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AMPEROMETRIC DETECTOR WITH A MANGANIN ELECTRODE FOR MICROCOLUMN LIQUID CHROMATOGRAPHY

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SUMMARY

The response of manganin and copper microelectrodes in amino acid solution was evaluated by using slow-scanning voltammetry. Enhancement of the complexation rate of the manganin electrode in relation to the copper electrode is explained on the basis of the properties of the electrode surface. Based on voltammetric measurements, manganin is suggested as a working electrode material for the confined wall-jet microflow-cell of an amperometric detector. The detector performance was tested under the conditions of reversed-phase microcolumn liquid chromatography using selected amino acids and dipeptides as the solutes. Employing flow-rates up to 1 μl/s, an increase in response corresponding to the 1/3 power of the flow-rate was found, which indicates that a diffusion-limited electrode reaction occurs. The minimum detectable concentrations of the amino acids and some dipeptides in the ranges 0.04–0.08 and 0.1–0.25 μM, respectively, appear promising for application of this detector to trace analysis.

INTRODUCTION

The analysis of complex mixtures by liquid chromatography necessitates sensitive and selective detection, especially when the substances to be determined cannot be detected directly and sensitively by optical or electrochemical detectors, *e.g.*, the amino acids.

A promising method of detection of underivatized amino acids (AH) was suggested, employing amperometric detection at a copper working electrode[1–4]. The detection is based on the complexation of cupric ions released from the working electrode with amino acids according to the overall reaction:

$$Cu + 2AH \rightarrow Cu(A)_2 + 2H^+ + 2\,e^- \quad (1)$$

Good selectivity, reliability, reproducibility, linearity of response, simplicity of operation and resistance to electrode poisoning were among the advantages claimed for this method. However, the detection limits in the range of a few nanograms for conventional columns[2,4] and a few tenths of a nanogram for microbore columns[2]

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proved to be a drawback when compared[2] with optical detection of amino acid derivatives.

The detector performance is dependent on the mobile phase composition and, especially, on the nature of the buffer anion[1,2]. The optimum results were achieved with phosphate buffer[1,2] which was used also in further works[3–5]. However, phosphate ions can also have a negative effect on amino acid detections. The side reaction of phosphate and cupric ions decreases the concentration of free cupric ions available for complexation with amino acids. Further, the side reaction increases the background current since the detection of amino acids occurs at potentials where the limiting current of the buffer anions is reached[1]. This limiting current is flow-rate dependent[1,2], and can thus give rise to increased electrode-current noise.

In spite of the reasons mentioned above, the origin of higher detection limits was ascribed to a slow complexation reaction between the amino acids and cupric ions[1,2,4]. On the other side, for a copper amalgam drop electrode reacting with a solution of glycine, a pure diffusion-controlled anodic current was found[6]. This is in agreement with the fact that the complexation rate of free cupric aquo ions is very high, with a rate constant greater than 10^8 s^{-1} (refs. 7 and 8). Hence, it is the electrode surface that seems to be responsible for slowing down the overall electrode reaction (eqn. 1).

In the present work, the resistance alloy manganin, composed of 84% Cu, 12% Mn and 4% Ni[9], is suggested as an electrode material for the detection of complexing solutes. The manganin electrode was studied by using slow-scanning voltammetry and microcolumn liquid chromatography. A confined wall-jet microflow-cell equipped with a manganin working electrode was tested for the detection of amino acids and dipeptides.

EXPERIMENTAL

Apparatus

Voltammetry. Voltammetric measurements were carried out on an PA 3 polarographic analyzer (Laboratory Instruments, Prague, Czechoslovakia) equipped with a three-electrode cell consisting of a main compartment, a platinum-foil auxiliary electrode and a saturated calomel reference electrode. Voltammograms were recorded on a 4103 XY recorder (Laboratory Instruments) using a scanning rate of 1 mV/s.

The working microvoltammetric electrodes were made of copper or manganin wires, 0.15 mm in diameter (Kablo, Hostivař, Czechoslovakia), sealed with epoxide resin in a glass capillary. The end of the capillary with a cross-section of metallic wire was polished with metallographic paper and allowed to equilibrate with the solution for 5 min at the starting potential prior to every measurement. The microelectrodes used are not significantly affected by movement in the solution. When comparing the diffusion layer thickness of a stationary disk electrode[10,11] with that of a rotating disk electrode[12], it appears that the steady-state current density observed at the disk microelectrode of 75 μm in radius is equivalent to that observed at a rotating disk electrode operated at 1.2 rps. Although this rotation speed is relatively slow the use of slow voltage scanning ensures potentiostatic conditions of measurements[13].

The pH values of the background electrolyte, mobile phase and amino acid

solutions were measured by employing an OP-208/1 pH meter (Radelkis, Budapest, Hungary).

Chromatography. The microcolumn reversed-phase liquid chromatography was performed on a laboratory-built chromatograph. The mobile phase was pumped by a SP 8700 solvent-delivery system (Spectra-Physics, San Jose, CA, U.S.A.) operated under a constant-pressure mode. The mobile phase flow-rate was monitored with a calibrated capillary and a stop-watch. A sample 0.75 μl in volume was introduced by a laboratory-made four-port injection valve described previously[14]. A CGC glass microcolumn, 150 mm × 1 mm (TESSEK, Prague, Czechoslovakia), was packed with Silasorb SPH C_{18}, 7.5 μm (Lachema, Brno, Czechoslovakia) by the viscosity packing technique[15]. An EMD 10[16] amperometric detector with enhanced range of polarizing voltage was used to detect amino acids and dipeptides. The platinum working electrode of the EMD 10 microflow-cell was therefore replaced by the manganin electrode of 0.75 mm in diameter. The effective cell volume was calculated to be 20 nl. The detector cell was polarized continuously with a voltage of +0.2 V throughout all the measurements. The metallic appearance of the surface of the working electrode was maintained while being used in the detector for weeks. The chromatograms were recorded on a TZ 4200 line recorder (Laboratory Instruments).

Chemicals

The D,L-amino acids used were glycine, valine, leucine, phenylalanine and tryptophan (Lachema). The DL dipeptides were glycylvaline, glycylleucine and leucylglycine (Serva, Heidelberg, F.R.G.). The mobile phase was prepared from analytical grade chemicals (Lachema).

RESULTS AND DISCUSSION

Voltammetry at the microdisk electrodes

The voltammograms obtained are summarized in Fig. 1. Curves 1 and 3 show the current–voltage dependences for copper and manganin electrodes immersed in the background electrolyte. Curves 2 and 4 result from a $1.2 \cdot 10^{-3}$ *M* solution of glycine and curve 5 from a $1.2 \cdot 10^{-4}$ *M* solution of glycine in the background electrolyte. The potentials are referred to the saturated calomel electrode (SCE), whose potential is 0.242 V *vs.* the normal hydrogen electrode[17] (NHE). Comparison of curves 1 and 2 in Fig. 1 shows that with the copper electrode the limiting current is obtainable only at such potentials where a considerable increase in background current occurs. Also, the half-wave potential, which is about −0.07 V *vs.* SCE, together with the wave shape indicate the strong influence of the electrode kinetics.

This is more evident if the half-wave potential for reversible dissolution of metal (Me) in a solution of complexing ligand[18] (X) is calculated:

$$E_{1/2} = E^0_{Me} + \frac{RT}{zF} \cdot \ln \frac{2^{p-1}D'}{pD^*\beta_p[X]^{p-1}} \qquad (2)$$

Here, p is the number of ligands in the complex, [X] is the concentration of ligands, E^0_{Me} is the standard potential of the metal/ion electrode, z is the number of electrons exchanged in the electrode reaction, β_p is the cumulative stability constant of the

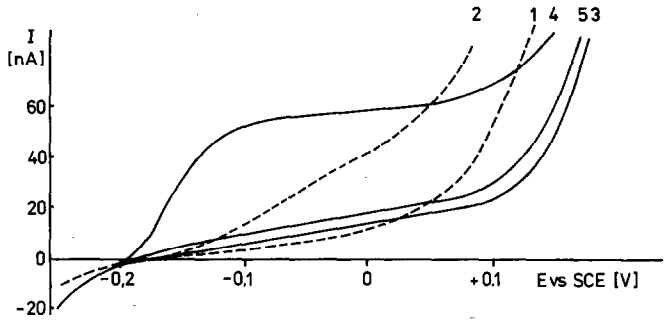

Fig. 1. Voltammograms on copper (1,2) and manganin (3,4,5) micro-disk electrodes, r = 0.075 mm, in 25 mM acetate, pH 7.5. Scan rate: 1 mV/s. Glycine concentrations: 0 (1,3), 1.2 (2,4), 0.12 mM (5).

complex, D' and D^* are the diffusion coefficients of the ligand and complex, respectively; other symbols have their usual meanings. Considering the influence of the protolytic equilibrium on eqns. 1 and 2, approximating $D'/D^* = 1$ and taking $\log \beta_2 = 15$[19], $pK_1 = 9.6$[19], $E^0_{Me} = 0.337$ V[20], $z = 2$, $p = 2$ and pH 7.5, the half-wave potential of reversible copper dissolution in a $1.2 \cdot 10^{-3}$ M solution of glycine appears to be +0.042 V *vs.* NHE or −0.200 V *vs.* SCE.

The theoretical value of the limiting current, I_d, can be calculated from the following equation[10,11]

$$I_d = \frac{z}{p} \cdot 4FD'C_X a \tag{3}$$

where C_X is the total concentration of the ligand and a is the radius of the disk microelectrode. Taking $z = 2, p = 2, C_X = 1.2 \cdot 10^{-3}$ M, $D' = 1.0 \cdot 10^{-5}$ cm^2/s[21] and $a = 7.5 \cdot 10^{-3}$ cm, we obtain $I_d = 35$ nA. This value is in good accord with the difference between curves 1 and 2, Fig. 1, at high potentials.

The response of the manganin electrode differs considerably from that of the copper electrode in the same solutions, see Fig. 1, curves 3 and 4. Mainly, its half-wave potential is about 85 mV closer to the half-wave potential calculated for a reversible electrode reaction. This indicates a considerably more rapid reaction proceeding on the electrode surface. A more detailed comparison can be made on the basis of the equation for irreversible voltammetric wave[22]:

$$\frac{I}{I_d - I} = \frac{\delta \cdot k^0}{D'} \cdot \exp[(1 - \alpha)\frac{z_r F}{RT} \cdot (E - E^{0\prime})] \tag{4}$$

Here, I is the electrode anodic current expressed in terms of a function of variable potential E, δ is the diffusion layer thickness, k^0 is the heterogeneous electrode reaction rate constant at potential $E^{0\prime}$, which potential can be set equal to the reversible half-wave potential, with the approximation $D'/D^* = 1$; z_r is the number of electrons involved in the rate-determining step of the electrode process and is usually equal to unity, α is the transfer coefficient which can be taken as equal to 0.5 to a first approximation. The potential difference of 85 mV indicates that the rate constant of

the surface reaction at the manganin electrode is, under identical conditions, about five times that at the copper electrode.

Secondly, the slope of the tangent at the inflection of the voltammetric wave is much steeper than that obtained on the copper electrode. This again indicates the higher reversibility of the reaction at the manganin electrode. However, the analysis of the wave slope[23,24] gives the number of electrons transferred as 1.6 instead of 2 for a purely diffusion-controlled reaction. Both the slope and the half-wave potential still indicate a considerable influence of the surface reaction at the manganin electrode.

Thirdly, the limiting current of the manganin electrode amounts to 42–45 nA (see Fig. 1, curve 4) which is 1.2–1.28-fold higher than that of the copper electrode. This can be explained by the anodic dissolution of minor components of manganin (Mn, Ni) without their appreciable complexation with the amino acid. The stability constants of the complexation of Mn^{2+} and Ni^{2+} with amino acids are by several orders of magnitude lower when compared to those of Cu^{2+}[19]. It follows from the weight composition of manganin[9] that the copper content is 82 atom.%. Since all components of manganin form bivalent ions in solution, the limiting current of the manganin electrode should be greater by the factor $f = 1/0.82 = 1.22$ in comparison with the equation for the dissolution of pure copper (eqn. 3).

Curve 5, Fig. 1, indicates a ten-fold decrease in limiting current with a ten-fold dilution of the amino acid solution, which is promising for detector response linearity.

The more rapid surface reaction at the manganin electrode can probably be explained by the following effects of minor components of the alloy.

Copper, manganese and nickel are totally miscible in the solid phase, which leads to the formation of mixed crystals in the alloy[25]. The alloy components are scattered statistically in the mixed crystal lattice[25]. Since copper represents the main component of the mixed crystals, the dissolution potential of the alloy is determined by the equilibrium between metallic copper and cupric ions in the solution[25]. Manganese and nickel have substantially more negative equilibrium potentials than copper, which leads to their rapid release from the manganin crystal lattice. Hence, the release of minor components regenerates crystal edges, acting as copper crystallization centres. The increase in their surface concentration can enhance anodic oxidation of copper alloy.

The second reason is the change in the properties of the surface oxide layer. Mainly, the electric conductivity of the surface film on metals has a strong influence on the metal dissolution[26]. The comparison of diagrams of electrochemical equilibria in aqueous solution[27,28] shows the possibility of the coexistence of copper, manganese and nickel oxides under the experimental conditions used. Pure copper is known to be covered with a bilayer of cuprous and cupric oxides if placed in contact with neutral aqueous solutions[29,30]. While these oxides are poor electric conductors, the manganese oxides are fair ones[31]. Moreover, the introduction of copper ions into the lattice of manganese oxides leads to an increase in conductivity by up to several orders of magnitude[32]. The conductivity enhancement of the surface oxides on the manganin electrode can facilitate the more rapid electrode reaction, which requires oxidation of cuprous compounds to cupric ones within the surface oxide layer prior to the complexation with the ligand.

It follows that the acceleration of both single-electron processes, *i.e.*, oxidation

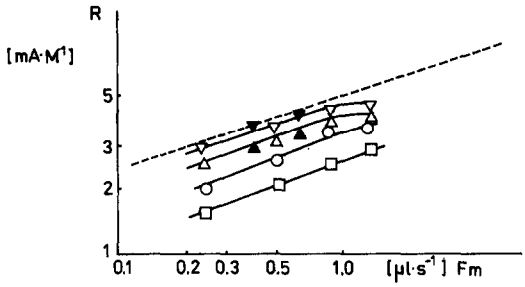

Fig. 2. Influence of the flow-rate on the response of the EMD 10 flow cell with the manganin electrode, $r = 0.375$ mm. Cell: confined wall-jet, volume 20 nl, polarized with +0.2 V. Mobile phase: 25 mM acetate, pH 7.5. Column: glass CGC 150 mm × 1 mm, packed with Silasorb SPH C_{18}, 7.5 µm. Solutes: ▽, ▼, valine; △, ▲, leucine; ○, phenylanaline; □, tryptophan. – – – –, Response calculated using eqn. 5, see text.

of metallic copper to cuprous oxide and its further conversion into cupric compounds, can speed up the anodic dissolution of manganin in relation to pure copper. Although the explanation suggested above is more or less speculative, it supports, together with the experimental evidence, the preference for a manganin working electrode in the flow-through amperometric detector, *e.g.*, for liquid chromatography.

Microcolumn liquid chromatography with amperometric detection

The compounds detected were separated on the microcolumn using a mobile phase of the same composition as the background electrolyte used for voltammetric measurements.

The manganin working electrode was used in the microflow-cell described previously[16]. The geometry of this cell is close to that of a confined wall-jet flow-through cell[33].

In the case of a diffusion-controlled electrode reaction, an increase in response, $R = I_d/C_X$, according to the 1/3 power of the flow-rate, F_m, was predicted for this type of cell geometry[33]. In the present case, considering that manganin contains 82

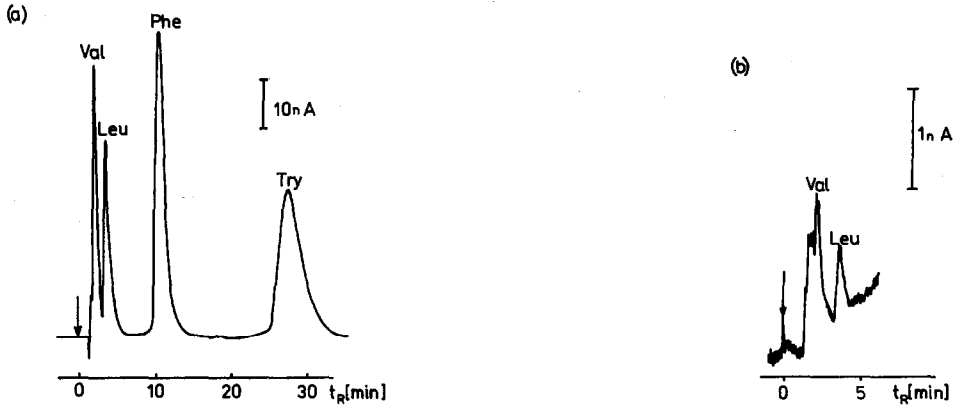

Fig. 3. Separation of selected amino acids. Sample volume: 0.75 µl. Sample concentrations: (a) Val, 0.5; Leu, 0.5; Phe, 1.5; Try, 2.5 mM; (b) Val, 0.01; Leu, 0.01 mM. For other conditions see Fig. 2.

atomic % copper so that $f = 1/0.82 = 1.22$, the dependence takes the following form

$$R = 1.22 \cdot 3.15\, F(z/p)\, (F_m D'^2\, r^4 b^{-2})^{1/3} \quad (5)$$

where b is the cell thickness and r is the radius of the disk electrode. The predicted dependence of R on F_m is shown in Fig. 2. The values $D' = 0.6 \cdot 10^{-5}$ cm^2/s (from ref. 1), $z = 2, p = 2, r = 0.375$ mm and $b = 0.05$ mm were taken for the calculation. The responses obtained from the chromatograms for valine, leucine, phenylalanine and tryptophan are depicted in the same figure. The injections of 1 mM and 0.02 mM solutions are marked with open and filled symbols, respectively. Fitting of these points to the lines common for respective amino acids indicated good linearity, as found earlier for the copper electrode[1,2,4]. With the exception of the highest flow-rates used, the dependences of log R *vs.* log F_m found are parallel to the predicted line. This indicates a diffusion-limited electrode reaction. Mutual differences between the lines for respective amino acids can be explained by the diffusion coefficients of these acids; the average value of $0.6 \cdot 10^{-5}$ cm^2/s obviously is not appropriate for all substances studied. The somewhat lower overall response found can probably be explained by the cell design. The surface opposite the working electrode is not planar, but conical, with an angle of 5°, which alters the flow pattern slightly.

A typical chromatogram of amino acid separation using the detector described is shown in Fig. 3a. Fig. 3b shows the trace detection of amino acids. The peak-to-peak noise amounts to 0.1 nA, while the signal-to-noise ratios are 12 and 8 for valine and leucine, respectively; the corresponding concentrations in the peak maxima are 0.25 and 0.2 μM. With a signal-to-noise ratio, S/N = 2, the minimum detectable concentrations are 0.04 and 0.05 μM for the respective amino acids. Similar values can also be calculated from the responses obtained with the higher concentrations injected, see Table I. In this table, the detection parameters obtained are compared with those found in the literature[2].

Several important features can be drawn out of the data shown. The advantage of the manganin electrode can be seen from a comparison of the electrode areas and the responses. In spite of its 15 times smaller area, the response is about twice as great as that of the copper electrode. This suggests a more rapid electrode reaction at manganin than at copper, as also deduced from the voltammetric measurements. Further, the background current density is about three times smaller on the manganin electrode, which correlates with the use of acetate rather than phosphate as the mobile phase, see Introduction.

The greatest practical profit from the manganin electrode follows from the decrase in the minimum detectable concentrations by about one order. Simultaneously, the rapid electrode reaction facilitates construction of a microflow-cell with an effective volume down to 20 nl without deterioration of the response and detections limits.

It was shown that a number of compounds other than amino acids such as dicarboxylic acids[5] and choline[4] can be amperometrically detected at a copper electrode. All these compounds are difficult to detect directly with high sensitivity, *e.g.*, by UV detection. The short-chain peptides also rank among this class of compounds[3,4]. It is seen (from Fig. 4) that sensitive amperometric detection of dipeptides is possible at the manganin electrode. The calculated responses are 0.8, 1.5 and 1.9

TABLE I

PARAMETERS FOR AMPEROMETRIC DETECTION OF AMINO ACIDS ON MANGANIN AND COPPER ELECTRODES

Parameter	*Ref. 2*	*This work*
Column diameter (mm)	1.1	1.0
Mobile phase	25 m*M* phosphate pH 7.2 with 10% methanol	25 m*M* acetate pH 7.5
Flow-rate (ml/min)	0.13	0.06
Cell		
Configuration	Confined wall-jet	Confined wal-jet
Volume (μl)	<1	0.02
Electrode		
Composition	Copper	Manganin
Area (mm^2)	7	0.45
Background current (nA)	700	15
Peak-to-peak noise (nA)	0.6	0.1
Response (mA/*M*)		
Valine	2.28	4.5
Leucine	1.8	4.0
Phenylalanine	2.52	3.5
Tryptophan	1.44	2.6
Minimum detectable concentration (μM)		
Valine	0.5	0.04
Leucine	0.7	0.05
Phenylalanine	0.5	0.06
Tryptophan	0.8	0.08

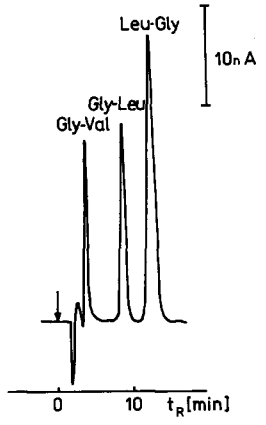

Fig. 4. Separation of selected dipeptides. Sample concentrations: Gly–Val, 0.5; Gly–Leu, 0.5; Leu–Gly, 1.0 m*M*. For other conditions see Fig. 3.

mA/M for Gly-Val, Gly-Leu and Leu-Gly, respectively. These values indicate that the minimum detectable concentrations of these substances are lower than 0.3 μM.

CONCLUSIONS

The behaviour of manganin found in the voltammetric study with the stationary microdisk electrode is confirmed with the microflow-cell HPLC detector. The microdisk electrode is easy to handle, the quantitative interpretation of the results is simple and theoretically well founded. The microdisk electrode can be recommended as a tool for optimization of the flow-cell electrode material.

The detector described was shown to detect amino acids at concentrations down to 0.04 μM. When considering the peak volume of 20–30 μl, typical of microcolumns, the detection limits appear to be about 1 pmol. Similarly, sensitive detection can be achieved for dipeptides and, according to preliminary experiment, also for other complexing solutes, *e.g.*, aromatic phenolic acids and aromatic amino acids.

The discussion of the properties of the manganin electrode leads to the conclusion that the alloy composition used need not be optimal either qualitatively or quantitatively. Further research into the electrode material composition may provide interesting results.

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