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High-Performance Liquid Chromatographic Determination of Naphtholsulfonic Acids

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Abstract

A HPLC-method for the quantitative analysis of mixed solutions of 2-naphthol-5,7-disulfonic acid and 2-naphthol-7-sulfonic acid was developed. A complete reproducible separation of these compounds in a very short time could be reached by using a reversed-phase column.

Introduction

Naphtholsulfonic acids are manufactured in large quantities as intermediates for the synthesis of azo dyes. Only few methods are known to carry out the analysis of mixtures of sulfonic acids by high-performance liquid chromatography (HPLC) [1–3]; usually ion-exchangers are used as column packings to separate such mixtures. This paper deals with the separation and quantitative determination of two naphtholsulfonic acids (2-naphthol-5,7-disulfonic acid and 2-naphthol-7-sulfonic acid) in aqueous solution on a reversed-phase column packing.

Experimental

Materials

2-naphthol-7-sulfonic acid was recristallyzed twice from water and once from ethanol; 2-naphthol-5,7-disulfonic acid and 2-naphthalenesulfonic acid (the latter to be used as internal standard, see following section) were recristallyzed three times from water. Thin-layer chromatographic analysis of each of the sulfonic acids showed the content of aromatic byproducts to be less than 0.5%. The sodium salts of the sulfonic acids were used for the analysis tests; distilled water and reagent grade organic solvents were used for the mobile phases.

Equipment

The liquid chromatograph consisted of a diaphragm reciprocating pump (Orlita DMP), a capillary tubing followed by a Bourdon-tube as a pulse-dampening device, a pre-column (25 cm × 5 mm) filled with Cellite® for the filtration of the mobile phase, an injection port, the separating column (25 cm × 7 mm) and a spectrophotometer (Perkin-Elmer LC 55) as detector. The separating column was slurry-packed with μ-Bondapak® C 18 (fully porous particles with a chemically bonded layer of octadecylsilane). The analysis samples (1.5 μl) were injected with a microsyringe.

Results

As the naphtholsulfonates are only weakly retained onto the lipophilic stationary phase, we attempted the use of strongly polar solvent systems as mobile phases. A complete separation of the two naphtholsulfonates in a very short time (less than 5 min) was obtained using a methanol-water mixture (10% methanol). The chromatograms were evaluated quantitatively by the internal standard technique [4]. Several carboxylic acids were first tested as standards, but they had to be discarded since they showed serious band broadening and/or tailing. Good results were obtained by using sodium 2-naphthalenesulfonate: Fig. 1 shows the chromatogram of a solution of 2-naphthol-5,7-disulfonate (0.13 mg/cm³, peak 1), 2-naphthol-7-sulfonate (0.13 mg/cm³, peak 2) and 2-naphthalenesulfonate (0.12 mg/cm³, peak 3). As all three compounds were eluted close to each other with high symmetrical peaks, the peak-height rations were measured for the quantitation of the chromatograms; the content of naphtholsulfonate

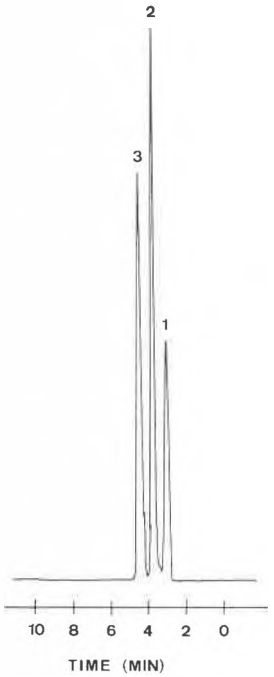

Fig. 1: Separation of the sodium sulfonates on μ-Bondapak C 18. Mobile phase, water/methanol (9/1); flow rate, 1.2 cm³/min; pressure, 25 bar; detection, UV at 228 nm.
(1) 2-naphthol-5,7-disulfonate
(2) 2-naphthol-7-sulfonate
(3) 2-naphthalenesulfonate

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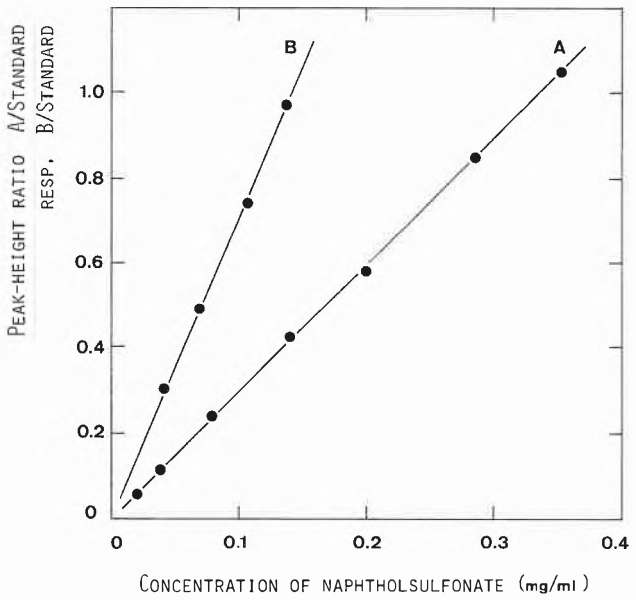

Fig. 2: Calibration curves for 2-naphthol-5,7-disulfonate (A) and for 2-naphthol-7-sulfonate (B).

was determined from corresponding calibration curves (Fig. 2). This method proved to be rather unsensitive to the variations which occur when injecting the samples by hand with a microsyringe; the analysis of 4 samples with a content of 0.16 mg/cm³ 2-naphthol-7-sulfonate showed a standard deviation $\sigma = 1.9\%$.

Acknowledgement

The authors wish to thank Dr. J. Schreiber for his advice during this work.

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Selective Transport, Coupled to a Counterflow of Protons, of Ions Across Neutral Carrier Membranes *

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Abstract

Neutral carrier membranes can transport ions with high selectivity. A specific transport of calcium ions under zero-current conditions has been realized by adding proton carriers to the membrane phase and exposing it to a *p*H gradient. The reported experimental results are in accord with a simple theory.

Neutral ionophores such as valinomycin [1, 2] have been reported to selectively complex ions and to transport them across natural membranes [3], artificial bilayers [4], or bulk membranes [5] when a transmembrane potential gradient is applied. Similar behavior is observed for a series of synthetic ionophores of various selectivities [5, 6]. This was confirmed by electrodialysis experiments on carrier membrane cells where, as a rule, the most preferred cation is transported with a transference number of close to unity [5]. The fact that the total electrical current is carried by complexes of this ion documents the highly selective nature of this cation transport.

Antibiotics of the monensin/nigericin group [1] undergo deprotonation at *p*H values in the physiological range and therefore constitute electrically charged ligands. Metal ions having been complexed can easily be carried across membranes by a direct coupling of their flux to a counterflux of protons induced by a transmembrane *p*H gradient [7]. The same mechanism of zero-current ion transport is not possible for uncoupled neutral carrier membrane systems because interactions between uncharged ionophores and hydrogen ions are virtually absent.

Here we report on neutral-carrier-mediated ion transport driven by a transmembrane *p*H gradient. Coupling between ion flux and driving force (*p*H gradient) was achieved by adding a proton carrier. This permits the whole range of ion specificity of neutral carriers to be utilized in zero-current ion transports.

To elucidate the experimental results presented in this work, we shall first discuss the simplified membrane model outlined in Fig. 1. Here it is assumed that the uptake of cations I^{z+} into the lipophilic membrane is facilitated by the formation of 1:n complexes with neutral carriers, IS_n^{z+}, and by subsequent association between these cationic complexes and negatively charged ligands R^-. The species IS_nR_z thus formed are transported across the interior of the membrane. The primary energy source for this transport under zero-

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current conditions comes from the simultaneous countertransport of a second sort of ions J^+ (e.g. protons being driven by a *p*H gradient) which are carried through the barrier as complexes JR. Since there exists a closed-circuit flux of the carriers S and R^- (e.g. proton carriers) within the membrane, the net transport of ions I^{z+} is finally coupled to the facilitated diffusion of species J^+.

The countertransport system shown schematically in Fig. 1 is similar to the membrane systems reported by *Cussler* et al. [7] and others [8], except that here neutral carriers are introduced as the cation-specific principles. In fact, the theoretical description of the ion fluxes turns out to be basically the same as was set forth by *Cussler*'s group [7] (for z = 1 and n = 0) and was extended by *Erne* et al. [8]. The only formal difference consists in that here the distribution of the cations I^{z+} across the membrane/solution interfaces will be characterized by a complex distribution parameter, $K_i = \beta_{is} c_s^n k_i$, where β_{is} is the stability constant of the complexes IS_n^{z+}, c_s the concentration of free carriers S in the membrane, and k_i the distribution coefficient of free cations I^{z+} [9]. Accepting the model assumptions specified earlier [8, 10], we obtain the following relations for the steady-state fluxes of monovalent cations (z = 1):

$$J_i = \frac{D\, c_r^{tot}}{d} \frac{a_i'}{a_i' + K_{ij}\, a_j'} - \frac{D\, c_r^{tot}}{d} \frac{a_i''}{a_i'' + K_{ij}\, a_j''} \quad (1)$$

$$J_j = -J_i \quad (2)$$

In Equation (1) the flux density J_i of ions I^+ across the membrane is given as a function of the ion activities a_i and a_j present in the external solutions (′ resp. ″, see Fig. 1). Evidently, the flux is proportional to the diffusion coefficient D, to the total concentration c_r^{tot} of anionic sites in the membrane, as well as to the reciprocal of the membrane thickness d. The factor K_{ij} can be considered to be a measure of the selectivity of the membrane for the ion J^+ relative to the ion I^+ and is given as

$$K_{ij} = \frac{K_{jr}\, k_j}{K_{ir}\, K_i} \quad (3)$$

where K_{jr} is the stability constant of the complexes JR, k_j is the distribution coefficient of free cations J^+, and K_{ir} is the equilibrium constant for the association reaction between cationic species IS_n^+ and ligands R^-. The corresponding results for a divalent-ion carrier transport system (z = 2) read:

$$J_i = \frac{D\, c_r^{tot}}{2\, d} \left[\sqrt{1 + \frac{1}{4} K_{ij} \frac{a_j'^2}{a_i'}} - \sqrt{\frac{1}{4} K_{ij} \frac{a_j'^2}{a_i'}} \right]^2 - \frac{D\, c_r^{tot}}{2\, d} \left[\sqrt{1 + \frac{1}{4} K_{ij} \frac{a_j''^2}{a_i''}} - \sqrt{\frac{1}{4} K_{ij} \frac{a_j''^2}{a_i''}} \right]^2 \quad (4)$$

$$J_j = -2\, J_i \quad (5)$$

$$K_{ij} = \frac{(K_{jr}\, k_j)^2}{2\, c_r^{tot}\, K_{ir}\, K_i} \quad (6)$$

It may be recognized that the decisive parameter K_{ij} can generally be identified with the equilibrium constant of the basic ion-exchange reaction

$$IS_nR_z(m) + z\, J^+(aq) \rightleftarrows I^{z+}(aq) + z\, JR(m) + n\, S(m)$$

Accordingly, the value of K_{ij} is expected to decrease (a) with increasing selectivity of the neutral carriers S for the cations I^{z+}, and (b) with decreasing affinity of the negatively charged carriers R^- for the counterions J^+, i.e., with decreasing pK_A value in the case of proton carriers. For an efficient ion pumping, the ligand R^- should be a relatively weak complex-former. Indeed, if the value of K_{ij} were too high, the membrane would be capable of pumping only a small number of ions. The species JR would then become predominant throughout the membrane, which, in terms of Equations (1) and (4), corresponds to the situation where $a_i' << K_{ij} a_j'^z$ and $a_i'' << K_{ij} a_j''^z$. A too low value of K_{ij}, on the other hand, would be deleterious in that the species IS_nR_z would tend to predominate in the membrane. Again, this would heavily reduce the membrane-internal concentration gradients which are a prerequisite for diffusion. The predicted trends are nicely illustrated in Fig. 2 where theoretical results are given for the countertransport system 10^{-3}M I^{z+} | membrane | 10^{-3}M J^+. The activity a_i'' on the side where the cations I^{z+} leave the carrier membrane was computed by using the conditions

$$V \frac{da_i''}{dt} = A\, J_i \quad (7)$$

and

$$V \frac{da_j'}{dt} = -A\, J_j \quad (8)$$

V is the volume of the electrolyte compartments on each side of the membrane, and A is the active area of the membrane. Fig. 2 clearly demonstrates that membrane systems of the type shown in Fig. 1 will definitely

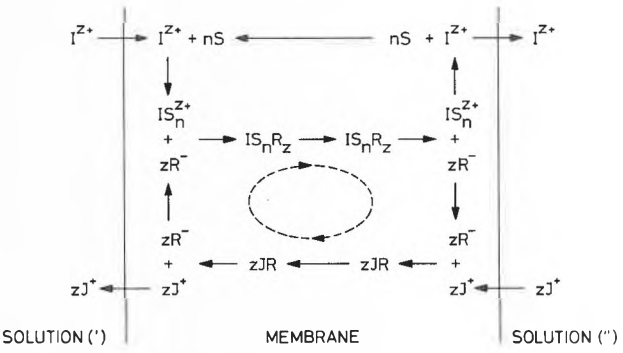
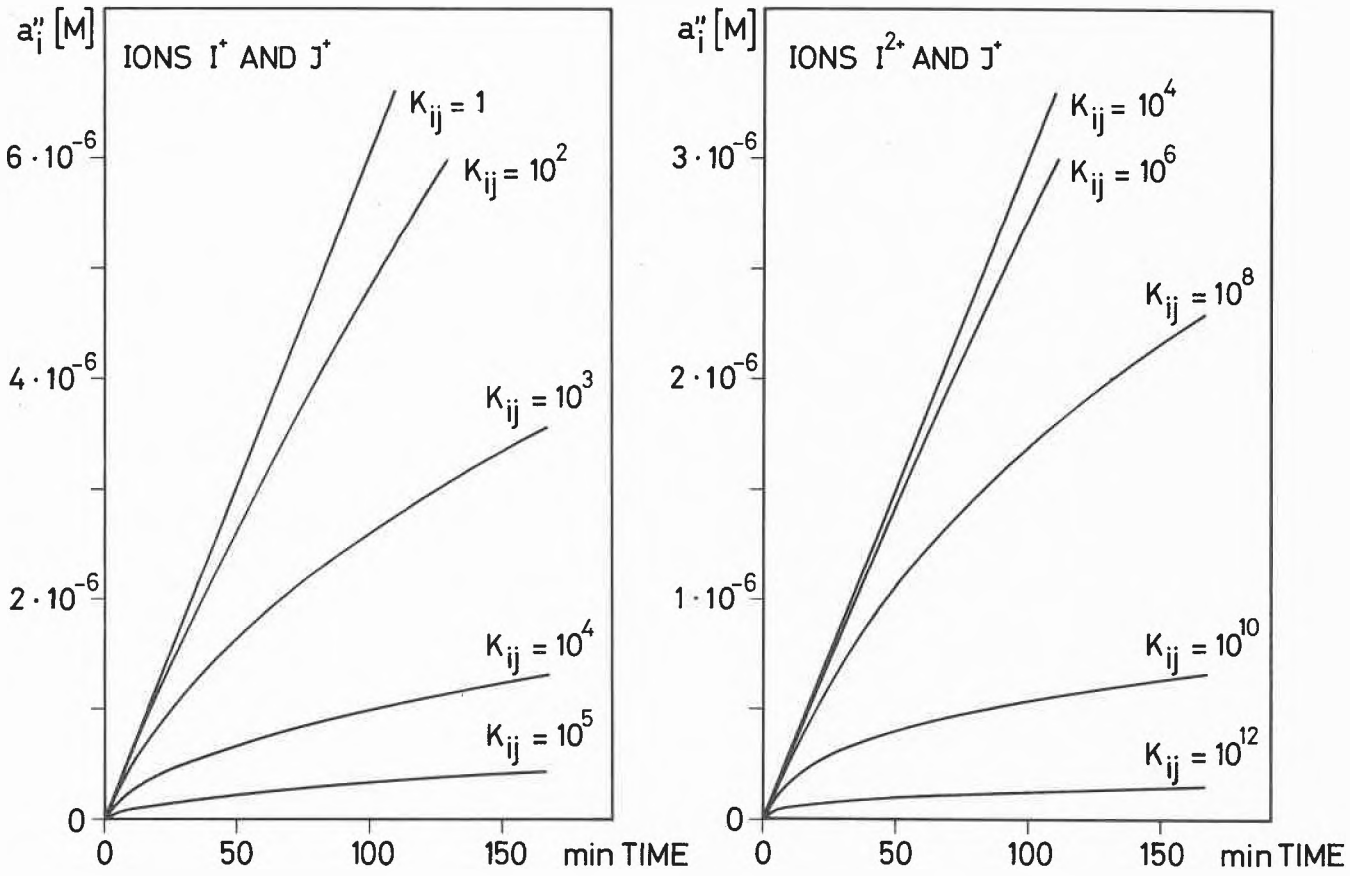
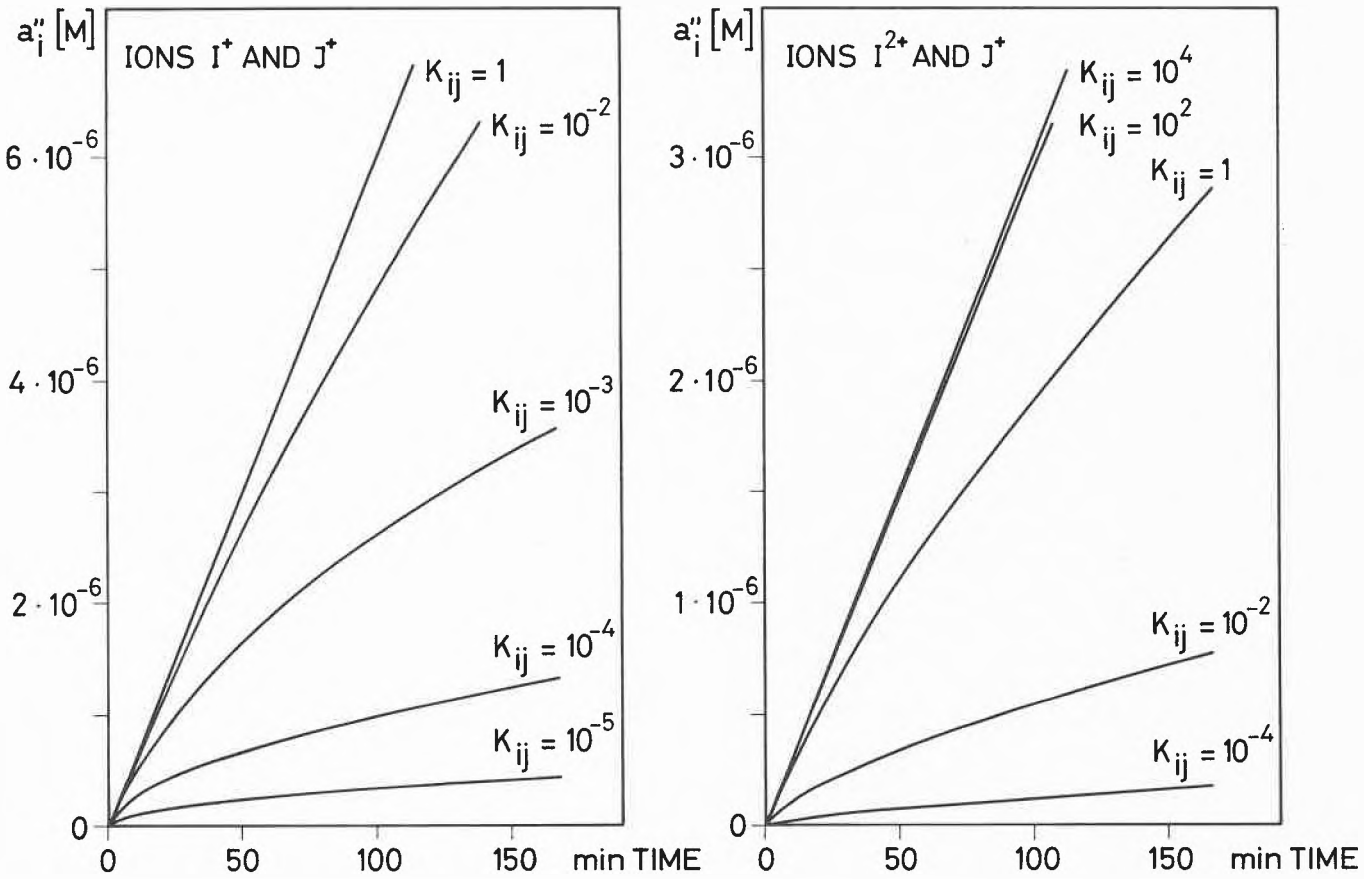


Fig. 1: Mechanism of Coupled Ion Transport Across Neutral Carrier Membranes.



a: Effect of high values of K_{ij} on the transport rate.



b: Effect of low values of K_{ij} on the transport rate.

not transport large amounts of ions if the values of the parameter K_{ij} are either too high or too low. In contrast, maximal transport rates, given by $A\ J_i = A\ D\ c_r^{tot}/z\ d$ [mol s^{-1}], can be realized for intermediate values of K_{ij}.

To examine the theoretical predictions, experiments were carried out on solvent polymeric membranes containing the calcium-specific neutral carrier **1** [9] and various proton carriers (uncouplers of oxidative phosphorylation, **2**–**5**, or tetraphenylborate **6**, see Fig. 3). The membrane compositions were ~1 wt.-% ligand **1**, ~0.4 wt.-% of compound **2**, **3**, **4**, **5**, or **6**, ~64 wt.-% o-nitrophenyl octyl ether, and ~34.5 wt.-% PVC [12]. Each membrane (active area: 0.2 cm^2, thickness: 0.0015 cm) was interposed between two aqueous electrolyte solutions of 20 cm^3 volume. One solution contained 10^{-2}M $CaCl_2$ and $4 \cdot 10^{-4}$M KOH (pH = 10.5), and the other solution was 10^{-2}M KCl and 10^{-3}M HCl (pH = 3.0). The concentrations of Ca^{2+} ions arising in the acidic solution were determined by flameless atomic absorption spectroscopy [12].

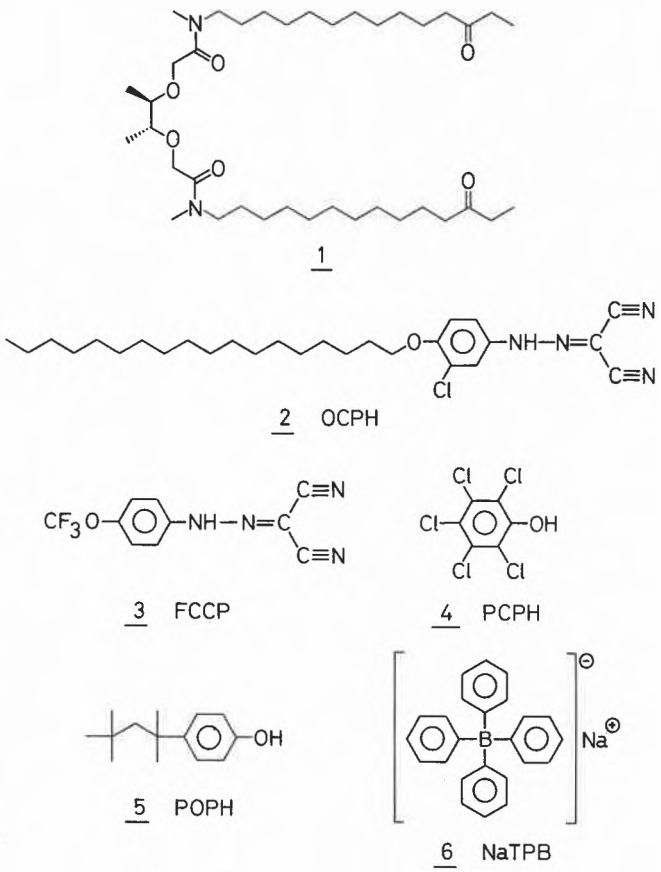


Fig. 3: Structures of the Ligands Discussed.

◀ *Fig. 2:* Calculated Ion Transport for the System 10^{-3}M I^{z+} | membrane | 10^{-3}M J^+.
The activities a_i'' were calculated as a function of the time using $A/V = 10^{-2}$ cm^{-1}, $D = 10^{-7}$ cm^2 sec^{-1}, $d = 10^{-2}$ cm, and $c_r^{tot} = 10^{-2}$ M [11].

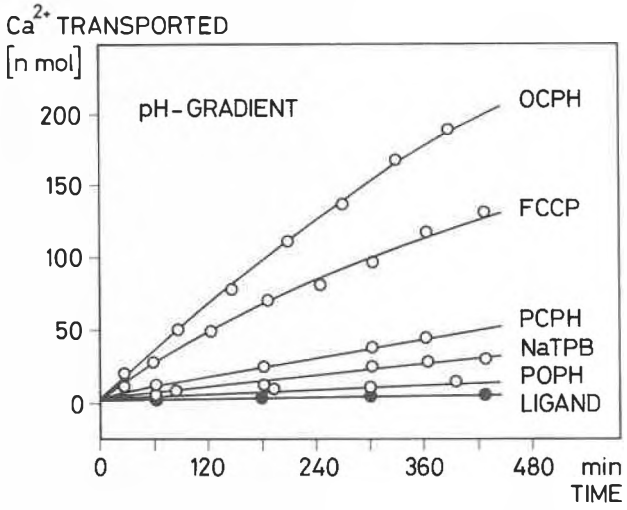


Fig. 4: Ca^{2+}-Transport Induced by a pH Gradient. Results for membranes containing ligand **1** and lipophilic components **2** to **6** are indicated by open circles. Full circles denote results obtained in the presence of ligand **1** only.

Fig. 4 shows the results obtained for the different membranes. The number of nanomoles of transported Ca^{2+} ions is given as a function of the time. Evidently, the highest transport rates were achieved when the membrane contained proton carriers having a pK_A value of around 6 (OCPH: 6.1 [13], FCCP: 5.7 [14]). The use of either more basic ligands (POPH: $pK_A \cong 10.0$ [12]) or of ligands that bind protons more weakly (PCPH: 5.0 [14], NaTPB) led to a considerable slowing of the calcium countertransport. These remarkable findings are in accord with the theoretical curves in Fig. 2; the corresponding K_{CaH} values are expected to decrease in the order POPH > > OCPH $\gtrsim$ FCCP > PCPH > > TPB.

One curve in Fig. 4 refers to a solvent polymeric membrane containing the calcium-selective ligand **1** only. This experiment clearly indicates that the unmodified neutral carrier membrane is not capable of pumping ions under zero-current conditions at all. Hence, the simultaneous presence of a proton carrier proves to be indispensable for the coupling of the ion fluxes, as has been suggested in Fig. 1. For evident reasons, a high pH gradient across the membrane will favor the coupled transport. Fig. 5 confirms that the absence of this driving force indeed results in a serious reduction of the ion fluxes. In this experiment, the diffusion of calcium ions (and the concomitant counterflow of protons) was driven solely by the Ca^{2+}-concentration gradient.

To demonstrate the pronounced ion specificity of the "ion pump" developed here, experiments were carried out using the Ca^{2+}-carrier/FCCP membrane in contact with 10^{-2}M solutions of different metal chlorides MCl_z (pH = 10.5). The acid solution on the other side of the membrane was the same as in Fig. 4. The results of these experiments are given in Fig. 6. Whereas the

membrane was found capable of pumping a considerable number of Ca^{2+} ions, virtually no transport could be realized for Ba^{2+}, Mg^{2+}, or Na^+ ions. The attempts at transporting K^+ across the same membrane were also unsuccessful [12]. The ion selectivity documented in Fig. 6 can again be rationalized on the basis of the model discussed before. It was shown in Fig. 2a that a too high value of the parameter K_{MH} can severely limit the transport process. Now, this decisive parameter is related to the value K_{CaH} as follows:

$$K_{MH} = (K_{CaH}/K_{CaM})^{z/2} \quad (9)$$

where K_{CaM} characterizes the selectivity of the carrier membrane for the ion M^{z+} relative to Ca^{2+}. Since the reported selectivities of membranes based on ligand **1** are on the order of $K_{CaBa} < 10^{-3}$ and $K_{CaM} \approx 10^{-5}$ ($M^{z+} = Mg^{2+}$, Na^+, or K^+) [9], one immediately finds that $K_{MgH} > K_{BaH} >> K_{CaH}$. Hence the observed ion fluxes should reflect the selectivity sequence $Ca^{2+} >> Ba^{2+} > Mg^{2+}$ (see Fig. 2a), which fact is corroborated by the experimental results. By similar arguments, one can give a cogent explanation for the selectivity order $Ca^{2+} >> Na^+, K^+$ that was realized in the present ion transport experiments.

In conclusion, we have demonstrated that highly selective ion transports can be performed on neutral carrier membranes under zero-current conditions. This was accomplished by adding a lipophilic weak acid (proton carrier) to the membrane phase and by applying a transmembrane *p*H gradient. It is conceivable that membrane systems of the type introduced here may be exploited for selective ion separations, so much the more since at present neutral carriers with considerable specificity for Li^+, Na^+, K^+, NH_4^+, Ca^{2+}, Ba^{2+}, UO_2^{2+}, Cd^{2+}, or other ions are available [9, 15].

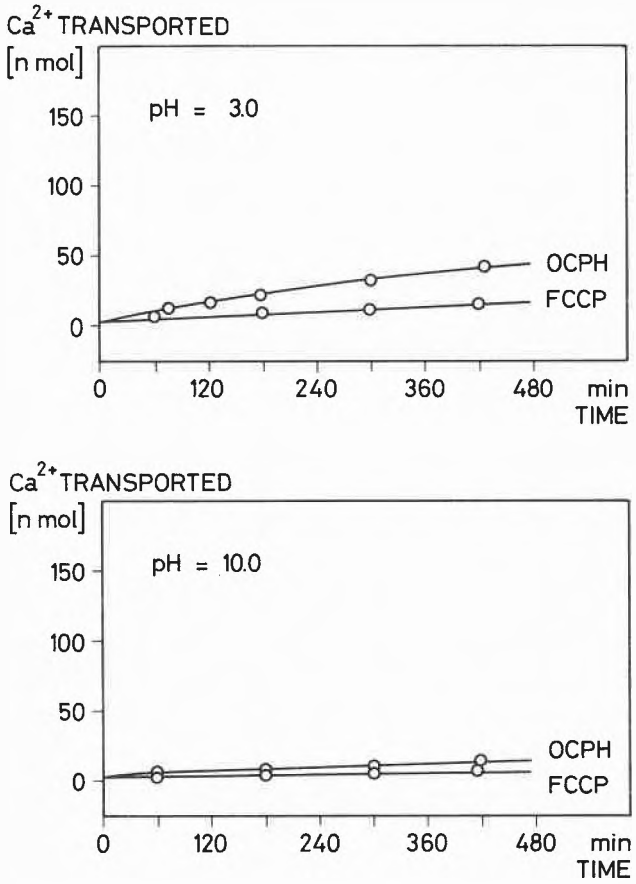


Fig. 5: Ca^{2+}-Transport in the Absence of a *p*H Gradient. Except for the pH values of the aqueous solutions, the experimental conditions are identical to those described for Fig. 4.

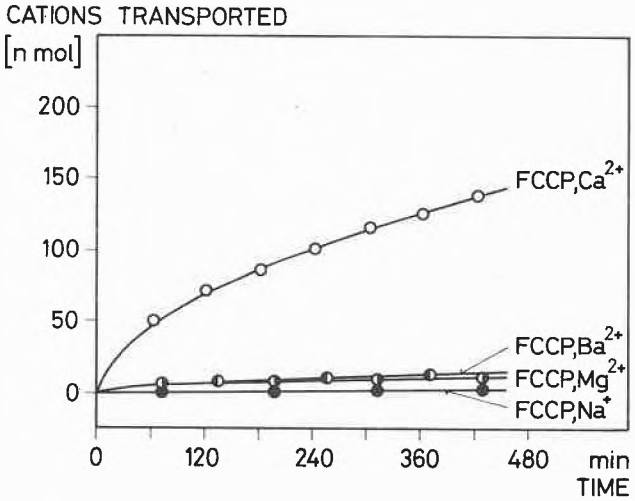


Fig. 6: Selectivity of the Ion Transport in a *p*H Gradient.

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10 The basic assumptions were (i) transport mechanism according to Fig. 1, (ii) equilibrium in respect to the extraction and complexation reactions, (iii) zero-current steady-state, (iv) electroneutrality, (v) identical diffusion coefficients for all species in the membrane.

11 Figure 2a was presented earlier in the context of a Mg^{2+}-carrier transport system [8]. There, the scale of the ordinate was by mistake given in units of 10^{-7} instead of 10^{-6} nanomoles.

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