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# Simultaneous amperometric and potentiometric detection of sugars, polyols and carboxylic acids in flow systems using copper wire electrodes

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**Abstract**

Copper wire electrodes, used for amperometric and potentiometric detection in flow injection analysis and high-performance liquid chromatography, have been developed for the 'simultaneous' detection of sugars, polyols and carboxylic acids. Sugars and polyols were oxidised at +0.5 V (versus Ag|AgCl|0.1 *M* $Cl^-$) in 100 m*M* NaOH and citric acid was potentiometrically detected at a copper wire in Milli-Q water. Citric acid, acetic acid, glucose, fructose, glycerol and ethanol were separated by ion-exclusion chromatography with water as eluent and passed through the potentiometric detector then the amperometric detector after addition of 100 m*M* NaOH. The amperometric detector gave detection limits of 5, 5, 2 and 80 pmol for glucose, fructose, glycerol and ethanol, respectively, with a linear response over 3 decades ($10^{-6}$–$10^{-3}$ *M*). For the potentiometric detector, the detection limits were 10 and 50 pmol for citric acid and acetic acid and linearity was in range of $10^{-5}$ *M*–$10^{-3}$ M. Compared with a universal detector such as refractive index, the reported combination electrochemical detector shows high sensitivity and low detection limits.

*Keywords:* Copper electrodes; Amperometic detection; Potentiometric detection; Detection, LC; Polyols; Carboxylic acids; Carbohydrates

## 1. Introduction

The development of electrochemical detection methods in HPLC for the determination of carbohydrates and carboxylic acids has received considerable attention in recent years because of their importance in beverages and foods, where they are both found [1,2]. Carbohydrates and carboxylic acids are difficult to detect by conventional spectrophotometric methods because they lack suitable chromophores or fluorescent groups. Refractive index can be used for the detection of these compounds, but with limited sensitivity [3]. Chemical derivatization has been used to enhance the sensitivity and detection limit of spectrophotometric detection. The disadvantage of this process is that it is time consuming and other reagents present in the samples can interfere with the analysis [4]. A simple and sensitive detection method for the HPLC of these compounds is therefore highly desirable.

Electrochemical detection (potentiometry and amperometry) has been recognised as a useful method for the detection of carbohydrates and carboxylic acids following separation by HPLC [5]. A number of methods based on electrochemical oxidation using

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various metallic electrodes have been reported for the detection of carbohydrates following HPLC [1,6]. Fouling of gold and platinum electrodes may be overcome by pulsed amperometric detection which combines amperometric detection with alternate anodic and cathodic polarization to clean and reactivate the electrode surface. However constant potential amperometry is preferred because of its instrumental simplicity and inherent sensitivity. Metals such as Cu [7], Ni [8] and Ag [9], as well as chemically modified electrodes [10] have been developed for the electrocatalytic oxidation of carbohydrates at alkaline media. From cyclic voltammetry studies it has been shown that glycine and ethanol, and carbohydrates such as glucose are oxidised in 100 m*M* NaOH solution above +0.5 V vs. AgCl|Ag [11]. The use of a copper-based electrode for the amperometric detection of carbohydrates following separation by anion-exchange chromatography has been described recently [12,13].

In contrast to amperometric detection, potentiometric detection in HPLC using ion-selective electrodes has received less attention. Heterogeneous membrane ion selective electrodes (ISEs) with PVC containing a quaternary ammonium salt and a lipophilic macrocyclic pentamine, were used to detect carboxylic acids after separation by ion-exclusion chromatography. The detection limits were at least 100 times lower than the values obtained with UV detection [14].

A copper electrode has been reported for indirect-redox potentiometric detection in ion chromatography [15]. This electrode can provide a response to solutes which complex copper ions more or less strongly than eluent components. Passage of such a solute past the electrode causes a change in the local concentration of copper ion near the electrode, and hence a change in potential.

In this paper, we report the use of copper electrodes for potentiometric detection of carboxylic acids and the amperometric detection of carbohydrates after separation by ion-exclusion chromatography. The copper electrodes have been evaluated by both voltammetry and flow injection analysis, and the application of this combination detector is demonstrated in ion-chromatography for the analysis of organic anions and neutral compounds.

## 2. Experimental

### 2.1. Instrumentation

Voltammetric measurements were performed on BAS 100 B electrochemical analyzer (Bioanalytical Systems, West Lafayette, USA) with a three-electrode cell using a copper wire working electrode, an Ag|AgCl|0.1 *M* $Cl^-$ reference electrode and platinum wire auxiliary electrode. A Teflon tube allowed purging of the solution with nitrogen. During the measurements nitrogen was passed over the solution.

A home made potentiometric flow-through detector incorporating a copper wire indicator electrode was connected to a millivolt meter and interfaced to a personal computer. Amperometric measurements were performed using a Waters (Model 480) electrochemical detector, a home made amperometric flow-through electrochemical cell [16] with a Cu wire electrode, Ag|AgCl reference electrode and platinum wire auxiliary electrode interfaced to a personal computer. The arrangement of cells and electrodes is shown in Fig. 1. Flow injection and liquid chromatography experiments were carried out with Waters 510 pump equipped with a U6K injector and a Waters refractive index detector. Post column addition of 100 m*M* NaOH was accomplished by a peristaltic pump delivering solution at 0.5 ml $min^{-1}$. It would have been better to use an HPLC pump, but the noise was reduced by employing a 2-m restric-

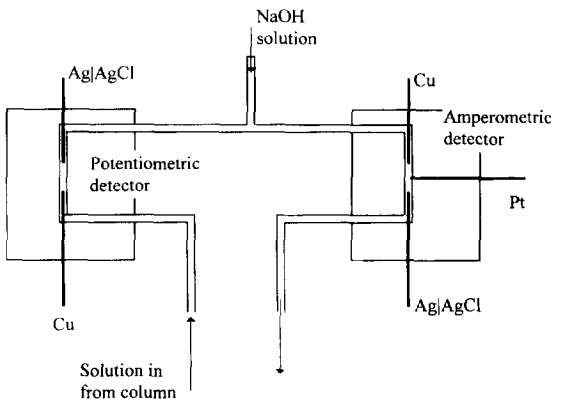


Fig. 1. Diagram of the cells and electrode arrangement for the simultaneous detection of carboxylic acids (potentiometric cell) and carbohydrates (amperometric cell).

tion tube. A mixing 'T' was connected to the exit of the potentiometric detector. Chromatographic separation of tested solutes was performed with an ion-exclusion column (Bio-RAD, HPX-87H) with Milli-Q water at a measured pH of 5.5 as eluent, operating at 30°C.

### 2.2. Chemicals

Standard solutions of sugars, polyols and carboxylic acids were prepared from analytical grade reagents in doubly distilled water. Eluents were treated by passage through a Millipore Milli-Q water purification system (Bedford, MA, USA). Prior to use, all eluents were filtered through a 0.45-μm membrane filter and degassed in an ultrasonic bath. Solutions of sodium hydroxide were protected from carbon dioxide and oxygen by purging with high purity nitrogen. Experiments were performed by using 100 m*M* NaOH as electrolyte unless otherwise specified.

### 2.3. Electrode preparation

The copper wire electrode (3 cm×0.5 mm) used for potentiometric and amperometric detection was polished with alumina. For potentiometric detection, it was conditioned in water prior to use. For voltammetry the electrode was held at −1.0 V vs. Ag|AgCl for 3 min in 100 m*M* NaOH solution before voltammetric scans were initiated. In most cases, the electrodes were cycled briefly from 0.0 to +0.6 V in 100 m*M* NaOH before use in flow injection systems. This usually served to decrease background currents and the length of time required for the background to become stable.

## 3. Results and discussion

### 3.1. Cyclic voltammetry

Cyclic voltammetry was used to study the electrochemical oxidation of carbohydrates at the copper electrode in alkaline solution. The behaviour of the copper electrode in deaerated 0.1 *M* NaOH solution was examined by sweeping the potential between −1.2 V to +1.2 V at a scan rate of 100 mV $s^{-1}$. Voltammograms obtained at a copper electrode in NaOH electrolyte, and with added glucose and glycerol are shown in Fig. 2. Oxidation of copper occurs at potentials greater than 0.0 V and the oxides formed are reduced showing two distinct peaks −0.65 V ($Cu^{II} \rightarrow Cu^{I}$) and −0.95 V ($Cu^{I} \rightarrow Cu^{0}$). Above +0.5 V, a transition to $Cu^{III}$ occurs. In general, our results agree well with previous studies of copper electrodes in alkaline solution [17,18]. Addition of 100 m*M* glucose and glycerol solutions results in enhancement of the anodic current above +0.55V. It has been proposed that $Cu^{III}$ participates in the electrocatalytic oxidation of organic compounds [12,19], and this is supported by our findings for glucose and glycerol.

The anodic currents varied linearly with the concentration of the tested solutes.

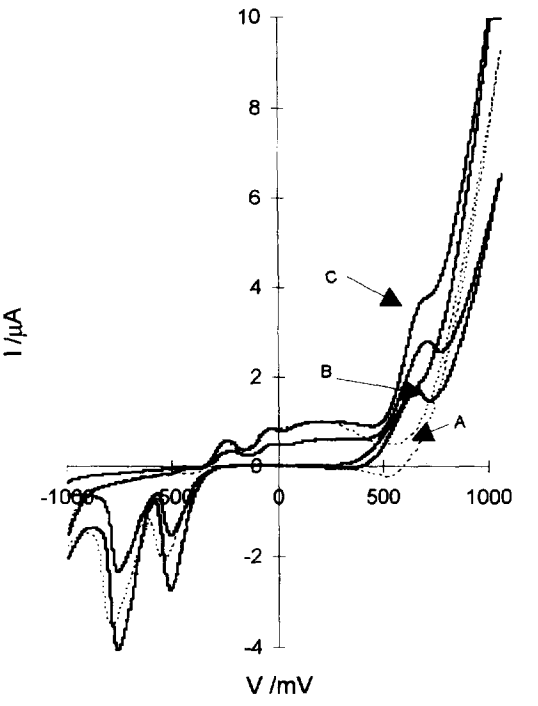


Fig. 2. Cyclic voltammograms at a copper electrode, scan rate of 100 mV $s^{-1}$ (A) electrolyte 100 m*M* NaOH (dotted line), (B) electrolyte 5 m*M* glucose in 100 m*M* NaOH, (C) electrolyte 5 m*M* glycerol in 100 m*M* NaOH.

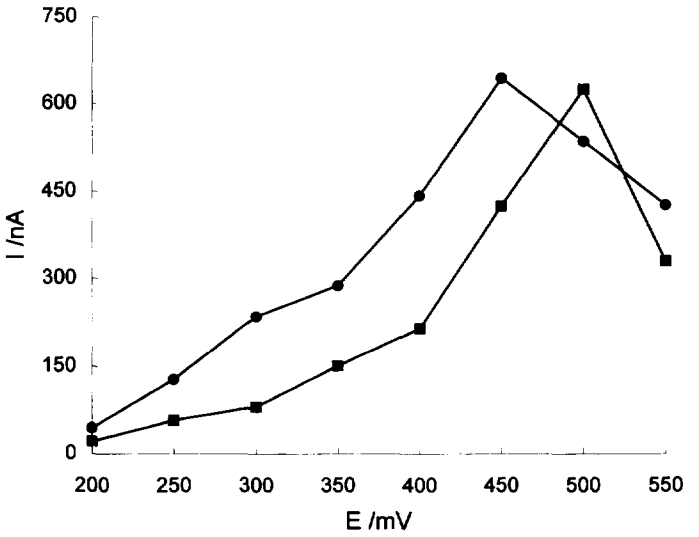


Fig. 3. Hydrodynamic votammograms of glucose and glycerol at a copper electrode in a FIA system. Carrier: 100 m*M* NaOH, flow-rate: 0.5 ml $min^{-1}$, injection: 10 μl×1 m*M*.

### 3.2. Flow injection detection

Fig. 3 shows the hydrodynamic voltammograms of glucose and glycerol at a copper electrode obtained under flow injection conditions with injections of 10 μl of 1 m*M* glucose and glycerol in 100 m*M* NaOH at different applied potentials. The copper electrode shows a maximum response at +0.45 V for glycerol and +0.5 V for glucose which is somewhat less than the cyclic voltammetric peaks (Fig. 2).

The response exhibits a maximum as a result of the background oxidation current which increases with applied potential [21]. A similar result was observed at a copper chemically modified electrode [22]. At higher potentials, too, the response was less precise. Therefore, to maintain electrode stability and a low background current, an applied potential of +0.45 V was selected for subsequent FIA and chromatography experiments.

The FIA current at a copper electrode for 10-μl injections of glucose in 100 m*M* NaOH is shown in Fig. 4. Repeatable peaks were obtained at +0.45 V and linearity between current and concentration was observed over a range $10^{-6}$ to $10^{-3}$ *M* with a sensitivity of 37.9 nA $nmol^{-1}$ for a 20-μl injection. Five injections of glucose (10 μl×0.5 m*M*) gave a mean current of 242 nA with a relative standard deviation of 2% ($n=5$). The copper electrode proved to be stable and was used up to one week before it was repolished.

A typical potentiometric response of the copper electrode for 10 μl injections of citric acid is shown in Fig. 5, using Milli-Q water as carrier. Negative FIA peaks were obtained as a result of the decrease in $Cu^{2+}$ concentration at the copper electrode from complexation by citric acid [15]. The calibration plot exhibits the expected Nernstian behaviour with a slope of −28.9 mV $pCu^{-1}$ and linearity was in the range of $10^{-5}$ *M*–$10^{-3}$ *M*. The electrode potential saturates at high concentrations of citric acid. The reproducibility of the potentiometric response was evaluated by successive injections of 10 μl of 1 m*M* citric acid which gave a mean potential of 30 mV with a R.S.D. of 3% ($n=5$). The copper electrode was used for 2 weeks without any change in response.

### 3.3. Chromatographic detection

The goal of this work is to develop a sensitive and stable electrochemical LC detector for carboxylic acids and carbohydrates. Conventional amperometric or potentiometric LC detectors used separately do not offer simultaneous detection of sugars, polyols

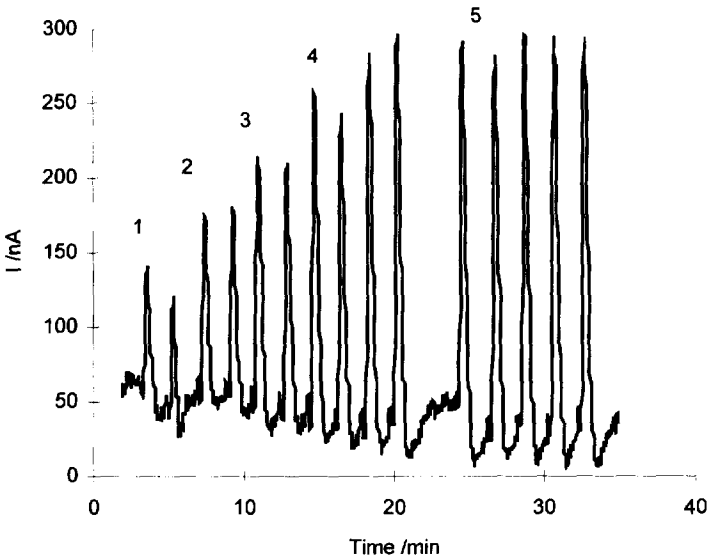


Fig. 4. FIA oxidation currents of glucose at a copper electrode. Figures on peaks are injected amounts in nmol. The last five peaks are replicate injections of 5 nmol. Applied potential: +0.50 V vs. Ag|AgCl, carrier: 100 m*M* NaOH, flow-rate: 0.5 ml $min^{-1}$, injection volume 20 μl.

and carboxylic acids. Ion-exclusion chromatography may be used for the separation of sugars, polyols and carboxylic acids, and is therefore a candidate for use with the proposed copper electrochemical detectors.

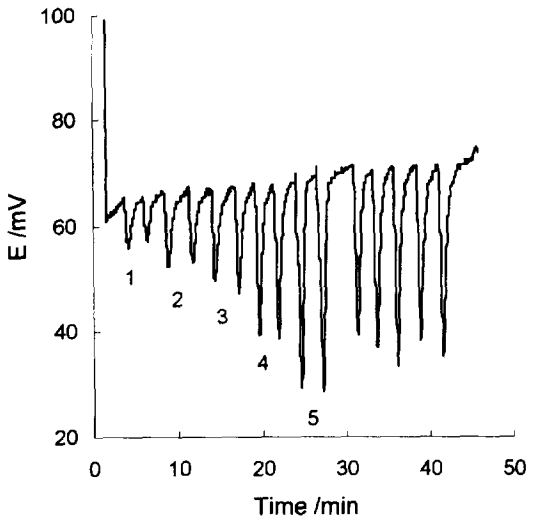


Fig. 5. FIA potentiometric response to citric acid at a copper electrode. Figures on peaks are injected amounts in nmol. The last five peaks are replicate injections of 4 nmol. Carrier Milli-Q water, flow-rate: 0.5 ml $min^{-1}$, injection volume 10 μl.

In ion-exclusion chromatography, the replacement of eluent ions by solute does not occur in the same manner as for ion-exchange chromatography. Retention of solutes is governed by a combination of the Donnan exclusion effect, size exclusion from the pores of the stationary phase and reversed-phase interaction with the unfunctionalised regions of the resin [15]. In general, the best conditions for chromatographic separation may be different from those required for optimum detector sensitivity. In this system, a fast and a efficient resolution of solutes separated by ion-exclusion chromatography requires dilute acid as a eluent [15], the electro-oxidation of carbohydrates requires alkaline medium and the best potentiometric response for carboxylic acids is expected for eluents which are moderately weak in their complexation of copper [2,15,20]. Therefore, dilute solutions of acids or water have been used as the eluent for the separation of carbohydrates and carboxylic acids in ion-exclusion chromatography and as the carrier for potentiometric detection, with post column addition of NaOH as the electrolyte for the amperometric detector. This combination gave good chromatograms and high sensitivity and low detection limits for both detectors.

To achieve optimum conditions for the chromatog-

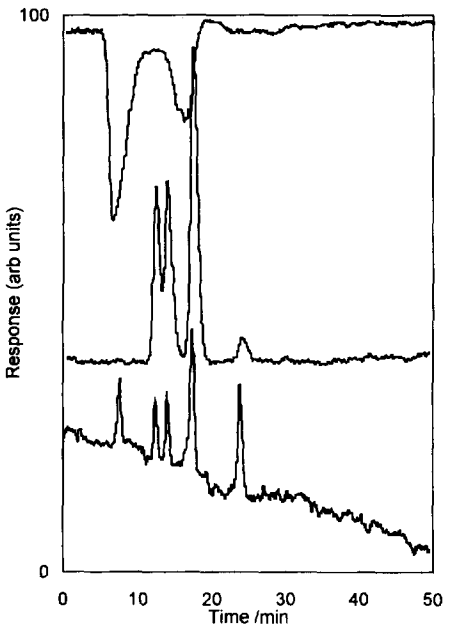


Fig. 6. Chromatography of a mixture of 1 m*M* each of (in order of elution) citric acid, glucose, fructose, acetic acid, glycerol and ethanol. Eluent, Milli-Q water, flow-rate: 0.5 ml $min^{-1}$. Injection volumes 10 μl for electrochemical detection, 20 μl for RI detection. Upper curve: potentiometric detection of citric acid and acetic acid at a copper electrode. Middle curve: amperometric detection of glucose, fructose, glycerol and ethanol at a copper electrode. Post column addition of 100 m*M* NaOH at flow-rate: 0.5 ml $min^{-1}$. Applied potential +0.5 V vs. Ag|AgCl. Lower curve: refractive index detector (sensitivity: RI×0.5). Acetic acid and glycerol coelute.

raphy and detection sensitivity, different eluents were examined, including dilute solutions of phosphoric and sulfuric acids, and water buffered to various pH values. In each case the flow-rate was also optimised. It was found that the optimum conditions for the chromatographic separation were not the same as those for sensitive detection of the analytes by both amperometric and potentiometric detectors. Therefore it was necessary to compromise in order to select suitable conditions for both separation and detection. The top two curves of Fig. 6 show the simultaneous chromatograms obtained for glucose, fructose, glycerol, ethanol, citric acid and acetic acid in a carrier of Milli-Q water, with post-column addition of 100 m*M* NaOH before the amperometric detector. A negative potentiometric response to citric acid and acetic acid was observed in agreement with previous reports [2,20], and the amperometric detector responded to glucose, fructose, glycerol and ethanol. The lowest curve in Fig. 6 is a chromatogram of the same mixture obtained from a refractive index detector in a separate experiment with the same chromatographic conditions. Both amperometric and potentiometric responses were more sensitive that that of the RI detector and successfully resolved co-eluting acetic acid and glycerol. It is noted that the potentiometric peaks were broad, even though this cell was first to detect ions off the column. The slow response of metal wire electrodes has been noted and is ascribed to the slow kinetics of interaction between the ions and $Cu^{2+}$ at the electrode surface [15].

Calibration of the amperometric detector was linear from $10^{-6}$ to $10^{-3}$ *M* for sugars and polyols and the electrode potential *vs.* log *C* for the potentiometric detector was linear from $10^{-5}$ to $10^{-3}$ *M*. The detection limits (3 $\sigma_{blank}$) of different detectors are listed in Table 1 together with retention times of the test analytes. The detection limits of the electrochemical detectors were much better than that of the RI detector under the conditions used in this study.

Table 1
Detection limits in ion-exclusion chromatography using different detectors

| Compound | Amperometric (pmol) | Potentiometric (pmol) | RI (pmol) | Retention time (min) |
|---|---|---|---|---|
| Citric acid | – | 10 | 1000 | 9.0 |
| Acetic acid | – | 50 | 2000 | 19.5 |
| Glucose | 5 | – | 1000 | 12.5 |
| Fructose | 5 | – | 1000 | 14.0 |
| Glycerol | 2 | – | 5000 | 18.5 |
| Ethanol | 80 | – | 2000 | 25.0 |

Experimental conditions are described in Fig. 6.

## 4. Conclusion

This study has demonstrated the simultaneous electrochemical detection of carboxylic acids and carbohydrates. The proposed system overcomes the limitations of conventional electrochemical LC detection employing a single detector. The proposed electrochemical detection technique (both amperometry and potentiometry) offers higher sensitivity and lower detection limits than RI combined with simplicity and low cost. This novel electrochemical detector could be coupled with other separation mechanisms such as reversed-phase, ion-exchange and ion-interaction chromatography by use of suitable electrodes.

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