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# Potentiometric detection of organic acids in liquid chromatography using polymeric liquid membrane electrodes incorporating macrocyclic hexaamines

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**Abstract**

Potentiometric detection employing coated-wire electrodes was applied to the determination of organic acids in liquid chromatography (LC). Poly(vinyl chloride)-based liquid membranes, incorporating lipophilic macrocyclic hexaamines as neutral ionophores were used as electrode coatings. The selectivity and sensitivity of the macrocycle-based electrodes were found to be superior to an electrode based on a lipophilic anion exchanger (a quaternary ammonium salt). Sensitive detection was obtained for the di- and tricarboxylic acids tartaric, malonic, malic, citric, fumaric, succinic, pyruvic, 2-oxoglutaric and maleic acids after separation in reversed-phase LC. Detection limits (signal/$4\sigma_{noise}$=3) of 6 pmol for malonic acid and 2 pmol for maleic acid were attained. The detection was explained using a molecular recognition model. The hexaamine-based potentiometric electrodes had a 1-s response time at 1 ml $min^{-1}$ flow-rates. They were stable for at least 4 months, with an intra-electrode variation of 3.2% ($n$=5). 

*Keywords:* Electrochemical detection; Detection, LC; Membranes; Electrodes; Carboxylic acids; Hexaamines

## 1. Introduction

In the past 10 years, a growing interest has been noted for the determination of organic acids in biological, clinical and food samples. Organic acids are intermediates in the most important metabolic pathways of carbohydrates, lipids and proteins. Precise measurement of organic acids present in biological fluids is essential in order to diagnose certain metabolic disorders [1]. Carboxylic acids can be found as natural components or as preservation additives in various foodstuffs [2–5]. The knowledge of the organic acid content and composition of food and beverages is important to assess the quality, stability and nutritive value of the product, to monitor the fermentation process and to validate the authenticity of juices. The determination of carboxylic acids has also been widely used in environmental

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chemistry [6]. For example, the determination of carboxylic acids in rainfall samples is important for proper monitoring of atmospheric chemistry [7]. Carboxylic acids are also used extensively for manufacturing technical products. The quality control of these products requires the determination of the carboxylic acid composition [8].

As carboxylic acids are generally non-volatile, their determination analysis with gas chromatography requires derivatisation. However, the derivatisation process is often quite tedious, time-consuming and decreases the reproducibility and repeatability of the analysis [9]. Currently, the most widely used methods for the determination of carboxylic acids include liquid chromatographic techniques based on reversed-phase (RP) [2,3], ion-exchange [7,10], ion-exclusion [3] and ion-pair [5,11] mechanisms. Owing to its high separation ability, capillary electrophoresis has also become a powerful technique for the determination of carboxylic acids [12]. Several detection methods have been employed in liquid chromatography (LC) for the detection of carboxylic acids. Refractive index detection has a limited sensitivity and suffers from interferences [13]. Pre- and post-column derivatisation with a suitable chromophore or fluorophore have often been used in order to improve the UV and fluorescence detection sensitivity of carboxylic acids [14]. However, the derivatisation procedures are often time consuming and other reagents present in the samples can interfere with the analysis [15]. A simple and sensitive detection method for carboxylic acids in LC is therefore highly desirable.

Electrochemical detection has been recognised as a useful method for the detection of carboxylic acids following separation by LC. Suppressed conductivity is considered to be the traditional method of detection for ion chromatography. Amperometric detection is limited as most carboxylic acids are electroinactive. Indirect amperometric detection of electroinactive carboxylic acids was possible at conducting polymer-based electrodes [16]. Potentiometric detection has been used in conjunction with various separation methods for the determination of organic acids. Chen and co-workers employed a tungsten oxide electrode [17] and a metallic copper electrode [11,18] for the detection of organic acids following separation by reversed-phase, ion-interaction and ion-exclusion chromatography. Isildak [19] used a tubular poly(vinyl chloride) (PVC) matrix membrane electrode for the determination of inorganic and organic anions in ion chromatography. In our group, potentiometric detection of organic acids was recently carried out with coated-wire liquid membrane electrodes [20], conducting polymer coated electrodes [21] and an ion-selective field effect transistor [22]. In these studies, the applied membrane coatings for the potentiometric detection of organic acids were anion-exchange materials, having no specific interaction with the analytes. The sensitivity that can be reached with membrane-based potentiometric detection is dependent on the difference in interaction energy of the analyte ion and the buffer ion with the membrane components, and on the membrane's perm-selectivity [23]. In order to optimise this difference in interaction energy, the present study examines the addition of molecular recognition compounds (macrocyclic polyamines) to the membrane. Macrocyclic polyamines were designed as selective polyoxyanion receptor molecules [24–27]. In their multiprotonated form, they form stable complexes with polycarboxylates via ion–ion interactions.

Two lipophilic derivatives of a macrocyclic hexaamine were tested in this work. The potentiometric response properties of the macrocyclic hexaamine-based electrodes were compared to those of an electrode containing a non-selective quaternary ammonium salt as anion exchanger.

## 2. Experimental

### *2.1. Reagents*

All the chemicals used were of analytical-reagent grade. Dioctylphthalate (DOP) and high-molecular-mass PVC were obtained from Janssen (Geel, Belgium). *O*-Nitrophenyl octyl ether (*o*-NPOE), methyltridodecylammonium chloride (MTDDACl) and tetrahydrofuran (THF) were purchased from Fluka (Buchs, Switzerland). Two alkylated macrocyclic hexaamines were synthesised by one of us (M.P.). First, a macrocyclic hexaamine was prepared according to the method described in Ref. [28]. Next, this hexaamine was *N*-substituted with hexadecyl

(host 1, Fig. 1a) or with oximidodecyl chains (host 2, Fig. 1b). To obtain the different alkylated hexaamines, the following general procedure was applied. One mmol of the unsubstituted polyamine was dissolved in 50 ml of dry acetonitrile under an argon blanket. The acetonitrile used was freshly distilled over $CaH_2$. Five mmol of anhydrous potassium carbonate per nitrogen atom was added to this mixture while stirring. The potassium carbonate was freshly calcined and finely ground. After that, 1.05 mmol of the alkylating agent, either hexadecyl iodide or oximidodecyl iodide, per nitrogen atom was added to the suspension. The suspension was then heated and magnetically stirred under argon at reflux for 16 h. It was filtered off while still hot to avoid precipitation of the product. The filtrate was evaporated to dryness under reduced pressure and crystallised from hexane.

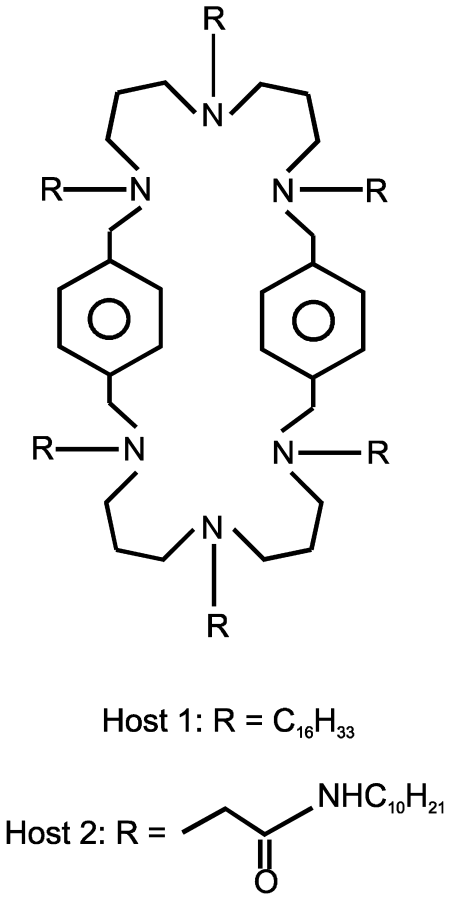


Fig. 1. Structures of the macrocyclic hexaamines: host 1 and host 2.

The eluent used in reversed-phase chromatography was prepared by dilution of a 89% (w/w) phosphoric acid solution, obtained from UCB (Leuven, Belgium). It was prepared daily and filtered through a 0.45-μm cellulose acetate membrane filter (Alltech, Deerfield, IL, USA). The eluent was constantly degassed during the analysis with a degasser. Organic acids were purchased from Merck, UCB, Acros, CRB, Aldrich, Fluka, Sigma and LBC. These organic acids included tartaric, malonic, malic, maleic, lactic, citric, fumaric, succinic, pyruvic and 2-oxoglutaric acids. Stock solutions of these analytes and of mixtures of them were prepared in the eluent solution. Dilutions of the stock solutions in the running eluent were made daily and were filtered (0.45 μm) before injection.

### *2.2. Electrode construction*

The potentiometric sensors used in this study were of the so-called ''coated-wire type''. The sensor membrane is deposited directly onto an electrically conductive support. Two different types of liquid PVC-based membranes were prepared, containing either a lipophilic macrocyclic hexaamine (host 1 or host 2) or a classical anion exchanger (MTDDACl) as the anion-sensing element.

Membranes containing a lipophilic macrocyclic hexaamine were composed of 1% ionophore (host 1 or host 2), 33% PVC and 66% DOP or *o*-NPOE. A 300-mg amount of this membrane mixture was dissolved in 3 ml THF. The quaternary ammonium salt-based membrane consisted of 6% MTDDACl, 65% DOP or *o*-NPOE and 29% PVC. A 350-mg amount of this composition was dissolved in 3.5 ml THF. The membrane mixtures were freshly used after mixing and coated onto cylindrical glassy carbon electrodes (3 mm diameter) mounted in plastic bodies. These electrodes were first polished with a 5-μm grid polishing sheet (3M) to obtain a clean surface. The coated-wire sensors were prepared by casting three consecutive layers of the membrane cocktail, at an interval of 5 min, with a Pasteur pipette. Each layer was formed by application of 40 μl of the membrane cocktail. The THF was allowed to evaporate under atmospheric conditions for at

least 2 h. Membranes with a thickness of ~100 μm were obtained in this way. Prior to use, the anion-sensitive electrodes were conditioned with the running eluent in the LC system until a stable baseline was obtained (~2 h).

### 2.3. Instrumentation

LC was performed using a SP8810 isocratic pump (Spectra Physics, San Jose, CA, USA), a Valco injector (10-μl loop) and a reversed-phase column (LiChrospher 100-5 RP-8, 125×4 mm I.D., Merck). The detection unit consisted of a laboratory-made large-volume wall-jet type flow cell in which both the indicator and reference electrode were placed [29] (see Fig. 2). The column effluent was directed perpendicularly towards the sensitive membrane of the coated-wire electrode (the indicator electrode). The distance from the LC tubing outlet [polyether ether ketone (PEEK) tube, 100 μm I.D., Alltech] to the electrode was 100 μm. The membrane potential was measured against an Orion 800500 Ross reference electrode using a high impedance amplifier (internal resistance $10^{13}$ Ω, Knick, type 87 F). The detection signals were amplified 10 times with a laboratory-made amplifier and recorded on a PC 1000 data acquisition system from Thermo Separation Products (San Jose, CA, USA).

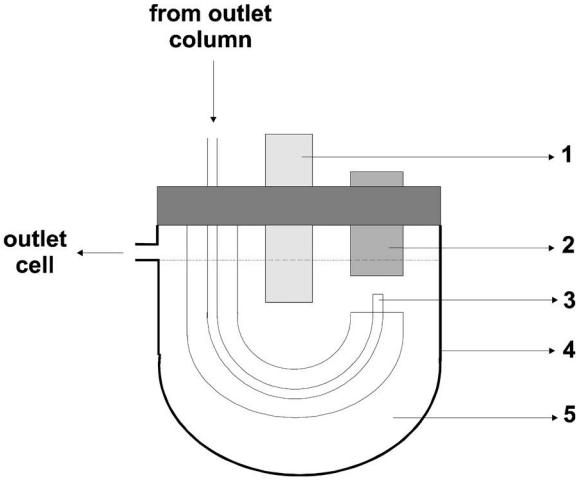


Fig. 2. Schematic presentation of the laboratory-made large-volume wall-jet detector: (1) reference electrode, (2) coated-wire electrode, (3) LC tubing outlet, (4) large-volume cell in glass and (5) eluent solution.

## 3. Results and discussion

### 3.1. Optimisation of the membrane composition in ion-suppression chromatography

Liquid membrane-based electrodes have the advantage that their selectivity can be tuned at will by introducing the right molecular recognition compounds into the membrane. In this study the response properties of two lipophilic derivatives of a macrocyclic hexaamine (see Fig. 1) will be examined. Analogous macrocycles were designed as selective polyoxyanion receptor molecules [24–27]. Macrocyclic penta- and hexaamines specifically interact with polycarboxylates having two carboxylate groups separated by a short distance such as succinate, malate, citrate, malonate, and maleate. They are reported to be inert towards other dicarboxylates like fumarate, asparate or glutarate and also towards the monocarboxylates acetate and lactate [25]. Their remarkable complexation properties are only manifested in their fully protonated forms, i.e., at relatively low pH. Eluents of low pH are required to separate organic acids via ion-suppression. Therefore, the combination of hexaamine-based electrodes with this separation technique seemed to be a good choice. It was shown previously [21] that an aqueous phosphoric acid solution allowed a good separation and sensitive potentiometric detection of organic acids in reversed-phase chromatography. The same eluent was therefore used to perform RP separations in this study. The macrocyclic hexaamines did not show a significant interaction with the phosphoric acid eluent. This was somewhat surprising in view of the reported response of macrocyclic amines to the $HPO_4^{2-}$ anion [29].

First, two membrane electrodes containing host 1 as a molecular recognition compound were investigated. The two membranes contained either DOP or *o*-NPOE as a plasticiser. A test mixture of seven carboxylic acids of analytical interest was separated. The chromatogram obtained with the membrane coating containing *o*-NPOE is shown in Fig. 3a. The repeatability of the chromatographic analysis was

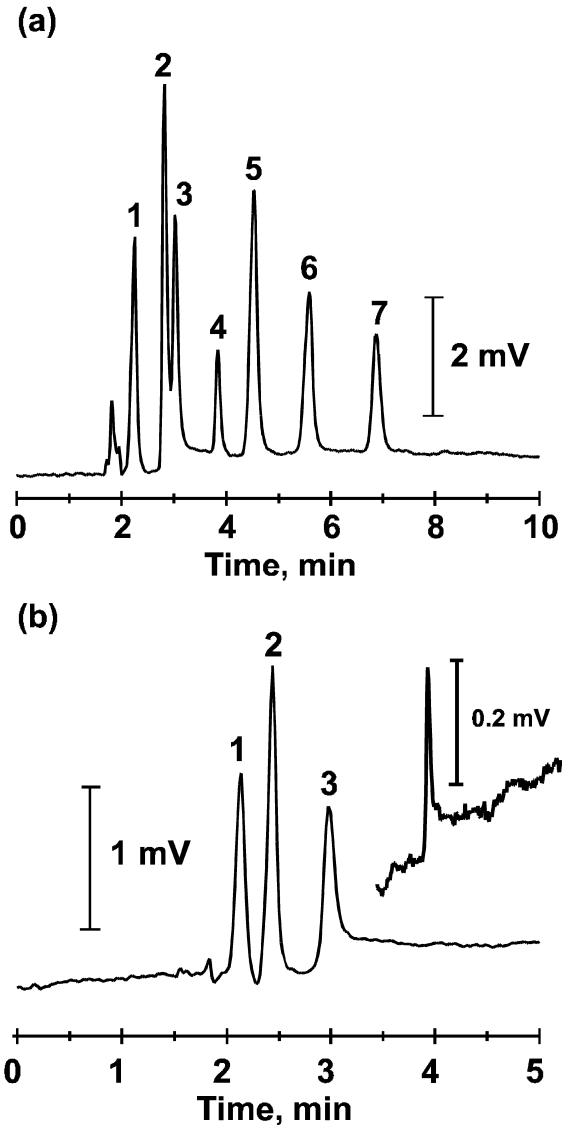


Fig. 3. (a) Chromatogram obtained using a coated-wire electrode incorporating host 1 and *o*-NPOE. Injected concentration and peak identification: (1) $5\cdot10^{-4}$ *M* tartaric acid, (2) $4\cdot10^{-5}$ *M* malonic acid, (3) $2\cdot10^{-4}$ *M* malic acid, (4) $5\cdot10^{-3}$ *M* lactic acid, (5) $5\cdot10^{-4}$ *M* citric acid, (6) $5\cdot10^{-4}$ *M* fumaric acid and (7) $1\cdot10^{-3}$ *M* succinic acid. Chromatographic conditions: column, LiChrospher 100-5 RP-8, 125×4 mm I.D.; eluent, 1 m*M* $H_3PO_4$; flow-rate, 0.5 ml $min^{-1}$; injection volume, 10 μl. (b) Chromatogram obtained using a coated-wire electrode incorporating host 1 and *o*-NPOE. Injected concentration and peak identification: (1) $1\cdot10^{-4}$ *M* pyruvic acid, (2) $1\cdot10^{-4}$ *M* 2-oxoglutaric acid and (3) $2.5\cdot10^{-6}$ *M* maleic acid. Chromatographic conditions as in (a). Insert: chromatogram of $5\cdot10^{-7}$ *M* maleic acid.

examined by repeated injection ($n=11$) of a $10^{-4}$ *M* solution of the test mixture. The standard deviations for the retention times of tartaric, malic, lactic, citric, fumaric and succinic acids were determined. They were calculated to be 0.50%, 0.40%, 0.40%, 0.27%, 0.26% and 0.19%, respectively. A stronger potentiometric response towards all tested acids was observed for the PVC-based liquid membrane containing the plasticiser *o*-NPOE, compared to that containing DOP. The more polar plasticiser *o*-NPOE increases the polarity of the membrane to a larger extent than DOP does. This facilitates the penetration of the polar carboxylate anions into the membrane. The relative responses of the different acids were comparable for membranes containing *o*-NPOE or DOP. Fig. 3a shows that malonic and malic acids were more responsive. On the grounds of these observations, only membranes containing *o*-NPOE as a plasticiser were investigated for further study. A membrane containing host 2 as ionophore and *o*-NPOE as a plasticiser showed a similar selectivity and sensitivity as the membrane containing host 1 and the same plasticiser (results not shown).

The performance of a membrane based on an anion exchanger (MTDDACl) was compared to the performance of a macrocyclic hexaamine (host 1 or host 2)-based membrane. All tested carboxylic acids provoked a higher potentiometric response at the membranes containing either host 1 or host 2 when compared to the anion exchanger membrane. This was most notable for malonic and malic acids. In contrast to the macrocyclic hexaamine-based electrode, membranes based on MTDDACl exhibited the same selectivity and sensitivity when *o*-NPOE or DOP was used as a plasticiser. Generally, a very stable baseline was obtained for chromatograms recorded with the macrocyclic hexaamine-based electrodes. In contrast, a potential drift was always noted in the chromatograms where the quaternary ammonium salt-based membrane electrode was used.

Fig. 3b shows a chromatogram of a mixture of three carboxylic acids employing a liquid membrane electrode based on host 1. The selectivity and sensitivity of this liquid membrane electrode was compared to that of the MTDDACl-based electrode. The anion exchanger membrane showed a comparable sensitivity for the three carboxylic acids. The liquid membranes based on the macrocyclic hexa-

amine exhibited a differentiated potentiometric response towards the tested acids. It was possible to detect maleic acid with a 40-fold higher sensitivity with this electrode as compared to the MTDDACl-based electrode: see Table 1.

Table 1 lists the detection limits for all tested carboxylic acids, obtained with the different anion-selective coated-wire electrodes. The detection limit was calculated as the lowest concentration of analyte for which the corresponding signal is larger than 12 times the standard deviation of the noise, $\sigma_{noise}$. The sensitivity of the MTDDACl-based membrane electrode was always lower than the sensitivities of the macrocyclic hexaamine-based sensors, except for the monocarboxylate lactate. From this table we can conclude that the addition of macrocyclic polyamines to the membrane, improves the detection sensitivity for di- and tricarboxylic acids. The macrocycles form complexes with these compounds and facilitate their extraction in the membrane phase. Each of the two negatively charged groups of the dicarboxylate substrates interacts with a triammonium unit of the hexaamine receptor. This results in the formation of strong ion–ion interactions [27]. The effect was more pronounced for the detection of malonic, malic and maleic acids. Detection limits of 6, 30 and 2 pmol, respectively, were attained for these acids with the membrane electrode incorporating host 1.

Molecular modelling using MM2 (CambridgeSoft, Cambridge, MA, USA) shows that the dianions malonate, malate and maleate fit well in the dimensions of the macrocycle ring structure: see Fig. 4. Calculated ion–ion interaction enthalpies in the gas phase are strongly negative (such calculations were also performed very recently by Kane et al. to predict sensor responses for the detection of gaseous substances [30]). Although molecular modelling could explain the high responses noted for malonate, malate and maleate, it was difficult to use it to explain their relative response order. Any model that

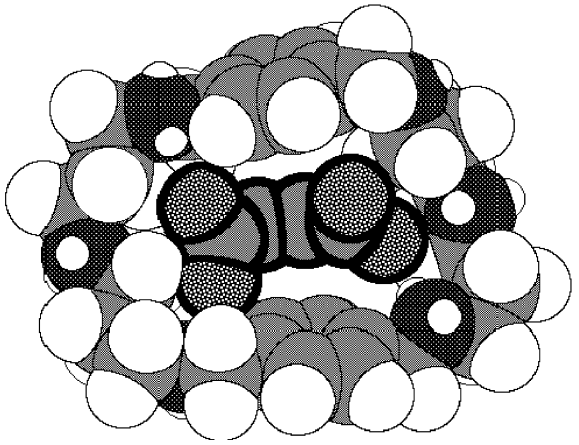

Fig. 4. Complex formation between maleate and the macrocyclic hexaamine as calculated by MM2. The lipophilic tails of the macrocycle were omitted for reasons of clarity. The six nitrogen atoms of the macrocycle are shown in black. Oxygen atoms of the organic acid are shaded. Hydrogen atoms are shown in white.

Table 1
Detection limits (nmol injected, signal/$4\sigma_{noise}=3$) for potentiometric detectors in ion-suppression chromatography

| Acid | Host 1 | | Host 2, *o*-NPOE | MTDDACl | |
|---|---|---|---|---|---|
| | *o*-NPOE | DOP | | *o*-NPOE | DOP |
| Tartaric | 0.05 | 0.07 | 0.03 | 0.2 | 0.2 |
| Malonic | 0.006 | 0.03 | 0.004 | 0.2 | 0.2 |
| Malic | 0.03 | 0.09 | 0.02 | 0.2 | 0.3 |
| Lactic | 3 | 2 | 2 | 1 | 2 |
| Citric | 0.05 | 0.09 | 0.03 | 0.2 | 0.1 |
| Fumaric | 0.2 | 0.2 | 0.08 | 0.3 | 0.3 |
| Succinic | 0.3 | 0.7 | 0.2 | 0.7 | 0.9 |
| Pyruvic | 0.1 | n.d.[a] | 0.1 | 0.2 | n.d. |
| 2-Oxoglutaric | 0.1 | n.d. | 0.1 | 0.1 | n.d. |
| Maleic | 0.002 | n.d. | 0.003 | 0.08 | n.d. |

Electrodes based on a macrocyclic hexaamine (host 1 or host 2) or on an anion exchanger (MTDDACl), and containing either *o*-NPOE or DOP as a plasticiser, were employed.

[a] n.d., Not determined.

wants to account for this will have to include dissociation equilibria of the diacids to their dicarboxylate form, hydration energies and interaction energies with membrane components other than the macrocycles.

### 3.2. Potentiometric detector characteristics

#### 3.2.1. Response time

The response times of the coated-wire anion-selective electrodes were measured in a flow injection analysis set-up, using 1 m*M* $H_3PO_4$ as the solvent carrier. We determined the $t_{90}$ response time, as recommended by IUPAC [31]. $t_{90}$ is defined as the time required to reach 90% of the signal steady-state value. We injected rectangular concentration pulses of $10^{-3}$ *M* malic acid, using a 500-μl injection loop (pulse time of 15 s). The response time of the electrodes increases when the flow-rate is lowered, as illustrated in Fig. 5. This is caused by an increased thickness of the diffusion layer near the membrane.

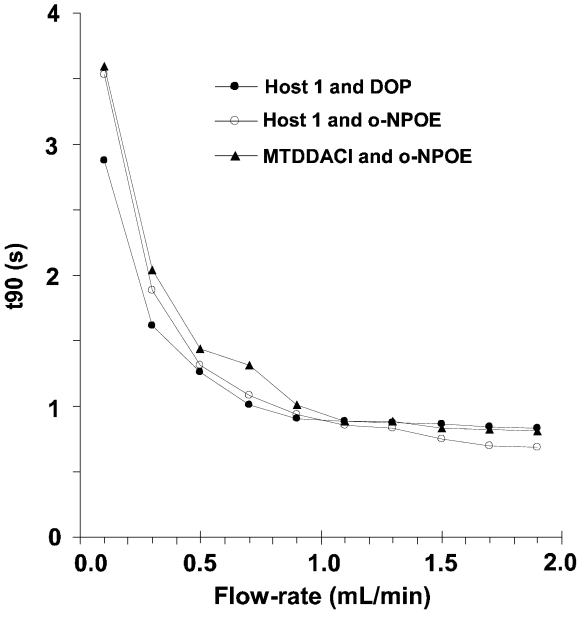


Fig. 5. Response time as a function of flow-rate for a coated-wire electrode containing the indicated anion-sensing element and plasticiser. Measurements were done in a flow-injection analysis set-up. Solvent carrier: 1 m*M* $H_3PO_4$. Concentrations plugs of $1{\cdot}10^{-3}$ *M* malic acid were injected.

The tested coated-wire electrodes all showed a response time of 1 s at a flow-rate of 1 ml $min^{-1}$.

#### 3.2.2. Calibration curves

Calibration curves for tartaric, malonic, malic, lactic, citric, fumaric and succinic acids, showing peak height (in mV) versus the logarithm of the injected concentration, are presented in Fig. 6. A logarithmic relationship was observed between the injected concentration of malonic acid and the peak height for concentrations higher than $2.5{\cdot}10^{-6}$ *M*. For the remaining acids this logarithmic relationship was noted for concentrations higher than $2.5{\cdot}10^{-5}$ *M*. At lower concentrations the calibration curves showed a linear dependence of the signal versus the concentration injected, as was theoretically predicted by our group in a previous publication [32].

#### 3.2.3. Electrode reproducibility and long-term stability

The intra-electrode reproducibility of the signal provoked at a hexaamine-based electrode was examined over a 1-day period. The relative standard deviation (RSD) of the peak heights was 1.4% ($n=10$).

When the hexaamine-based electrode was stored in the aqueous phosphoric acid eluent, it was found to be in good condition even after 4 months,

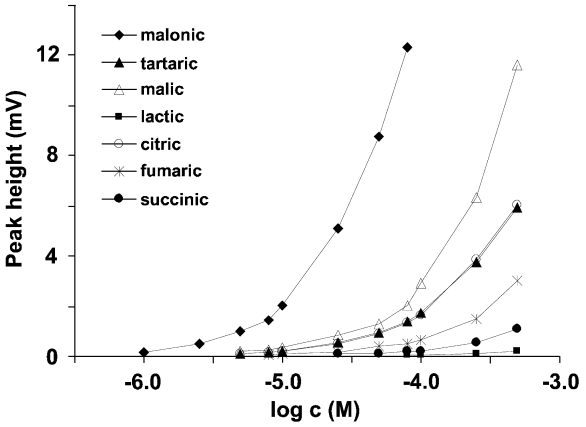


Fig. 6. Calibration curves of indicated organic acids for a coated-wire electrode incorporating host 1 and *o*-NPOE. Measurements were made under the chromatographic conditions described in Fig. 2.

indicating a good long-term stability of the electrode. The calculated RSD in this case was 3.2% ($n=5$).

### *3.3. Optimisation of the membrane composition in anion-exchange chromatography*

Macrocyclic polyamines are known to form stable complexes with polyanions (e.g., di- and tricarboxylates, phosphates, carbonates) at acidic and neutral pH in aqueous solution. In alkaline aqueous solution, these compounds exist as uncharged and inactive forms with respect to anions. For this reason it is not recommended to employ macrocycle polyamine-based electrodes in combination with anion-exchange chromatography, which demands strongly alkaline solutions as eluents. Therefore a copper (II) complex of host 1 was prepared, and tested as a potentiometric sensor in anion-exchange chromatography. The complex formation was carried out according to the procedure described by Pietraszkiewicz et al. [33].

A chromatogram obtained with a coated-wire electrode incorporating the copper (II) complex of host 1 is shown in Fig. 7. A test mixture of three carboxylic acids was separated on a latex-based anion-exchange column, employing 12 m*M* NaOH (pH 12.1) as the eluent. The three acids could be detected at a $1\cdot10^{-4}$ *M* level. Determination of the acids under the latter conditions is clearly less sensitive than determination under ion-suppression conditions. Probably, the interference of hydroxyl ions is quite high, as these form complexes with the copper (II) ion [34].

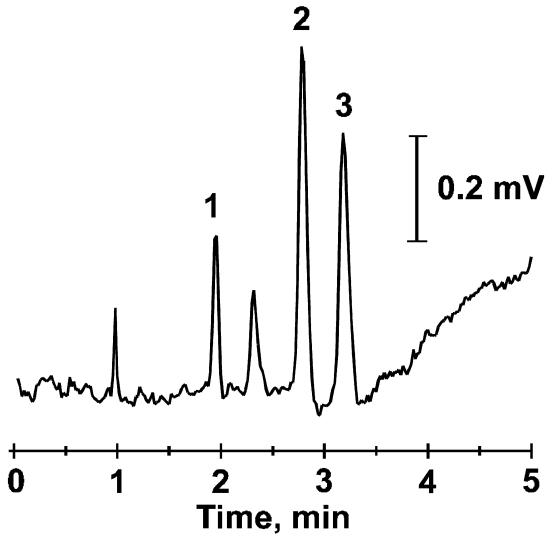


Fig. 7. Chromatogram of a mixture of three carboxylic acids obtained using a coated-wire electrode incorporating the Cu(II) complex of host 1 and *o*-NPOE. Column: Dionex Ion Pac AS11, 250×4 mm I.D. Flow-rate: 1.5 ml $min^{-1}$. Injection volume: 10 μl. Eluent: 12 m*M* NaOH. Injected concentration: $5\cdot10^{-4}$ *M* of each acid. Peak identification: (1) malic acid, (2) 2-oxoglutaric acid and (3) fumaric acid.

## 4. Conclusions

This study shows that potentiometric detection using PVC-based liquid membrane coated-wire electrodes, incorporating lipophilic macrocyclic hexaamines, can be employed successfully in conjunction with ion-suppression LC for the detection of carboxylic acids. The lowest detection limits were obtained for maleic and malonic acids which showed the strongest interaction with the macrocyclic hexaamines. The high stability of these complexes is attributed to the good fit of these dicarboxylic substrates in the hexaamine receptor. Sensitive detection of ionic organic substances with appropriate molecular recognition compounds can lead to the construction of sensitive sensors for flow-analysis and for batch-analysis. Further work is in progress to exploit this principle to other organic ions.

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