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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF ORGANIC ACIDS WITH POTENTIOMETRIC DETECTION USING A METALLIC COPPER ELECTRODE

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SUMMARY

The use of a metallic copper wire electrode for potentiometric detection of organic acid anions in ion-exchange chromatography is described. These anions were detected by changes in electrode potential resulting from complexation of copper(I) or copper(II) ions at the electrode surface. The direction of this potential change, and hence the direction of the peak produced, was found to depend on the relative strengths of copper complexation between the injected ligand and the eluent ligand. Calibration relationships between the electrode potential and the amount of injected solute were studied and were observed to depend on the type of solute and the amounts injected. Several separations obtained using the copper wire electrode detector are presented as examples. Included are separations of glycinate, glutamate, and oxalate, and of acetate, lactate, formate, succinate, and benzoate.

INTRODUCTION

Electrochemical detectors which employ potentiometric measurements have been widely applied to flow analysis, yet have found only limited usage in high-performance liquid chromatography (HPLC). For HPLC, amperometric or conductimetric detectors have been preferred over potentiometric detectors[1] because the latter type has been considered to suffer from drawbacks in the areas of sensitivity, response time and reproducibility. In addition, these detectors may often be too selective for general use in HPLC.

An early application of potentiometric detection in HPLC was the use of a silver–silver chloride microelectrode for the ion-exchange determination of halide ions in the presence of other inorganic anions[2]. In this work, the authors also proposed the use of a lead amalgam electrode for the detection of sulphate and phosphate ions, and also the use of a liquid-state chloride ion-selective electrode for detection of nitrate and perchlorate ions.

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Ion-selective membrane electrodes have been used as potentiometric detectors in HPLC for both direct[3–6] and indirect[7–9] detection of eluted ions. In the direct detection methods, the electrode responds selectively to particular ions. Examples of this approach include the detection of nitrite and nitrate using a liquid-state membrane nitrate electrode[3], the detection of cyanide and sulphide using a silver sulphide electrode[4], the detection of monovalent cations using coated-wire electrodes[5], and the detection of anions and cations using non-selective ion-exchange membranes[6]. In contrast, indirect detection involves use of the ion-selective electrode to monitor an eluent ion which varies in concentration when a sample ion elutes. This is illustrated in the determination of amino acids by reversed-phase or ion-exchange HPLC, wherein post-column addition of copper ions was used in conjunction with a copper ion-selective electrode[7]. Here, copper complexation by eluted amino acids resulted in a potential change at the electrode, allowing indirect detection of the amino acids. In a similar manner, a chloride ion-selective electrode has been used with chloride containing eluents in gel permeation chromatography for the indirect detection of fluoride, sulphate, acetate, oxalate, citrate, iodide and thiocyanate[8].

Two further reported examples of indirect potentiometric detection in HPLC are the detection of eluted carboxylic acids with a glass pH electrode[9] and the detection of transition and rare earth metal ions with a cupric ion-selective electrode[10]. In the latter application, post-column addition of copper(II) EDTA was employed, and reaction of this solution with eluted metal ions caused displacement of copper(II) ions which were then detected at the electrode.

In previous papers[11–16], we have reported the use of a metallic copper electrode for the detection of copper complexing ligands in continuous-flow analysis[11], reversed-phase HPLC[12], flow-injection analysis[13–15] and ion chromatography[16], using both direct and indirect detection methods. The aim of the present study was to investigate the utility of a metallic copper wire electrode for detection of organic acids in ion-exchange HPLC. Aliphatic and aromatic carboxylic acids and several amino acids were included in this study.

EXPERIMENTAL

The HPLC equipment consisted of a Waters Assoc. (Milford, MA, U.S.A.) Model M45 solvent pump and Model U6K injector. The potentiometric flow-through detector incorporating a copper wire electrode has been described previously[13]. This detector was connected to a Radiometer (Copenhagen, Denmark) pHM 62 pH/millivolt meter, interfaced to a Houston Instruments (Austin, TX, U.S.A.) Omniscribe recorder. The column used was a Vydac (Separations Group, Hesperia, CA, U.S.A.) low capacity anion-exchanger (type 302 IC 4.6), 250 × 4.6 mm I.D. All parts of the chromatographic system in contact with the eluent were either stainless steel or polypropylene. Prior to use, the copper wire electrode was removed from the cell, briefly immersed in concentrated nitric acid and then rinsed with distilled water. The detector cell was then reassembled and eluent was pumped through the cell until a stable baseline potential was attained.

The reagents used were: potassium hydrogen phthalate, potassium dihydrogen orthophosphate, sodium lactate (70% solution) and sodium propionate from Ajax Chemicals (Sydney, Australia); citric acid and malonic acid from Merck (Darmstadt,

F.R.G.); glycine from BDH Biochemicals (Poole, U.K.); glutamic and salicylic acid from Koch-Light (Colnbrook, U.K.); potassium oxalate, succinic acid and chloroacetic acid from May & Baker (Dagenham, U.K.); maleic acid from Aldrich (Milwaukee, WI, U.S.A.); potassium benzoate from Pfalz and Bauer (Stamford, U.S.A.) and sodium fumarate, sodium formate and sodium acetate from BDH (Sydney, Australia). All these reagents were used without further purification. Eluents were prepared in distilled and deionised water and were filtered through a 0.45-μm membrane filter and degassed before use. The pH of each eluent was adjusted with sodium hydroxide.

RESULTS AND DISCUSSION

Detector response in various eluents

Our previous studies[13–15] have shown that in continuous-flow or flow-injection applications, a metallic copper electrode can be used for the direct potentiometric detection of copper complexing ligands. Under conditions where formation of a single complex species of copper with the determined ligand predominated, the relationship between the copper electrode potential and the total ligand concentration was found to be Nernstian[14]. The detector response in flow-injection measurements or in chromatographic determinations resulted from differences in complexation strength between components of the carrier solution or eluent and the ligand or ligands present in the injected sample. Eluted species may form stronger or weaker copper complexes than the ligand present in the eluent, resulting in either an increase or a decrease in the observed electrode potential. Hence both positive and negative peaks can be expected, and we have used the arbitrary convention that a positive peak corresponded to the elution of a stronger complexing ligand than that present in the mobile phase. In fact, this situation gave a *decrease* of electrode potential, however the recorder polarity was arranged so that a positive peak was displayed.

The most frequently used eluents in single column anion chromatography are benzoate, phthalate and sulphobenzoate[17], and these have been used for the separation of both inorganic and organic anions. Similar results can be expected using citrate as eluent, provided that the concentration is adjusted to allow for the high affinity of citrate for anion-exchange resins. Orthophosphate (at pH 3.2) has also been used for the separation of several organic acids on an amino column[18]. In the present study, the response of the electrode detector to eluted organic acid ions was examined using the following eluents: 2 m*M* potassium phthalate at pH 4.0, 2 m*M* potassium orthophosphate at pH 7.0 and 1 m*M* sodium citrate at pH 6.5. These eluents are similar with respect to their elution strengths, but differ with regard to copper ion complexation. The values of side-reaction coefficients $\alpha_{Cu(II)(L)}$ calculated using stability constants taken from Smith and Martell[19] are 1.3, 2.8 and 690 for the phthalate, orthophosphate and citrate eluents, respectively. These values are consistent with the experimentally observed potentials of the copper electrode in these solutions, which were −143, −151 and −211 mV (*vs.* Ag/AgCl in 0.1 *M* KCl in agar gel), respectively.

The detector response towards some organic acid anions was examined by injection of 100-μl volumes of 10 m*M* solutions of each acid, adjusted to pH 6.0 ± 0.5. In several cases smaller samples volumes were used, as indicted in the caption

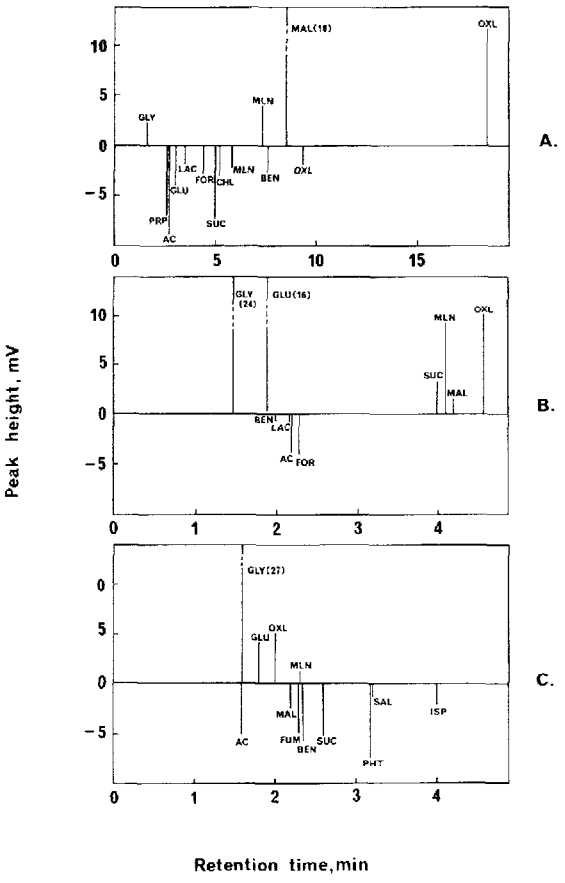

Fig. 1. Schematic presentation of experimentally obtained retention times and peak heights for organic acids using ion-exchange separation with (A) 2 m*M* potassium hydrogen phthalate, (B) 2 m*M* potassium orthophosphate or (C) 1 m*M* sodium citrate as eluent. Conditions: Column, Vydac 302 IC; flow-rate, 2 ml min^{-1}; injection volume, 100 μl of 10 m*M* solutions of each acid, except in the case of orthophosphate eluent, where 25-μl (malonate and maleate), 5-μl (glycinate and glutamate) or 2.5-μl (oxalate) volumes were used. Solute identities: AC = acetate, BEN = benzoate, CHL = chloroacetate, FOR = formate, FUM = fumarate, GLU = glutamate, GLY = glycinate, ISP = isophthalate, LAC = lactate, MAL = maleate, MLN = malonate, OXL = oxalate, PRP = propionate, SAL = salicylate, SUC = succinate.

to Fig. 1. The observed retention times and peak heights are schematically presented in Fig. 1, which also indicates whether the peaks were positive or negative. Values of the side-reaction coefficients $\alpha_{Cu(II)(L)}$ for the studied ligands are listed in Table I. These values were calculated using tabulated stability constants (determined at 25°C, ref. 19), under conditions of 0.1 *M* ionic strength and at the experimental pH values, using the assumption that the total concentration of the studied ligand at the peak maximum was equal to 1 m*M*. This assumption can only be strictly valid for one retention time, however a numerical value for ligand concentration was necessary to perform the calculations of $\alpha_{Cu(II)(L)}$ values. Nevertheless, Table I permits fairly accurate prediction of the experimental results presented in Fig. 1. When the calculated

TABLE I

VALUES OF SIDE-REACTION COEFFICIENTS, $\alpha_{Cu(II)(L)}$ FOR THE STUDIED LIGANDS

The values were calculated using a ligand concentration of 1 m*M* and stability constants taken from ref. 19.

	pH 4.0		*pH 7.0*		*pH 6.5*	
	Ligand	$\alpha_{Cu(II)(L)}$	*Ligand*	$\alpha_{Cu(II)(L)}$	*Ligand*	$\alpha_{Cu(II)(L)}$
Ligands complexing more strongly than eluent ligand	Oxalate	630	Oxalate	$1.7 \cdot 10^3$	Glycinate	$9.3 \cdot 10^3$
	Malonate	6.6	Glutamate	$1.1 \cdot 10^3$	Oxalate	$1.7 \cdot 10^3$
	Fumarate*	4.9	Glycinate	890		
	Glycinate	1.4	Malonate	170		
			Salicylate	18		
			Fumarate*	10		
			Maleate	3.4		
Eluent	2 m*M* Phthalate	1.3	2 m*M* Phosphate	2.8	1 m*M* Citrate	690
Ligands complexing less strongly than eluent ligand	Lactate	1.2	Phthalate	2.2	Glutamate	160
	Glutamate	1.1	Succinate	1.4	Malonate	160
	Acetate	1.0	Lactate	1.3	Fumarate*	10
	Benzoate	1.0	Acetate	1.0	Salicylate	6.3
	Chloroacetate	1.0	Benzoate	1.0	Maleate	3.1
	Formate	1.0	Formate	1.0	Phthalate	2.2
	Maleate	1.0			Succinate	1.4
	Propionate	1.0			Acetate	1.0
	Succinate	1.0			Benzoate	1.0

* Value calculated for copper(I) ions, *i.e.* $\alpha_{Cu(I)(L)}$.

value of $\alpha_{Cu(II)(L)}$ for a particular ligand was larger than that calculated for the eluent, then a positive peak was expected, according to the convention described earlier. Under opposite conditions, a negative peak was expected.

Comparison of Table I and Fig. 1 shows that, for the potassium phthalate eluent, the only discrepancy observed was maleate, which exhibited a large, reproducible positive peak instead of the expected negative peak. Of all the ligands studied, only two (maleic acid and fumaric acid) form complexes with cuprous ions, and these complexes are more stable than those formed with cupric ions[19]. Accordingly, fumarate gave a large positive peak with a retention time of 24 min (not shown on Fig. 1A). In the case of maleate at pH 4 and a total ligand concentration of 1 m*M*, complexation of copper(I) ions is very limited, however the magnitude of the measured detector response under these conditions suggests that the amount of free maleate in the eluent stream approaching the indicator electrode was sufficient for transient stabilization of cuprous ions at the copper electrode surface, producing a comparatively large potential change.

Other interesting features can be noticed for malonate and oxalate, both of which exhibited a positive and a negative peak (Fig. 1A). These two ligands are the only ones used in this study which are known to form copper complexes with both the free ligand and the singly protonated ligand[19]. At pH 4, 61% of total oxalate is

present as the singly protonated form and 39% exists as the deprotonated form: for malonate, the corresponding values are 92% and 2%, respectively. Although two peaks were observed for both oxalate and malonate, these peaks were very distorted in comparison to those obtained for other acids. The distorted shapes of the dual peaks were consistent with the elution of two rapidly interconverting species (*e.g.* $C_2O_4^{2-}$ and $HC_2O_4^-$), see the review by Keller and Giddings[20]. The appearance of dual peaks for oxalate and malonate was observed only for the phthalate eluent since at the higher pH values of the citrate and phosphate eluents, both oxalate and malonate exist predominantly in the deprotonated form.

In the orthophosphate eluent, complexation of copper ions by the eluent was only slightly stronger than for phthalate, but the higher eluent pH resulted in an increase of copper complexation by the studied ligands, due to increased fractions of the deprotonated form of each ligand. This resulted in an increase in the positive peak height of several of the studied ligands (*e.g.* glycinate and malonate). The observed discrepancy between experimental results and calculated data for succinate can be considered to be within the margins of error introduced by assuming a ligand concentration of 1 m*M* at the peak maximum, and also taking into account inaccuracies in the stability constant values involved in the calculations.

For the citrate eluent, agreement between predicted and observed peak direction was good for all ligands except glutamate and malonate. These ligands exhibited positive peaks, whereas the data in Table I suggested that negative peaks could be expected. The fact that these ligands eluted early in the chromatogram suggested that the likely source of error in the calculation shown in Table I could have been the assumed ligand concentration of 1 m*M*. Solute dispersion for early eluting peaks is noticeably less than for later peaks and in accordance with this, a ligand concentration of 3 m*M* was used in the calculations. The $\alpha_{Cu(II)(L)}$ values so obtained were 1030 for glutamate and 820 for malonate, and these results were consistent with the observation of positive peaks for these ligands. When the same approach was applied to other rapidly eluting species in Fig. 1C, the predicted peak directions were identical to those indicated in Table I.

Examples of separations

The experimental findings presented in Fig. 1 provide preliminary information regarding the application of this detection approach to the separation of various mixtures of organic species. Clearly, each particular application requires adjustment of such experimental factors as eluent concentration, pH or flow-rate in order to achieve optimal separations. Figs. 2–5 present some examples of the application of potentiometric detection with a metallic copper electrode. For convenience, the ligands have been grouped according to the observed peak direction, so that all peaks in a particular chromatogram are in the same direction. This grouping is, of course, quite arbitrary and chromatograms showing both positive and negative peaks may be produced.

The first example (Fig. 2) shows the separation of glycinate, glutamate and oxalate using phosphate eluent at pH 7. At this pH, all these ligands form stable copper(II) complexes, so sharp positive peaks were recorded. Fig. 3 shows the separation of *cis*- and *trans*-isomers of 1,2-ethylenedicarboxylic acid (*i.e.* maleate and fumarate), using phthalate eluent. As discussed earlier, these ligands produced large

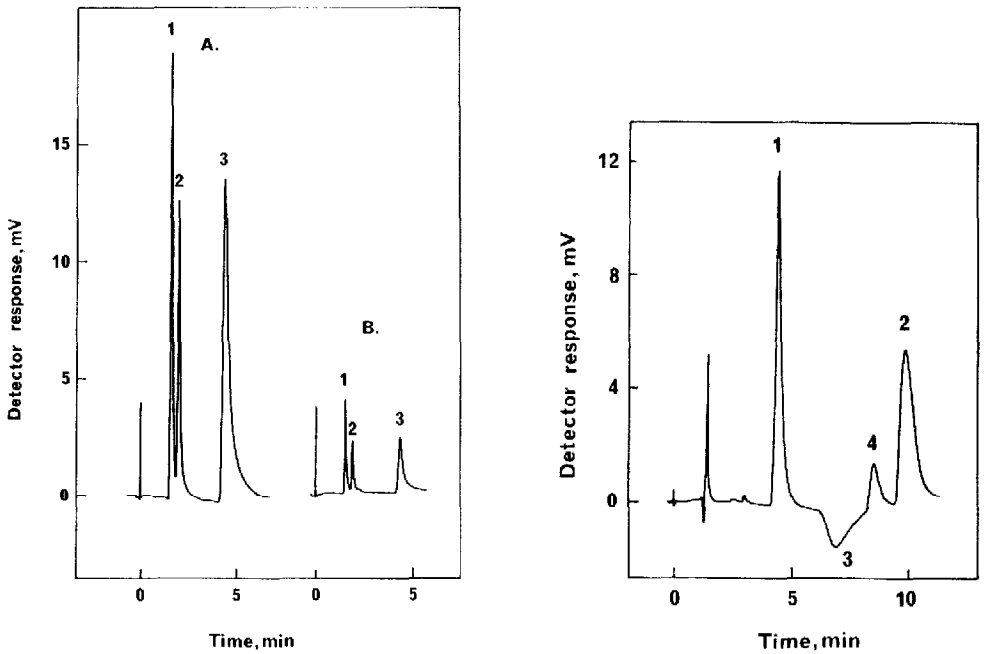

Fig. 2. Chromatogram of a mixture of glycinate (1), glutamate (2) and oxalate (3), obtained using 2 m*M* potassium orthophosphate at pH 7.0 as eluent. Injected amounts, (A) 50 and (B) 5 nmol of each ion. Other conditions as in Fig. 1.

Fig. 3. Separation of *cis*- and *trans*-isomers of 1,2-ethylenedicarboxylate using 5 m*M* potassium hydrogen phthalate at pH 4.0 as eluent. Injected amounts: 125 nmol of each compound. Peak identities: 1 = *cis* (maleate), 2 = *trans* (fumarate), 3 and 4 = system peaks. Other conditions as in Fig. 1.

positive peaks due to local stabilization of cuprous ions at the electrode surface. Also present in Fig. 3 are two "system" peaks of uncertain origin and the nature of these peaks is currently under study. Figs. 4 and 5 illustrate the detection of separated mixtures of ligands which form very weak complexes with copper(II) and accordingly produce negative peaks. The simple aliphatic acid anions formate, acetate, lactate, succinate and the aromatic acid anion benzoate were separated using 2 m*M* potassium hydrogen phthalate at pH 4.0 (Fig. 4), whereas three aromatic acid anions were separated with 1 m*M* sodium citrate at pH 6.5 (Fig. 5).

Calibration relationships

In previous reports of the application of potentiometric detection in liquid chromatography, the observed calibration relationships between solute concentration and electrode potential have differed significantly. These differences have generally arisen from the range of solute concentrations studied. In the range of large potential changes of the indicating electrode, Nernstian response applies and one can expect that the electrode potential value (and hence also the peak height) might be a linear function of the logarithm of the concentration of the analysed ion. Such a dependence has been reported[5] for the detection of monovalent cations using a coated-wire electrode. In the region of low activities of the determined species, much smaller sensi-

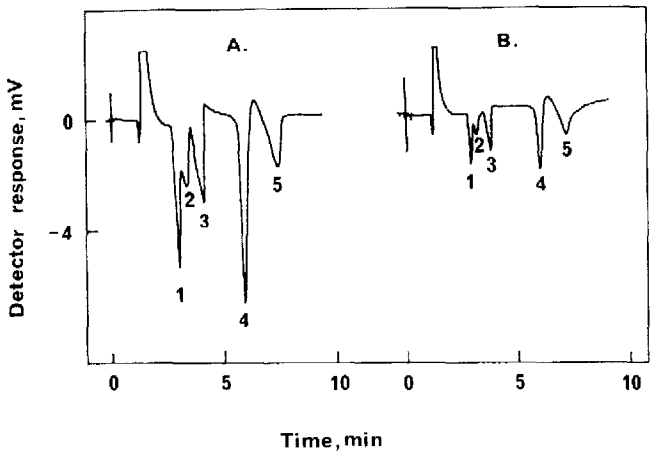

Fig. 4. Chromatogram of a mixture of acetate (1), lactate (2), formate (3), succinate (4) and benzoate (5), obtained using 2 m*M* potassium hydrogen phthalate at pH 4.0 as eluent. Injected amounts, (A) 0.4 μmole and (B) 0.1 μmole of each compound. Other conditions as in Fig. 1.

tivity of response is observed and a linear dependence of the potential value on concentration can result. In the case of solid-state membrane electrodes, this may be explained using an expansion of the mathematical function determining the electrode potential, together with appropriate simplifications and assumptions[21]. This approach can be used to justify linear relationships obtained in the determination of halides[2] and cyanide[4] with potentiometric detection. Linear dependence of peak height potential on solute concentration has also been observed using a membrane copper-sensing electrode in the determination of amino acids[7] and in cation chromatography with indirect potentiometric detection using a metallic copper electrode[16]. Alternatively, peak area and solute concentration have been shown to be linearly related when a chloride ion-selective electrode[8] or a liquid-state nitrate membrane electrode[3] were used for detection of eluted species.

In the present study, significant differences were observed regarding the detec-

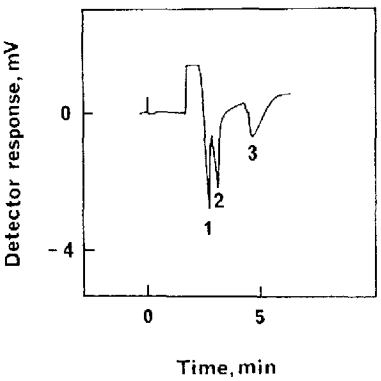

Fig. 5. Chromatogram of a mixture of benzoate (1), phthalate (2) and isophthalate (3), obtained using 1 m*M* sodium citrate at pH 6.5 as eluent. Injected amounts: 0.5 μmole benzoate, 0.25 μmole phthalate and 0.3 μmole isophthalate. Other conditions as in Fig. 1.

tion limits and characteristics of the calibration plots between ligands exhibiting positive or negative peaks. This is illustrated in Figs. 6 and 7, which show calibration plots for two separted mixtures. The first mixture contained anions which complex more strongly than the eluent ligand, and for low solute concentrations, a linear relationship between peak height and the amount of injected species was obtained (Fig. 6a). For larger injected amounts, a linear relationship between peak height and the logarithm of injected amount occurred, with a slope close to divalent Nernstian (Fig. 6b). Detection limits for the three determined ions in Fig. 6a were estimated to be approximately 0.7 nmol of injected species.

The second test mixture contained anions which formed weaker copper complexes with the eluent ligand and so produced negative peaks. These ligands did not give sensitive electrode response, as indicted in Fig. 7. Peak heights were not linearly related to solute concentration or the logarithm of concentration, except for acetate and succinate at relatively high concentrations (Fig. 7b). For low amounts of injected solute, a much better approximation to linearity was obtained by plotting peak area *vs.* amount of solute (Fig. 7c) with the exception of benzoate, which gave irregular results and was not included in Fig. 7c. The above results are similar to those reported by other workers[8]. When larger amounts of solutes were injected, all plots were curved. Detection limits calculated from Fig. 7c were 20 nmol for acetate, formate and succinate, and 45 nmol for lactate.

CONCLUSIONS

Detection of organic acid anions in ion-exchange chromatography is possible by using a potentiometric copper-wire electrode. The direction of the observed potential change for each solute anion can be rationalised in terms of the relative complexation strengths of copper by solute and eluent ligands. Calibration data indicates that the relationship between the electrode potential and the amount of injected solute is dependent on the type of ligand and the amounts injected.

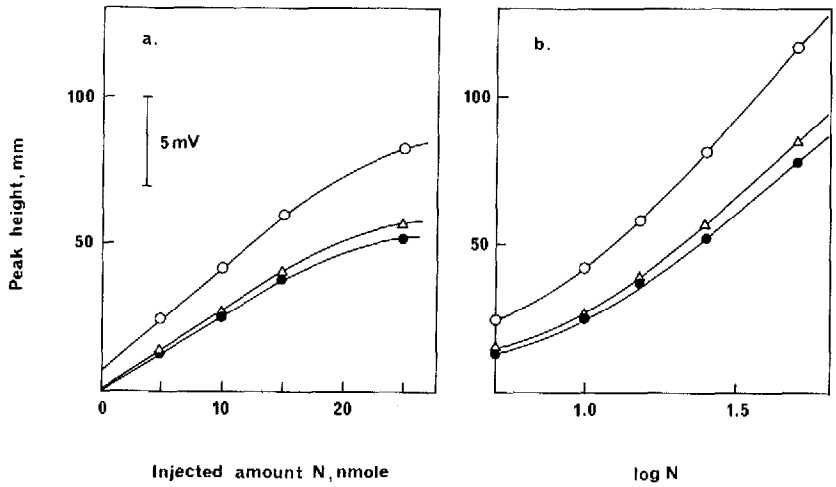

Fig. 6. Calibration plots of glycinate (○), glutamate (●) and oxalate (△). Conditions are as given in Fig. 2.

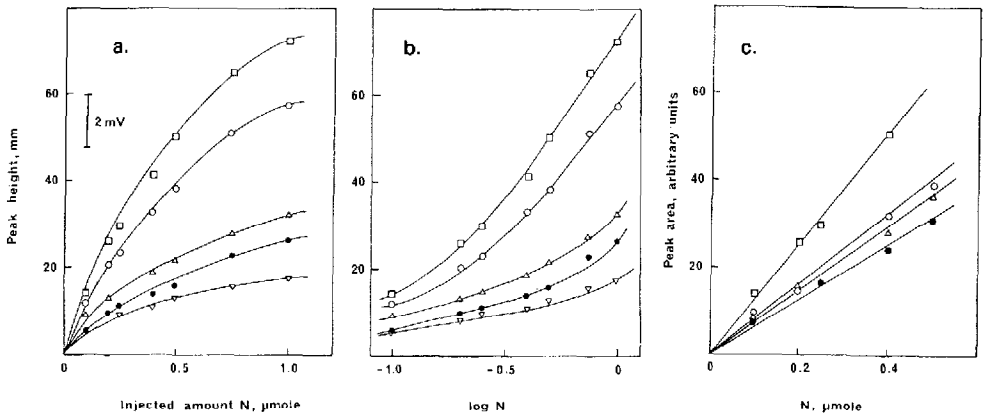

Fig. 7. Calibration plots for acetate (○), lactate (●), formate (△), succinate (□) and benzoate (▽). Conditions are as given in Fig. 4.

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