizers are implemented for ion-selective electrode fabrication. A simple spectroscopic method was developed for the fast and accurate determination of the ionic site concentration (covalently attached functionalized groups or impurities) in plasticized polymeric membranes and membrane plasticizers. The method is based on the determination of the degree of protonation of hydrogen ion-selective chromoionophores incorporated into these membranes or dissolved in the membrane plasticizers. In electroneutral membranes, the concentration of the positively charged, protonated ionophore and the total concentration of negative sites are equal. The method was applied for the determination of ionic sites (both positively and negatively charged) in PVC materials (different purity grade, and bearing various functional groups), polyurethanes (aliphatic, aromatic, and polycarbonate-based), and selected Fluka plasticizers (2-nitrophenyl octyl ether and 2-ethylhexyl sebacate). The technique proved to be appropriate for fast quantification of ionic impurities in hydrophobic, optically transparent materials.

All membrane sensors that develop a full Nernstian response for any or all species of a single charge sign (e.g., all +1 ions) must be permselective to the ions to be detected and measured.[1,2] In this case, permselectivity refers to the charge sign discrimination induced by ionic sites added or intrinsically present in the membrane. In practical terms, this condition means that cation-selective membranes contain fixed, or mobile, negatively charged, hydrophobic sites. For the detection of anions, the trapped sites are hydrophobic cationic species. Fixed sites are permanent in the membrane while the trapped mobile site concentration can change by salt extraction. The intrinsic sites are impurities present in the chemicals used for casting the membrane (e.g., polymer, plasticizer, or solvent) or provided by processing the polymeric materials.[3] They were determined as polymer-bound $ROSO_3^-$, RSO_3^-, and RCO_2^-.[4,5] The most often added negative and positive sites are tetraphenylborate (R^-) or quaternary ammonium ion (R^+) derivatives, respectively.

The importance of fixed and mobile anionic sites in ionophore-based solvent polymeric membranes was advocated very early,[6,7] and it was accepted that negative sites have beneficial influence on the performance of ionophore-based cation-selective sensors. Incorporated negative sites suppress salt coextraction (Donnan exclusion failure) from the bathing solution,[8] reduce the membrane resistance,[9,10] and shorten response time.[11] Site free membranes are unsuitable for ion-selective electrode applications.[12] In membranes with charged carriers, the charge sign of the sites should be opposite to that of the carrier.[13,14]

The molar ratio of the ionophore to negative sites influences the selectivity sequence among ions of different valences or forming complexes with different stoichiometries.[15−17] To set the

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(1) Helfferich, F. *Ion Exchange*; McGraw-Hill: New York, 1962.
(2) Hills, G. J. In *Reference electrodes*; Ives, D. J. G., Janz, G. J., Eds.; Academic Press: New York, 1961; Chapter 9, pp 411−433.

(3) Horvai, G.; Graf, E.; Toth, K.; Pungor, E.; Buck, R. P. *Anal. Chem.* **1986**, *58*, 2735−2740.
(4) van den Berg, A.; van der Wal, P.; Ptasinski, D.; Sudhölter, E. J. R.; Bergveld, P.; Reinhoudt, D. N. *Anal. Chem.* **1987**, *59*, 2827−2829.
(5) Ye, Q. S.; Horvai, G.; Toth, A.; Bertoti, I.; Botreau, M.; Duc, T. M. *Anal. Chem.* **1998**, *70*, 4241−4246.
(6) Morf, W. E.; Kahr, G.; Simon, W. *Anal. Lett.* **1974**, *7*, 9−22.
(7) Perrey, M.; Löbel, E.; Bloch, R. *J. Membr. Sci.* **1976**, *1*, 223−235.
(8) Boles, J. H.; Buck, R.; P. *Anal. Chem.* **1973**, *45*, 2057−2062.
(9) Nieman, T. A.; Horvai, G. *Anal. Chim. Acta* **1985**, *170*, 359−363.
(10) Lindner, E.; Gráf, E.; Niegreisz, Z.; Tóth, K.; Pungor, E.; Buck, R. P. *Anal. Chem.* **1988**, *60*, 295−301.
(11) Huser, M.; Gehrig, P. M.; Morf, W. E.; Simon, W.; Lindner, E.; Jeney, J.; Tóth, K.; Pungor, E. *Anal. Chem.* **1991**, *63*, 1380−1386.
(12) Buhlmann, P.; Yajima, S.; Thoda, K.; Umezawa, Y. *Electrochim. Acta* **1995**, *40*, 3021−3027.
(13) Amemiya, S.; Buhlmann, P.; Pretsch, E.; Rusterholtz, B.; Umezawa, Y. *Anal. Chem.* **2000**, *72*(7), 1618−1631.
(14) Schaller, U.; Bakker, E.; Spichiger, U. E.; Pretsch, E. *Anal. Chem.* **1994**, *66*, 391−398.
(15) Meier, P. C.; Morf, W. E.; Läubli, M.; Simon, W. *Anal. Chim. Acta* **1984**, *156*, 1−8.
(16) Eugster, R.; Gehrig, P. M.; Morf, W. E.; Spichiger, U. E.; Simon, W. *Anal. Chem.* **1991**, *63*, 2285−2289.
(17) Eugster, R.; Spichiger, U. E.; Simon, W. *Anal. Chem.* **1993**, *65*, 689−695.

optimal ionophore-to-site ratio, the concentration of intrinsic ionic sites in the membrane matrix (polymer and plasticizer) has to be known accurately.[18] This intrinsic site concentration is than complemented with added sites, depending on the ionophore concentration.

When the intrinsic site concentration is not known precisely, the added sites are incorporated into the membrane in large excess, compared to the intrinsic sites, to minimize the detrimental effect of this uncertainty on the membrane selectivity. However, this approach is not always feasible. To apply a large excess of the added sites, at least the charge sign and an estimated concentration range of the intrinsic sites in the polymeric material has to be known. This requirement is not necessarily fulfilled with novel materials implemented to improve the biocompatibility or lifetime[19] of solvent polymeric membranes.

The required ionophore-to-site ratio (for optimal selectivity behavior) also restricts the applicable excess of added sites compared to the intrinsic sites when the ionophore concentration is fixed in the membrane. An important example is when the optimal response of a sensor is achieved with low ionophore concentration.[20] In membranes with reduced ionophore concentration, there is not much room to adjust the total site concentration because it has to be lower than the ionophore concentration. Similarly, the total site concentration cannot be increased at will due to the limited solubility of the ionophore in the membrane plasticizer.

Finally, in some modified PVC materials (e.g., carboxylated PVC) with a large concentration of active groups susceptible to dissociation, the concentration of the dissociated fraction could vary between wide ranges. The concentration of this dissociated or charged fraction ($\equiv$site concentration) depends on the polarity of the membrane plasticizer and the chemical characteristics and concentration of the other membrane ingredients.[10,18,21,22]

The ion exchange capacity (the site concentration) of bis(2-ethylhexyl) sebacate (DOS) plasticized, ionophore-free, PVC−COOH membranes was reported as 0.27 mmol of COO^-/kg (0.2% of the total COOH content). When the same membranes were loaded with valinomycin, the ion exchange capacity was determined as 1.48 mmol/kg (1.1% of the COOH content).[10] By replacing the nonpolar DOS with the polar, 2-nitrophenyl octyl ether (o-NPOE) as membrane plasticizer, the dissociated fraction of PVC−COOH increases further. By studying the effect of the ionophore-to-site ratio on the selectivity of PVC−COOH-based potassium-selective membranes, Cosofret concluded that ∼6.75% of the total COOH content (9 mmol/kg) is in its dissociated form.[18,22] Finally, a highly basic ionophore can also facilitate the deprotonation of the COOH groups. The dissociated fraction of total COOH concentration was determined as 46 mmol/kg (35% of the COOH content) in o-NPOE-plasticized PVC−COOH membranes compounded with ETH 5294.[18,22]

The ionophore-to-site ratio may change during extensive use related to the limited dissolution of the ionophore or charged sites from the membrane into the aqueous sample. To address problems connected to the loss of membrane ingredients, recently we developed a chronoamperometric method to estimate the concentration changes of the ionophore and lipophilic additives in a mobile site containing solvent polymeric membranes.[28]

Due to the importance of charged sites in carrier-based membranes, several articles have dealt with the identification[3,4,23] and the quantitative determination[3,10,21,22,24−27] of ionic sites (impurities and built-in fixed and mobile sites) in solvent polymeric membranes and membrane materials. Unfortunately, most of these methods are quite cumbersome or provide only qualitative data. Early radiotracer and ion exchange measurements estimated the amount of intrinsic negative site concentration in plasticized PVC membranes to be between 0.05 and 0.6 mmol/kg.[10,23] Impedance measurements of solvent polymeric membranes and membrane solutions of different compositions identified the sources of intrinsic anionic sites.[3,10] Selectivity studies revealed that the total and the active, i.e., exchangeable, site concentration might differ.[10] The infrared absorption spectra of PVC membranes were used to gain further insight regarding the charged and uncharged nature of carboxyl groups in PVC−COOH membranes.[21] Selectivity studies were also utilized for the quantitative evaluation of site concentrations in poly(vinyl chloride)- and polyurethane-based membranes.[22,25] The concentration of anionic sites in commercially available high molecular weight PVC was determined as 0.063 mmol/kg.[25] In carboxylated PVC materials, the experimentally determined site concentrations depend on the membrane composition and can range between 0.27 and 46 mmol/kg.[10,22] The cationic site concentration in Tecoflex polyurethane was found to be 0.044 mmol/kg.[25]

In this work, we propose a very simple, spectrophotometric method for the determination of site concentration (impurities and intrinsic fixed and mobile sites) in plasticized membranes and membrane plasticizers using pH-sensitive chromoionophores. The simplicity of the method qualifies it for fast quality control or fast screening of existing and novel membrane materials. The technique might also be attractive in other areas for the determination of ionic impurities in optically transparent hydrophobic materials.

EXPERIMENTAL SECTION

Reagents. Chromoionophore I (ETH 5294, Lot 61543/1), chromoionophore II (ETH 2439, Lot 61544/1), potassium tetrakis-[3,5-bis(trifluoromethyl) phenyl]borate (KTFPB, Lot 47822/1), bis-(2-ethylhexyl) sebacate (dioctyl sebacate, DOS, Lot 48396/1), and 2-nitrophenyl octyl ether (o-NPOE, Lot 46752/1) were purchased from Fluka (Fluka Chemical Corp., Milwaukee, WI). The hydroxylated PVCs, ETH 3528 (PVC-OH) and ETH 5042 (PVC-CH_2−OH), were a generous gift of Prof. Erno Pretsch. The piperazine,

(18) Cosofret, V. V.; Erdosy, M.; Buck, R. P.; Kao, W. J.; Anderson, J. M.; Lindner, E.; Neuman, M. R. *Analyst* **1994**, *119*, 2283−2292.

(19) Lindner, E.; Buck, R. P. *Anal. Chem.* **2000**, *72*, 336A−345A.

(20) Ceresa, A.; Bakker, E.; Hattendorf, B.; Gunther, D.; Pretsch, E. *Anal. Chem.* **2001**, *73*, 343−351.

(21) Ma, S. C.; Chaniotakis, N. A.; Meyerhoff, M. E. *Anal. Chem.* **1988**, *60*, 2293−2299.

(22) Cosofret, V. V.; Buck, R. P.; Erdosy, M. *Anal. Chem.* **1994**, *66*, 3592−3599.

(23) Thoma, A. P.; Viviani-Naurer, A.; Arvanitis, S.; Morf, W. E.; Simon, W. *Anal. Chem.* **1977**, *49*, 1567−1572.

(24) Buck, R. P.; Toth, K.; Graf, E.; Horvai, G.; Pungor, E. *J. Electroanal. Chem.* **1987**, *223*, 51−66.

(25) Nägele, M.; Pretsch, E. *Microchim. Acta* **1995**, *121*, 269−279.

(26) Cosofret, V. V.; Erdosy, M.; Raleigh, J. S.; Johnson, T. A.; Neuman, M. R.; Buck, R. P. *Talanta* **1996**, *43*, 143−151.

(27) Qin, Y.; Bakker, E. *Anal. Chem.* **2001**, *73*, 4262−4267.

(28) Pendley, B. D.; Gyurcsanyi, R. E.; Buck, R. P.; Lindner, E. *Anal. Chem.* **2001**, *73*, 4599−4606.

modified aminated PVC (PVC-NH₂) was a generous gift of Prof. Robert Kusy (UNC, Chapel Hill).[29] High molecular weight poly-(vinyl chloride): Selectophore (PVC-HMW, Lot 371469/1), purum grade (PVC-purum, Lot 370852/1), and carboxylated PVC (PVC-COOH, Lot 311194/1) were from Fluka. The polyurethane products tested in this work were medical grade and obtained from Thermedics Polymer Products (Woburn, MA) as a gift: aliphatic polyether-based Tecoflex (SG80A and SG85A); aromatic polyether-based Tecothane (1074A), and aliphatic polycarbonate-based Carbothane (PC3575). All other chemicals were of at least reagent grade and were used as received. Water was purified using a Milli-Q Gradient A10 system (Millipore Corp., Bedford, MA).

Membranes. Membranes were prepared according to the procedure of Craggs et al.[30] However, the PVC-to-plasticizer ratio and the concentration of the embedded chromoionophore were altered from the most common values. These alterations were necessary because the accuracy of the proposed method is optimal when the concentrations of the sites and the chromoionophore are comparable (the same order of magnitude). The ionophore concentration was decreased from about 1% to 0.03−0.1% (w/w) (in the majority of the cases 0.065%), and the polymer-to-plasticizer ratio was selected between 1:1 or 2:1 instead of 1:2. For the same reason, the PVC-COOH-based membranes were often cast as mixtures of PVC-COOH and PVC-HMW. The analytical characteristics (response slope, selectivities, etc.) of these membranes with the uncommon composition were the same or very similar to those cast with the usual 1 wt % ionophore. Membrane thicknesses were ~150 μm.

In experiments where the site concentration of the membrane was extremely low, very small amounts of membrane components had to be weighed in. To minimize the error of weighing, first stock solutions were prepared by dissolving, ~1 mg of chromoionophore or ~1 mg of lipophilic salt additive in dioctyl sebacate (100−500 mg). To obtain the required amounts for the membrane preparation, precisely weighed amounts of these stock solutions were used.

Apparatus. A Pariss (LightForm Inc., Belle Mead, NJ, http://www.lightforminc.com) system or a Chem 2000 UV−visible spectrophotometer (Ocean Optics Inc., Dunedin, FL; www.OceanOptics.com) was used for determination of the ratio of the protonated and unprotonated chromoionophore in membranes and plasticizers. The Pariss imaging spectrometer was attached to a Nikon Eclipse E600 microscope (Southern Micro Instruments, Atlanta, GA; http://www.southernmicro.com), and it was controlled by a Labview-based operating software using Pentium III PC. The operating software automatically transformed the transmittance values into absorbance.

Procedures. The spectral properties of the freshly prepared membranes were determined using the Pariss system. The studied membranes were placed on a microscope slide, and 240 spectra were collected in a single snap along a selected line of ~400-μm length. The mean absorbance values measured at 660 and 535 nm were used to quantify the absorbance of the protonated and unprotonated chromoionophore in the studied membrane. Next, each membrane was placed inside of a separate 25-mL vial filled
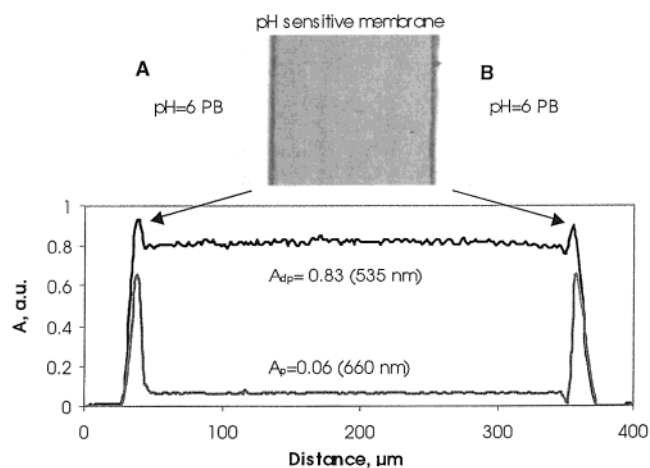

Figure 1. Concentration profiles of the protonated and deprotonated forms of ETH 5294 in a pH-selective membrane strip that was previously bathed on both sides in 0.05 M, pH 6 phosphate buffer. The concentration profiles were imaged with the Pariss optical microspectrometer Membrane composition: 2.22 mM/kg ETH 5294, PVC-HMW (Selectophore grade); DOS 1.2.

with 10⁻⁴ M HCl and were equilibrated for a minimum of 12 h. After equilibration, the membranes were taken out from the conditioning solution and placed back on a microscope slide. and the spectral properties were determined again. The peak evaluation and deconvolution routines of the Microcal Origin 6.0 software package were used to determine the absorbance ratio of the protonated and unprotonated forms of the chromoionophores from the recorded spectra.

Even though most of our spectroscopic measurements were made with the Pariss system, the proposed simple spectroscopic method can be used with any spectrophotometric device. The only difference in the procedure would be that instead of placing the studied membrane onto a microscope slide it has to be placed onto the inner wall of a cuvette at a height where the beam of light enters the cuvette. More details are given later in the Proof of Concept section.

For recording the concentration profiles of the protonated and unprotonated forms of ETH 5294 in an ion-selective membrane (Figure 1), a thin-layer electrochemical cell was placed onto the stage of the Nikon Eclipse E600 microscope equipped with the Pariss microspectrometer. In the electrochemical cell, a thin strip (~300 μm) of the studied membrane separated two solutions. In the experiment shown in Figure 1, a pH 6.0 phosphate buffer solution was placed on both sides of the membrane. After complete equilibration, 240 spectra (from 400 to 800 nm) were recorded across the cross section of the membrane (as a function of distance) with the imaging camera. The corresponding distance and absorbance values (measured at 535 and 660 nm) were used to generate Figure 1. The spectral imaging method was described in more detail previously.[31,32]

THEORETICAL SECTION

The phase boundary potential model[33] describes accurately the potentiometric response of carrier-based ion-selective electrodes.

(29) Kusy, R. P.; Whitley, J. Q.; Buck, R. P.; Cosofret, V. V.; Lindner, E. *Polymer* **1994**, *35*, 2141−2147.

(30) Craggs, A.; Moody, G. J.; Thomas, J. D. R. *J. Chem. Educ.* **1974**, *51*, 541−544.

(31) Lindner, E.; Gyurcsányi, R. E.; Buck, R. P. In *Membranes in Electroanalytical Chemistry. Membrane Based Chemical and Biosensors, Encyclopedia of Surface and Colloid Science*; Hubbard, A., Ed.; Marcel Dekker: New York, 2002; pp 3239−3264.

According to this model, in the Nenstian response range of the electrodes, there are no concentration gradients in the membrane bulk. The concentration of negative sites is matched with positively charged complexes of the ionophore, which sustains electroneutrality.[24] The fundamental statements of the model have been verified with optical measurements by determining the degree of protonation of a pH-sensitive chromoionophore in a KTFPB compounded pH-sensitive membrane at normal and extreme pH values.[33] Imaging the concentration profiles in the membrane cross section also clarified the correlation between potentiometric and optode responses.[34] In Figure 1, the concentration profiles of the protonated and unprotonated forms of ETH 5294 are shown in a pH-sensitive membrane strip that was equilibrated with a pH 6.0 phosphate buffer. As predicted, the concentrations of the two forms are constant across the membrane cross section and the ratio of the forms is determined by the concentration of negative sites in the membrane. Accordingly, in an ideally permselective membrane (complete exclusion of sample anions), with perfect proton selectivity and electroneutrality in its bulk, the negative site concentration can be determined by determining the concentration of the protonated form of a H^+-selective chromoionophore (e.g., ETH 5294). As long these assumptions prevail, the same method can be performed for the determination of negative sites in chromoionophore-loaded plasticizers. In the Nernstian response range of carrier-based membranes, all these conditions are fulfilled; i.e., the concentration of the protonated form of ETH 5294 (C_p) is equal to the concentration of anionic sites ($C_{site^-_{membr}}$) in the membrane:

$$C_p = C_{site^-_{membr}} \tag{1}$$

The optical spectrum of the membrane provides the absorbance values for the protonated (A_p) and unprotonated (A_{up}) forms of the chromoionophore that are a linear function of the respective concentration values (C_p and C_{up}):

$$A_p = C_p l \epsilon_p \quad \text{and} \quad A_{up} = C_{up} l \epsilon_{up} \tag{2}$$

where ϵ_p and ϵ_{up} are the molar absorption coefficients of the protonated and unprotonated forms of the chromoionophore and l is the optical path length (membrane thickness).

If the total concentration of the chromoionophore in the membrane (C_{tot}) is known, from the degree of protonation (α_H) the anionic site concentration can be calculated:

$$C_{site^-_{membr}} = C_p = C_{tot}\alpha_H = C_{tot}\frac{A_p/\epsilon_p}{A_p/\epsilon_p + A_{up}/\epsilon_{up}} \tag{3}$$

(32) Lindner, E.; Gyurcsányi, R. E.; Pendley, B. D. *Pure Appl. Chem.* **2001**, *73*, 17−22.
(33) Bakker, E.; Nägel, M.; Schaller, U.; Pretsch, E. *Electroanalysis* **1995**, *7*, 817−822.
(34) Lindner, E.; Zwickl, T.; Bakker, E.; Lan, B. T. T.; Tóth, K.; Pretsch, E. *Anal. Chem.* **1998**, *70*, 1176−1181.

Equation 3 simplifies by introducing the ratio of the two molar absorption coefficients ($f = \epsilon_{up}/\epsilon_p$):

$$C_{site^-_{membr}} = C_p = C_{tot}(A_p f/A_p f + A_{up}) \tag{4}$$

If the polymeric matrix is the sole source of anionic sites in the membrane, the negative site concentration in the polymer is calculated with eq 5, where w_{membr} and $w_{polymer}$ are the total mass of the membrane and the polymer, respectively:

$$C_{site^-_{polymer}} = (w_{membr}/w_{polymer})C_{site^-_{membr}} \tag{5}$$

When the site concentration has to be determined in membranes with positive sites ($C_{site^+_{membr}}$), the membrane is compounded with hydrophobic negatively charged sites (C_{R^-}) (e.g., KTFPB) in excess to the intrinsic positive sites. Next, this excess of negative sites is determined in the membrane quantitatively using the procedure described above. The concentration of positive sites in the polymer is then calculated from eq 6:

$$C_{site^+_{polymer}} = (w_{membr}/w_{polymer})(C_{R^-} - C_{site^-_{membr}}) \tag{6}$$

RESULTS AND DISCUSSION

Proof of Concept. First, the ratio of the absorption coefficients of the protonated (660 nm) and unprotonated forms (535 nm) of the ETH 5294 chromoionophore was determined in a DOS-plasticized PVC-HMW membrane. The ~150-μm-thick membrane was cast with a 1:2 polymer/plasticizer ratio and contained 100 mol % KTFPB with respect to the chromoionophore. A disk-shaped membrane segment (6 mm in diameter) was attached on the internal wall of a cuvette and was equilibrated with 1 M HCl solution. The 100 mol % KTFPB loading of the membrane and the high acid concentration in the bathing solution were chosen to facilitate complete protonation of the chromoionophore in the membrane. The absorption spectrum of the membrane was recorded intermittently until the equilibrium was reached, and the spectrum of the equilibrated membrane (protonated form) was saved. Next, the 1 M HCl solution in the cuvette was replaced with 1 M NaOH and the deprotonation of the chromoionophore was followed as a function of time. The absorption spectra were recorded continuously until the new equilibrium was established. The spectrum of the NaOH-equilibrated membrane (unprotonated form) was also saved. Then the protonation experiment was repeated and the unprotonated membrane (chromoionophore) was equilibrated in 1 M HCl bathing solution. From the recorded spectra, the ratio of the absorption coefficients (f) of the unprotonated and protonated forms was determined as 0.81 ± 0.02. This value correlates well with previously published data (0.79) in the PVC-based solvent polymeric membranes.[35]

It is important to note that ratio of the absorption coefficients is fairly constant although wavelength of the absorption maximums and the individual absorption coefficients for the unprotonated and protonated forms of ETH 5294 varied significantly in different solvents. In THF, $f = 0.73$ has been determined. The

(35) Bakker, E. Dissertation ETH 10229, ETH, Zurich, 1993.

literature value in methanol is $f = 0.74$.[35] Due to the robustness of the f determination, the method can be directly applied for different membrane formulations. The ratio of the absorption coefficients of the ETH 2439 chromoionophore is similarly stable. An f value of 0.45 has been determined in DOS-plasticized membrane. In THF, a ratio of 0.48 has been measured. The value reported in the literature in methanol is 0.47.[35]

To provide experimental proof for the practical applicability of the method, several membranes were cast with a given ETH 5294 or ETH 2439 chromoionophore concentration but different lipophilic anion contents (0−100 mol %). In these membranes, the total site concentration was determined from a single absorbance measurement. For the ETH 5294-loaded membranes, the correlation between the lipophilic anion concentration, calculated from the membrane formulation and from eq 3, using the absorbance values measured at 535 and 660 nm, is shown in Figure 2A. Between 0 and 65 mol % lipophilic anion concentrations, the correlation is close to perfect (slope ∼1.0, intercept ∼0.0). The deviations experienced at higher concentrations are attributed to the difficulties in determining the correct absorbance values of the unprotonated ionophore from the complex spectra of ETH 5294 when the protonated form is dominant. The highest accuracy is expected when the concentrations of the protonated and unprotonated forms of the chromoionophore are comparable (between 10 and 60%).

Larger separation between the absorbance maximums of the protonated and unprotonated forms of the ionophore or simpler spectra make the evaluation unambiguous even at higher site concentrations. As shown in Figure 2B, when the ETH 2439 chromoionophore was used for the site determinations, the correlation was found flawless in the whole concentration range (from 0 to 100 mol %).

The results shown in Figure 2 not only support the feasibility of the method but at the same time confirm our basic assumptions that the protonated ionophore concentration in the membrane is equal to the negative site concentration. In addition, they show that the selected experimental conditions were appropriate to reach equilibrium conditions in the membrane.

Anionic Impurities in Solvents and Plasticizers. Tracing the Presence and the Quantification of Ionic Sites in Chemicals Used for Membrane Casting. The spectrum of ETH 5294 in THF shows a single absorption maximum at 535 nm. This means that there are no protonated anionic impurities in the chromoionophore or in the THF. However, it is important to emphasize that this experiment informs only about anionic impurities, which are in their protonated form.

To determine the concentration of ionic impurities in the two most commonly used plasticizers, DOS and o-NPOE, ETH 5294 was dissolved in these plasticizers to give a concentration of 0.95 and 0.85 mmol/kg, respectively. Next, droplets of the respective solutions were transferred onto a microscope slide and their absorption spectra were recorded. No protonated chromoionophore could be detected in DOS. In contrast, the protonated ionophore concentration in o-NPOE was surprisingly high (0.188 mmol/kg o-NPOE). This suggests that there are no intrinsic anionic impurities with protons as counterions in the DOS, but the concentration of such impurities is relatively high in o-NPOE.
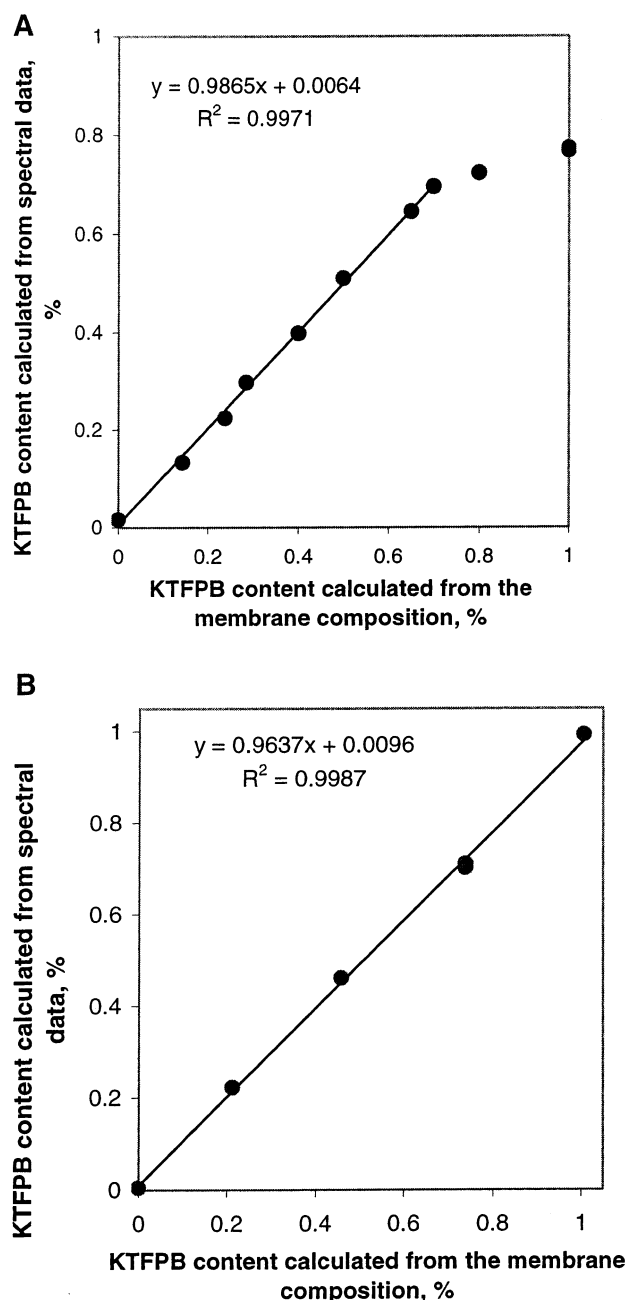
A

$$y = 0.9865x + 0.0064$$
$$R^2 = 0.9971$$

KTFPB content calculated from spectral data, %

KTFPB content calculated from the membrane composition, %

B

$$y = 0.9637x + 0.0096$$
$$R^2 = 0.9987$$

KTFPB content calculated from spectral data, %

KTFPB content calculated from the membrane composition, %

Figure 2. Correlation between the externally added lipophilic anion (KTFPB) concentrations in PVC−DOS (1:2) membranes calculated from the membrane formulation and from experimentally determined absorbance values using eq 3. ETH 5294 (A) or ETH 2439 (B) chromoionophores were used as "indicator" molecules in the spectroscopic determination.

Next, 200 μL of the DOS and o-NPOE solutions were vortexed with 4 mL of 10^{-4} M HCl solution, and the absorption spectra of organic phases were determined as a function of time. The spectrum of the DOS/ETH 5294 solution remained the same after the 10^{-4} M HCl treatment. However, the absorption maximum of the protonated form of ETH 5294 in the o-NPOE solution continuously decreased and after a one-week equilibration time it became undetectable (Figure 3). This means that the anionic impurities of o-NPOE are water-soluble and gradually leach out from the organic phase into the aqueous solution. This time-dependent loss of unaccounted anionic sites from o-NPOE
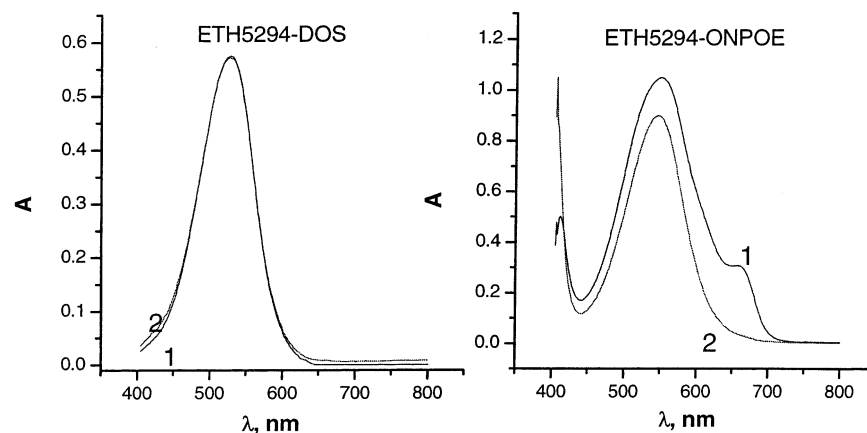
Figure 3. Absorbance spectra of the ETH 5294 chromoinophore dissolved in DOS (A) and *o*-NPOE (B) plasticizer. The ionophore concentrations were 0.95 and 0.85 mM/kg, respectively. The spectra were recorded with the freshly prepared plasticizer solutions (1) and after a one-week equilibration time with 0.1 mM HCl (2).

Table 1. Composition of Solvent Polymeric Membranes Screened for Anionic Sites

membrane	polymer, mg (%)	plasticizer, mg (%)	ETH 5294, mg (%)
purum PVC/DOS	139.90 (60.38)	91.64 (39.55)	0.156 (0.07)
ETH 5042(PVC-CH$_2$−OH)/DOS	122.38 (56.27)	94.96 (43.66)	0.147 (0.07)
PVC-COOH/DOS	138.47 (58.54)	97.90 (41.39)	0.174 (0.07)
Tecoflex SG85/DOS	135.95 (58.59)	95.95 (41.35)	0.152 (0.07)
ETH 3528 (PVC-OH)/DOS	68.96 (35.31)	126.17 (64.61)	0.146 (0.07)
aminated PVC (PVC-NH$_2$)/DOS	115.37 (55.01)	94.18 (44.91)	0.162 (0.08)
Selectophore grade PVC-HMW/*o*-NPOE	138.25 (55.15)	112.35 (44.82)	0.079 (0.03)
Selectophore grade PVC-HMW/DOS	137.04 (57.12)	102.82 (42.85)	0.075 (0.03)

plasticizer is reported here for the first time. The most recent results related to the detection limit of ionophore-based sensors suggest that such a prolonged leaching could lead to a biased result.[36−41]

Sites in Solvent Polymeric Membranes. The most common composition of ionophore-based membranes is 1 wt % or 10−20 mmol/kg neutral ionophore, ∼65 wt % plasticizer, and 33 wt % PVC-HMW. The intrinsic site concentration in these membranes is expected to be between 0.01 and 0.1 mmol/kg. Since the concentration of negative sites in the membrane is equal to the total concentration of protonated ionophore, as shown in Figure 1, in our method the concentration of the protonated form of a H$^+$-selective chromoionophore measures the site concentration in the membrane. The highest accuracy is expected when the concentration of the two forms of the ionophore (protonated and unprotonated) are comparable. To approach this required concentration range, the total concentration of the chromoionophore was drastically reduced and the percentage of the polymeric component was increased in the membranes. In our experiments, 0.065 wt % ionophore and 50−60 wt % polymer were typical. However, after an orientation experiment, one can fine-tune the membrane composition for the best accuracy and sensitivity.

In our procedure, all membranes were soaked overnight in 0.1 mM HCl before the spectroscopic measurement was performed. During this equilibration, the counterions of the anionic sites are selectively exchanged to protons. The H$^+$-selective chromoionophore ETH 5294 facilitates this selective ion exchange. The lack of perfect selectivity or compromised permselectivity can bias the results in a positive or negative direction, respectively. Since the only cations in the soaking HCl solution are H$^+$ ions,

the possibility of cation interference can be excluded. The probability of errors related to the coextraction of chloride ions was minimized by using dilute HCl solution (0.1 mM). As long the coextraction is negligible, the potentiometric response slopes are Nernstian. The calibration curves of ETH 5294 ionophore-based pH sensors are Nernstian between pH 3 and 10.[42] This was confirmed with our membranes cast with different polymer-to-plasticizer ratios (PVC-HMW to DOS, 1:3 and 3:1) and drastically reduced ionophore content (data not shown).

In this study, membranes made with different kinds of polymeric materials (PVCs and polyurethanes), relevant for the fabrication of potentiometric sensors, were investigated. The composition and optical spectra of various solvent polymeric membranes screened for intrinsic anionic sites are summarized in Table 1 and Figure 4. With the exception of the aminated PVC (PVC-NH$_2$) membranes, the concentration of the anionic sites in the plasticized polymeric membrane and in the polymeric material itself could be determined quantitatively from the spectral data. The intrinsic absorption of PVC-NH$_2$ in the visible region impedes

(36) Sokalski, T.; Ceresa, A.; Zwickl, T.; Pretsch, E. *J. Am. Chem. Soc.* **1997**, *119*, 11347−11348.
(37) Sokalski, T.; Ceresa, A.; Fibbioli, M.; Zwickl, T.; Bakker, E.; Pretsch, E. *Anal. Chem.* **1999**, *71*, 1210−1214.
(38) Sokalski, T.; Zwickl, T.; Bakker, E.; Pretsch, E. *Anal. Chem.* **1999**, *71*, 1204−1209.
(39) Mathison, S.; Bakker, E. *Anal. Chem.* **1998**, *70*, 303−309.
(40) Ceresa, A.; Sokalski, T.; Pretsch, E. *J. Electroanal. Chem.* **2001**, *501*, 70−76.
(41) Telting-Diaz, M.; Bakker, E. *Anal. Chem.* **2001**, *73*, 5582−5589.
(42) Cosofret, V. V.; Nahir, T. M.; Lindner, E.; Buck, R. P. *J. Electroanal. Chem.* **1992**, *327*, 137−146.
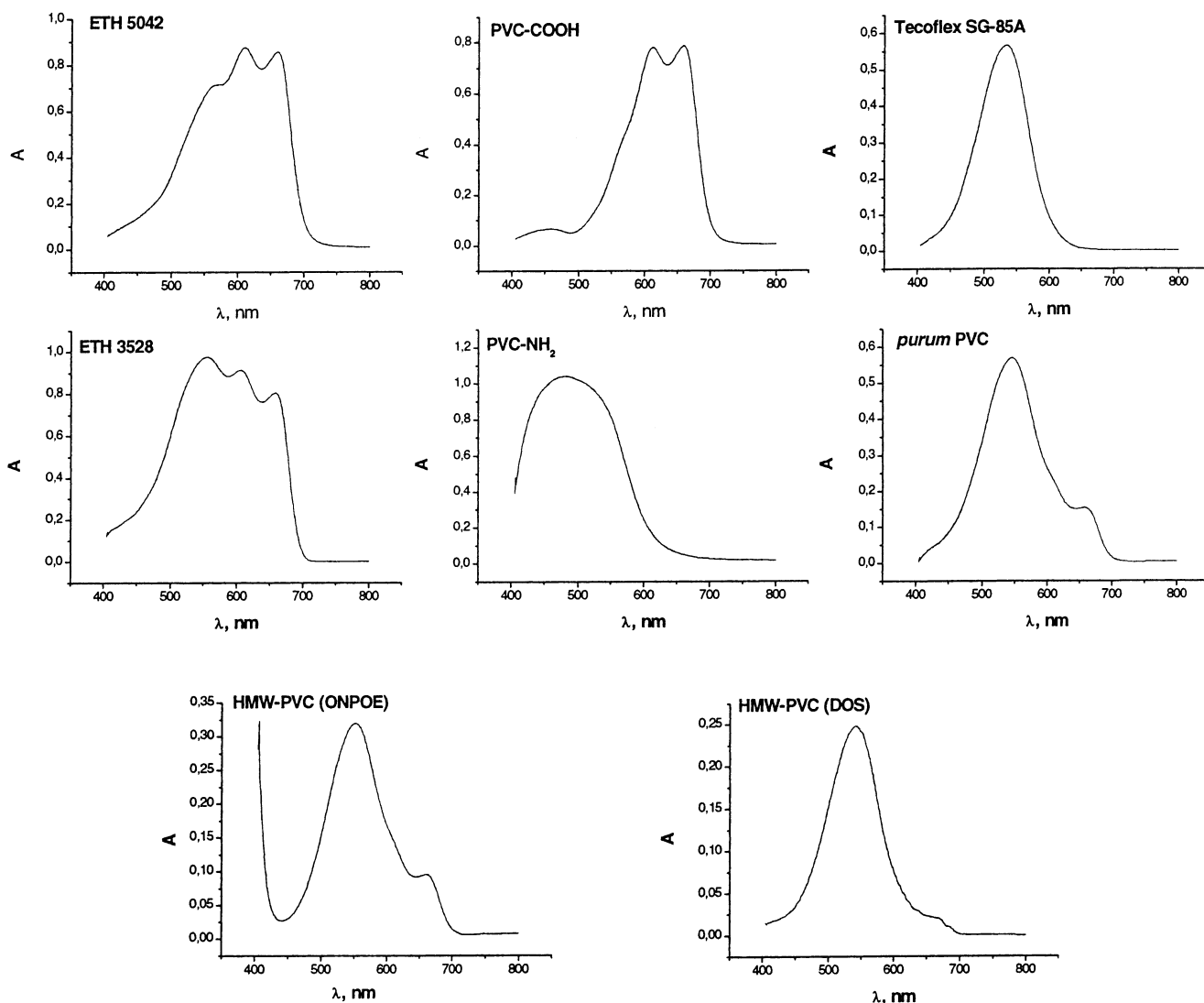
Figure 4. Optical spectra of the ETH 5294 chromoionophore in different membrane formulations. The membrane compositions are summarized in Table 1. The protonated form of the chromoionophore ETH 5294 is indicative for the concentration of anionic sites in the respective membranes.

Table 2. Anionic Site Concentrations in Different Polymeric Materials Used in Ion-Selective Membrane Formulations

polymeric material	anionic sites, mM/kg
Selectophore grade PVC-HMW (DOS)	0.06 ± 0.01
Selectophore grade PVC-HMW (*o*-NPOE)	0.16 ± 0.04
purum PVC	0.36 ± 0.01
PVC-COOH[a]	62.2 ± 9.4
ETH-5042 (PVC-CH$_2$−OH)	1.25 ± 0.05
ETH-3528 (PVC-OH)	1.78 ± 0.12
PVC-NH$_2$	0

[a] The anionic site concentration was determined with a pH-selective membrane having 0.14% PVC-COOH, 23.6% PVC-HMW, 76.1% DOS, and 1.26 mM/kg ETH 5294.

the accurate evaluation of the absorption maximum of the unprotonated form of the ionophore, and the concentration of the protonated form is below the detection limit of the method. The results suggest that no anionic impurities are present in PVC-NH$_2$ membranes.

Table 2 reviews the anionic site concentrations in different polymeric materials determined by the proposed spectroscopic method. These data must not necessarily coincide with previously determined site concentrations. Conductometric methods[10] can assess only the dissociated sites that contribute to the ionic conduction; i.e., the reported data may underestimate the total site concentration when the sites are undissociated or form strong ion pairs. The spectrophotometric method accounts for both the free sites and the sites in the form of ion pairs. Finally, the stability or the dissociation constant of the ionophore in the membrane also influences the experimentally determined site concentration values. The highly basic ETH 5294 chromionophore ($pK_a = 12.0$ in PVC-based membranes[35]) effectively deprotonates the functional groups with weak acid character, such as carboxylic groups in PVC-COOH, that are otherwise mostly undissociated in the membrane.[10,21] The influence of the membrane plasticizer and the incorporated ionophore on the experimentally determined site concentrations of ionophore-based membranes is summarized in Table 3.[10,22] The spectroscopic method advocated in this paper provides a mean value of 20.7 (RSD = 6.8%) mmol/kg site

Table 3. Concentration of Carboxylate Anionic Sites in Potassium and Hydrogen Ion-Selective, PVC-COOH-Based Solvent Polymeric Membranes Cast with Polar (o-NPOE) and Nonpolar (DOS) Plasticizers[a]

	plasticizer	
ionophore	o-NPOE	DOS
none		0.27 (0.2)[10]
valinomycin (K+ selective)	9.0 (6.75)[22]	1.48 (1.04)[10]
ETH 5294 (H+ selective)	46.0 (35.0)[22]	20.7 (14.7)[b]

[a] The concentrations are expressed as mmol/kg of membrane and as relative percentile of the total carboxylic group concentration (in parentheses). The limiting value is calculated from the carboxyl group concentration in carboxylated poly(vinyl chloride) (1.8%), the membrane composition, and the membrane density (1.064 g/mL DOS plasticized membranes with 1:2 polymer-to-plasticizer ratio). [b] Charged site concentration determined in this work.

concentration in DOS-plasticized, carboxylated PVC membranes when ETH 5294 is used as the membrane ionophore. The 20.7 mmol site/kg membrane (Table 3) corresponds to the 62.2 mmol site/kg PVC-COOH in Table 2.

This value could not be determined directly with regular composition membranes, cast with ∼33% PVC-COOH and ∼66% DOS because the proposed spectroscopic method requires that the ionophore concentration surpasses the charged fraction of the total COOH concentration. However, we could not use very high ionophore concentrations because they are incident to large absorbance values when the resolution of the spectroscopic method is crippled. Therefore, similar to the experiments shown in Figure 1, the site concentration was determined in PVC-HMW membranes with gradually increasing PVC-COOH content (0.14, 0.4, and 0.74%). In these experiments, the chromoionophore concentration was kept constant (1.26 mmol/kg), which determined the upper limit of the detectable site concentration. As expected, with increasing PVC-COOH content, the concentration of the protonated ionophore increased linearly. The charged site concentration was evaluated by extrapolating the site concentrations determined by 0.14, 0.4, or 0.74% PVC-COOH content to 33%. The extrapolation assumes that the degree of dissociation of COOH groups does not change considerably in the nonpolar, DOS-plasticized membranes.

As shown in Table 3, the charged site concentration can vary between 0.2 and 35% of the limiting value, the COOH group concentration in the membrane (143 mM). The site concentration in a valinomycin-loaded, PVC-COOH membrane was determined to be 5 times higher (1.48 mmol/kg) compared to the ionophore-free membrane (0.27 mmol/kg). In general, the degree of dissociation of the carboxylic groups is larger in polar, o-NPOE-plasticized membranes compared to nonpolar DOS-plasticized ones. Similarly, larger site concentrations were determined in membranes compounded with the highly basic ionophore (ETH 5294) compared to those cast with valinomycin. The limiting value is calculated from the carboxyl group concentration in carboxylated poly(vinyl chloride) (1.8%), the membrane composition, and the membrane density (1.064 g/mL DOS-plasticized membranes with 1:2 polymer-to-plasticizer ratio).

Determination of Positive Sites. Recently, polyurethane membranes challenged the dominance of PVC membranes in ion-selective electrodes[43,44] and there were contradicting papers on the charge sign of ionic sites in polyurethane membranes.[25,26]

In our studies, the Tecoflex SG85A revealed the absence of negative sites (Figure 4). Therefore, the different polyurethane membranes were screened for the presence of positive sites. The procedure used by Nägele and Pretsch[25] was adapted for the quantitative determination of positive sites in the solvent polymeric membranes. Lipophilic anions (e.g., KTFPB) were added to the membrane in excess to the intrinsic positive sites. Next this excess of negative sites was determined in the membrane quantitatively using the procedure described above. The concentration of positive sites in the polymer was calculated from the difference of lipophilic anion concentration incorporated into the membrane and the one determined from the spectroscopic experiment.

Values of 0.33 (RSD = 18%), 0.16 (RSD= 16%), 0.22 (RSD = 20%), and 0.02 mmol/kg (RSD = 39%) were obtained for Tecoflex SG85A, Tecoflex SG80A, Carbothane PC3575, and Tecothane 1074A, respectively. It should be noted that while the Tecoflex membranes were optically clear, the membranes made of Carbothane and Tecothane polymers demonstrated considerable light scattering.

Our spectroscopic method provided a significantly higher concentration of positive sites in the Tecoflex SG80A membrane (0.16 mM/kg) than the value (0.044 ± 0.006 mmol/kg) reported by Nägele and Pretsch. The reason for the discrepancy is not completely clear. Since different lots of Tecoflex SG80A were used in the two studies, it is possible that the fabrication technology of polyurethanes accounts for the difference. However, the use of o-NPOE as plasticizer by Nägele, can also be responsible for the divergent results. As we demonstrated above, o-NPOE contains a significant amount of anionic sites. The anionic impurities in o-NPOE (0.188 mmol/kg) can compensate for up to 0.376 mmol/kg positive sites in a polyurethane membrane with 65% o-NPOE and 32.5% Tecoflex content, as reported by Nägele. Since the negative sites of o-NPOE are gradually leaching out of the membrane, that makes it difficult to estimate the real concentration of positive sites in those experiments. However, the unaccounted negative sites of o-NPOE can only lower the experimentally determined positive site concentration.

CONCLUSION

A novel spectrophotometric method is proposed for the determination of ionic sites in plasticized polymeric membranes and membrane plasticizers. It is the simplest and least time-consuming among the existing methods. It allows fast and quantitative assessment of the concentration of fixed and mobile, negative and positive sites in solvent polymeric membranes and membrane plasticizers. The experimentally determined concentration of anionic impurities in high molecular weight PVC correlated well with the results of earlier methods. The range of materials that can be investigated by this method is likely to extend beyond those of potentiometric importance. However, the method is limited to materials (polymers, plasticizers, etc.) that are transparent in the visible spectral range.

(43) Cha, G. S.; Liu, D.; Meyerhoff, M. E.; Cantor, H. C.; Midgley, A. R.; Goldberg, H. D.; Brown, R. B. *Anal. Chem.* **1991**, *63*, 1666−1672.
(44) Lindner, E.; Cosofret, V. V.; Ufer, S.; Buck, R. P.; Kao, W. J.; Neuman, M. R.; Anderson, J. M. *J. Biomed. Mater. Res.* **1994**, *28*, 591−601.