this case. At higher concentrations there is barely sufficient conjugate to saturate all the immobilized antigen. With a second injection of the conjugate, the linear dynamic range can be increased by about 40% over a single injection double the size. The two-injection method also appears to decrease nonspecific interactions. An alternative to this would be to decrease the flow rate. However this also increases the probability of nonspecific interactions.

Experiments carried out using the same apparatus (*8*) indicate that the turnover of the enzyme is limited by mass transfer of oxygen to the immobilized conjugate at higher conjugate loadings. This can be alleviated to a significant extent by the use of oxygenated buffers, which lead accordingly to better detection limits.

It should be noted (Figure 4) that three injections of glucose were made to determine the amount of immobilized conjugate. If a calibration curve were prepared by using peak area from the first glucose injection, the correlation coefficient would be 0.900. This peak is approximately the same area as the combined area of peaks obtained for the second and third injections. The correlation coefficient and the accuracy increase when the area of the second or third injection is employed. The higher response for the first injection which is indicative of weakly or nonspecifically bound conjugate which is apparently displaced by the injection of glucose and the passage of hydrogen peroxide through the column. As mentioned before, the reactor regeneration step should be carried out with precise timing. The pH 2.0 buffer causes reversible denaturation of the bound antibody. Renaturation and restoration of the proper protein microenvironment takes time. The time allotted for this process has a strong effect on the precision of the measurement. For example if one waits 10 min, the precision is ±2–4%, whereas shortening the time to 5 min would give ±5–10% precision.

A sandwich assay has been employed to determine 1 amol (*10*) of substance. This involves an incubation time of 3 h. The possibility of improving the sensitivity of our system to this limit is being explored at the present time. This would involve slowing the flow rate, using more highly purified conjugate, increasing the detector sensitivity, and the utilizing oxygen-saturated buffers.

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Barium-Selective Electrodes Based on Neutral Carriers and Their Use in the Titration of Sulfate in Combustion Products

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Highly lipophilic, electrically neutral ionophores for Ba^{2+}-selective solvent polymeric membrane electrodes have been prepared. Sensors based on such ionophores exhibit a rejection of alkali metal ions of about 10^3. They are suitable for the titration of SO_4^{2-} with Ba^{2+} in nonaqueous systems. More than 100 titrations may be performed with no loss in the electromotive behavior of the electrodes.

Several Ba^{2+}-selective electrodes based on neutral carriers have been described (*1–5*). They are of considerable interest in the titration of sulfate ions with barium ions (*1–6*). It has been claimed (*3, 5*) that such sensors have very long lifetimes; however this still remains a limiting factor especially when titrations are performed in nonaqueous solvents. The lifetime of such an electrode depends to a large extent on the lipophilicity of the membrane components (*7*). In order to achieve a lifetime of 1 year for such sensors to continual use with a flowing aqueous sample, a partition coefficient, K, of the carrier between aqueous phase and membrane phase of about 10^5 to 10^6 is required (*7*). The carriers N,N,N',N'-tetraphenyl-3,6,9-trioxaundecanediamide (ETH 231) (*5*) and antarox CO-880 (*3*) used previously have partition coefficients of about $10^{2.6}$ and 10^3, respectively. We therefore designed Ba^{2+}-selective ionophores with considerably higher lipophilicities (*8*). We now report further representatives of such ionophores, the characteristics of the most attractive sensors based on these ionophores, and their use in the titration of sulfate.

EXPERIMENTAL SECTION

Reagents. All electrolyte solutions for the potentiometric measurements were prepared with doubly quartz distilled water and chlorides of high purity (pro analysis, E. Merck, Darmstadt, GRF; puriss. p.a., Fluka AG, Buchs, Switzerland). Poly(vinyl chloride), *o*-nitrophenyl octyl ether, dithiooxamide, L-cystein, DL-cystin, and sulfanilic acid were obtained from Fluka AG, Buchs, Switzerland. Dibenzyl disulfide, sulfonal, sulfamic acid, phenylthiourea, and S-benzylthiuronium chloride were from BDH Chemicals, Ltd., Parkstone, Poole, England. For ligand 2 see ref 8.

Synthesis. 2-{(2-[(-Dicyclohexylcarbamoyl)methoxy]phenoxy)ethoxy}-*N*,*N*-dicyclohexylacetamide (ligand 1). A 3.08-g portion (20.0 mmol) of 2-(2-hydroxyphenoxy)ethanol (*9*) in 50 mL of absolute benzene was slowly added to a suspension of 1.20 g (40.0 mmol) of NaH (80 wt %) in 30 mL of benzene. The solution was stirred for 30 min at room temperature. A solution of 12.08 g (40.0 mmol) of 2-bromo-*N*,*N*-dicyclohexylacetamide (*8, 10*) in 50 mL of benzene was then added dropwise to the solution over a period of 30 min. After the mixture was refluxed for 6 h, the NaBr was filtered off and the solvent was evaporated in vacuo. The residue was dissolved in $CHCl_3$, washed twice with 2 N NaOH and water, and dried over Na_2SO_4. After evaporation of the solvent and recrystallization from acetone 8 g of the pure product was obtained (mp 114–116 °C). Anal. Calcd for $C_{36}H_{56}N_2O_5$ (596.8): C, 72.44; H, 9.45; N, 4.69. Found: C, 72.78; H, 9.65; N, 4.68.

Bis(*N*,*N*-dicyclohexyl)-1,2-phenylenebis(oxy-2,1-ethanediyl)-bis(oxyacetamide) (ligand 3) was obtained by using the procedure described above: 1.98 g (10 mmol) of 1,2-bis(2-hydroxyethoxy)benzene (*11*), 6.04 g (20.0 mmol) of 2-bromo-*N*,*N*-dicyclohexylacetamide (*8, 10*), and 0.96 g (20 mmol) of NaH (50 wt %). The product was recrystallized from acetone (2.1 g of pure product, mp 105–107 °C). Anal. Calcd for $C_{38}H_{60}N_2O_6$ (640.9): C, 71.21; H, 9.43; N, 4.37. Found: C, 71.01; H, 9.61; N, 4.34.

1,2-Phenylenedioxybis(1,2-phenyleneoxy-*N*,*N*-dicyclohexylacetamide) (ligand 4) was obtained by using the procedure described above: 1.20 g (4.00 mmol) of pyrocatechol-bis(2-hydroxyphenyl) ether (*12*), 2.40 g (8.00 mmol) of 2-bromo-*N*,*N*-dicyclohexylacetamide (*8, 10*), and 0.24 g (8.00 mmol) of NaH (80 wt %). After chromatography on silica gel 60 (0.063–0.1 mm, Merck AG, Darmstadt, GFR) with chloroform as eluent, the product was recrystallized from ethanol and yielded 0.5 g of colorless crystals (mp 134–137 °C). Anal. Calcd for $C_{46}H_{60}N_2O_6$ (737.0): C, 74.51; H, 8.22; N, 3.77 (contains 0.25 mol of H_2O). Found: C, 74.40; H, 8.41; N, 3.74.

Electrode System. Cells of the type

Hg; Hg_2Cl_2, KCl (satd)|3 M KCl|sample solution||
membrane||internal filling solution, AgCl; Ag

were used at 20–21 °C. The external reference electrode was a double junction calomel electrode, Philips R44/2 SD-1 (N.V. Philips Gloeilampenfabrieken, Eindhoven, Netherlands). The internal filling solution was 10^{-2} M $BaCl_2$ for titrations and 10^{-1} M $BaCl_2$ for all other measurements.

Technical details of the emf measurements, computation of activity coefficients, corrections for liquid junction potentials, and evaluation of the selectivity factors using the separate solution technique (*13*) on 10^{-1} M solutions of the chlorides have been described (*5, 14, 15*). Membranes consisting of about 33 wt % poly(vinyl chloride) (PVC), about 66 wt % *o*-nitrophenyl octyl ether (*o*-NPOE), 1 wt % ligand, and 67 mol % (relative to the ligand) potassium tetrakis(*p*-chlorophenyl)borate (KT*p*ClPB) were prepared as described in ref 16.

Titrations. A Titroprocessor 636 (program version 102) and a Dosimat 635 of Metrohm AG (Herisau, Switzerland) were used. The buret volume was 5 mL; the titration solution (10^{-2} M $Ba(ClO_4)_2$ in 2-propanol) was added in steps of 25 μL; the emf was taken 5 s after each addition.

The combustions were performed according to the procedure originally proposed by Schöniger (*17*). The products were absorbed in 4 mL of distilled water to which 0.1 mL of H_2O_2 (30 wt %) was added and the mixture was shaken for 30 min. Before titration 25 mL of 2-propanol (30 vol %)/water and 50 μL of lithium acetate (1 M) were added to the absorption solution.

Lipophilicity Determinations. Lipophilicities (defined as the logarithm of the partition coefficient *P* between 1-octanol and water) were measured by a reversed-phase thin layer chromatography technique (*18–20*) using RPTLC-plates type KC18F (Whatman, Clifton, NJ), and 70 vol % and 90 vol % ethanol were used as mobile phase. By use of a series of calibration substances (see ref 21) the log *P* values were determined by interpolation or extrapolation of the linear calibration curve $\log P = f(R_M)$ where $R_M = \log(1/R_F - 1)$.

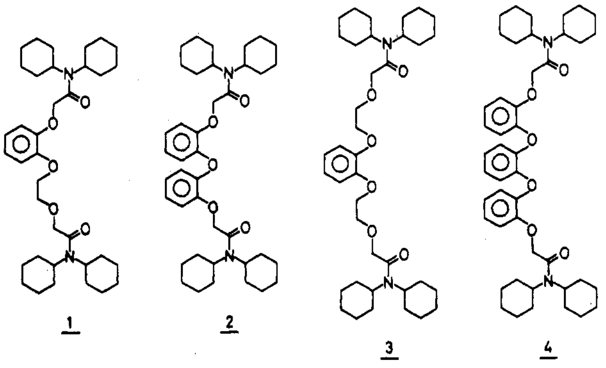

Figure 1. Constitutions of the carriers discussed.

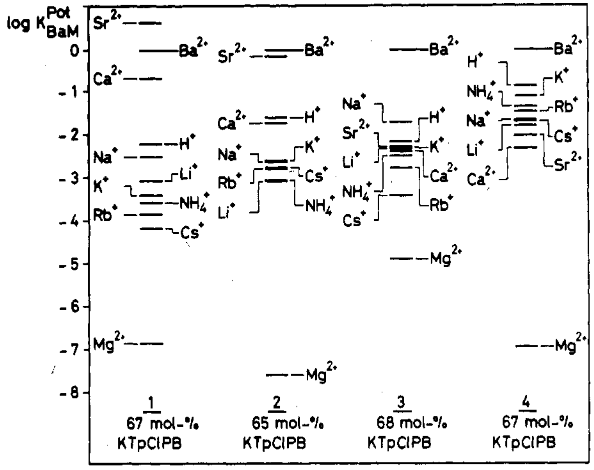

Figure 2. Comparison of the selectivity coefficients obtained with 0.1 M solutions of the chlorides on membranes with the carriers **1** to **4**.

Table I. Lipophilicity (log P_{TLC}) of Ionophores and Slope (*s*) of Electrode Response

ionophore	log P_{TLC}[a]	*s*,[b] mV
1	7.8 ± 0.5	30.1 ± 0.6
2	8.2 ± 0.4	29.7 ± 0.6
3	8.0 ± 0.6	29.9 ± 0.4
4	8.7 ± 0.5	28.8 ± 1.8
ETH 231	2.6 ± 0.3	
Antarox CO-880	(2.6–3.4) ± 0.3	

[a] Precision given: 95% confidence limits. [b] Theoretical value 29.1 mV (20 °C). Range: $10^{-5.5}$ to 10^{-1} M $BaCl_2$ (*N* = 5); standard deviation of slope is given. Membranes: 1 wt % ionophore, 33 wt % PVC, 66 wt % *o*-NPOE, ~67 mol % KT*p*ClPB (relative to ionophore).

RESULTS AND DISCUSSION

The selectivities induced by the ionophores 1–4 (Figure 1) in solvent polymeric membranes are given in Figure 2. Membranes based on 1 show a slight preference for Sr^{2+} whereas those based on 2–4 lead to sensors with selectivity for Ba^{2+} over all alkali and alkaline-earth cations. With all these membranes, the slopes of the electrode response are close to theoretical (Table I) and the lipophilicities are more than adequate for a lifetime of more than 1 year's continual use in aqueous sample solutions (*7*). The lipophilicities of the new ionophores are 10^4 to 10^5 times higher than those of the previously (*3, 5*) used ion carriers. Although 2 gives a poorer rejection of Sr^{2+} and Ca^{2+} than 3 and 4, membranes based on 2 are more attractive for the assay of sulfur in organic compounds by titration of sulfate with $Ba(ClO_4)_2$. This is due to

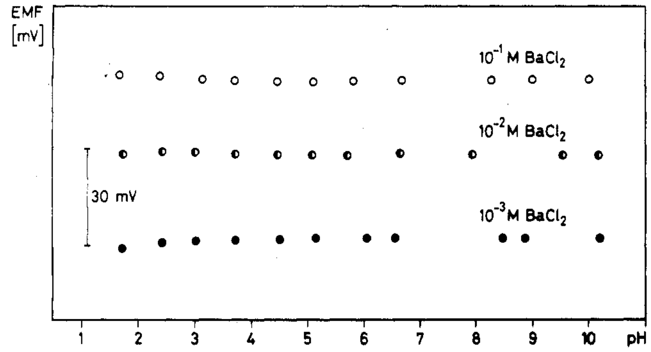

Figure 3. Dependence of the emf of a cell assembly with **2** on the pH of the sample solution for different $BaCl_2$ concentrations. The pH was adjusted by adding HCl and NaOH, respectively. No corrections for liquid junction potentials and activity changes were applied.

Table II. Titration of SO_4^{2-} with Ba^{2+}

sample,[a] μL (0.1 M H_2SO_4)	consumption,[b] mL (0.01 M $Ba(ClO_4)_2$ in 2-propanol)
150	1.503 ± 0.007 (*n* = 27)
25	0.257 ± 0.002 (*n* = 3)
15	0.145 ± 0.005 (*n* = 3)

[a] In 20 mL of 2-propanol (30 vol %)/water and 2.5 mM lithium acetate. [b] Number of titrations, *n*.

their higher selectivity over Na^+ and K^+. Previously reported sensors based on antarox CO-880 have selectivities (K_{BaM}^{Pot}) of 10^{-1} to 6×10^{-3} for M = Na^+, (1-2) × 10^{-2} for M = K^+, and 2×10^{-3} to 6×10^{-4} for M = Ca^{2+} (*3*). Selectivities of membranes based on **2** are superior in respect to Na^+ and K^+ but are poorer in respect to Ca^{2+}.

Electrodes with **2** as the ion-selective component have been studied in somewhat greater detail. The emf of such cell assemblies is only slightly affected by changes in the pH of the sample solution, within the range pH 1.5 to 10 (Figure 3). In aqueous $BaCl_2$ solutions the detection limit is $10^{-5.5}$ M. When the Ba^{2+} concentration is increased by a factor of 10 from 10^{-4} to 10^{-3} M, the final emf of the cell is reached within 0.3 mV in less than 1 min. In 0.1 M $BaCl_2$ solutions a drift of the emf of ≤0.05 mV/h was observed (20–21 °C).

The curve obtained by automatic titration of a 7.5×10^{-4} M solution of H_2SO_4 in 30 vol % 2-propanol/water ~2.5 mM aqueous lithium acetate buffer (pH ~4) with 10^{-2} M $Ba(ClO_4)_2$ in 2-propanol is shown in Figure 4. Table II shows that the standard deviation of single determinations for titrations of this concentration of SO_4^{2-} is 0.5%.

A determination of the partition coefficient of the ionophore between 30 vol % 2-propanol/water and the membrane phase and a subsequent assessment of the membrane lifetime based on available models (*7*) is difficult to achieve. However after more than 100 titrations, no significant loss in the slope of the electrode response was detectable.

Schöniger combustion (*17*) of 18 dibenzyl disulfide samples (1.3–3.6 mg, 26.03 wt % S) followed by titration of the products with 10^{-2} M $Ba(ClO_4)_2$ in 2-propanol showed a relative accuracy of the mean of 0.9% corresponding to an absolute accuracy in the assay of S of 0.2%. Linear regression of the consumed volume on the theoretical volume of $Ba(ClO_4)_2$ gave the following values: intercept, 0.03 ± 0.01 mL; slope, 0.989 ± 0.006; residual standard deviation, 0.015 mL; regression coefficient, 0.9994; regression range, 1.060–2.899 mL. On the basis of this calibration a total of 25 samples of *sulfonal*, *aminosulfonic acid*, phenylthiourea, *S*-benzylthiuronium chloride, dithiooxamide cystein, cystin, and *p*-aminobenzenesulfonic acid (0.5–3.8 mg) were combusted and the products titrated as described above. The mean absolute accuracy in the assay of the 25 samples was 0.14% (linear regression of found values on theoretical values: intercept, 0.000 ± 0.005 mL; slope, 1.000 ± 0.003; residual standard deviation, 0.011 mL; regression coefficient, 0.99987; regression range, 0.380–2.908 mL.

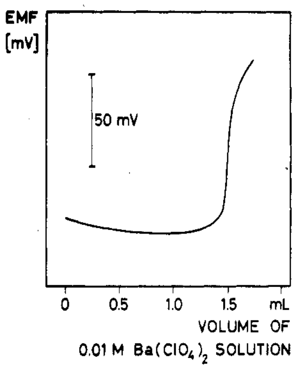

Figure 4. Titration of 20 mL of 7.5×10^{-4} M H_2SO_4 (30 vol % 2-propanol; ~2.5 mM lithium acetate; pH ~4) with 10^{-2} M $Ba(ClO_4)_2$ in 2-propanol.

In the Schöniger combustion technique, H_2O_2 is added to the absorption solution, in order to oxidize SO_2 to H_2SO_4 (*17*). If a Pb^{2+} selective solid membrane electrode is used as end point indicator (*22*), the excess H_2O_2 has to be removed before the titration. Since no interference from H_2O_2 was observed with the Ba^{2+}-selective liquid membrane electrode, the titrations can be carried out in the presence of the excess H_2O_2. This simplifies the overall procedure significantly.

Registry No. **1**, 98015-30-6; **2**, 96476-01-6; **3**, 98015-31-7; **4**, 98015-32-8; Ba, 7440-39-3; SO_4^{2-}, 14808-79-8; sulfur, 7704-34-9; 2-(2-hydroxyphenoxy)ethanol, 4792-78-3; 1,2-bis(2-hydroxyethoxy)benzene, 10234-40-9; pyrocatechol-bis(2-hydroxyphenyl) ether, 53181-24-1; 2-bromo-*N*,*N*-dicyclohexylacetate, 54700-62-8.

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