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ELECTROCHEMICAL DETECTOR FOR MICRO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND ITS APPLICATION TO THE DETERMINATION OF AMINOPHENOL ISOMERS

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SUMMARY

A flow thin-layer electrolytic cell using a glassy carbon electrode was designed and characterized for use in an electrochemical detector in micro high-performance liquid chromatography. The detector was utilized for the determination of aminophenol isomers separated on a micro ODS column. The linear dynamic range between peak current and concentration was about 10^3 (10 pg–10 ng) and the minimum detectable amount was about 10 pg for three isomers. The method was also applied to the determination of aminophenols present as impurities in high-purity aminosalicylic acid.

INTRODUCTION

Micro high-performance liquid chromatography (MHPLC)[1] is powerful new technique for the analysis of trace samples of biomedical origin. Previously only spectrophotometric detectors have been employed for the detection of trace compounds in eluents in MHPLC. Although spectrophotometric detectors are easy to use in MHPLC they have poor specificity.

Several different electrochemical techniques have been applied in detectors for ordinary HPLC. Recently a review paper with more than 70 references concerning electrochemical detection was published[2]. Amperometric detectors have been especially successful and some designs are already commercially available. However, none of them is designed for MHPLC, in which the detector should have a very small cell volume and dead volume. The active region of most amperometric detectors consists either of a tubular electrode or a thin-layer cell. The latter seems to be more suitable for MHPLC because of the ease with which a small cell volume and dead volume can be achieved.

In this paper, we describe an amperometric detector based on a thin-layer cell suitable for use in MHPLC and its application to the characterization of aminophenol isomers separated on a micro ODS column.

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EXPERIMENTAL

Apparatus

Fig. 1 shows schematically the micro high-performance liquid chromatograph using an electrochemical detector employed in this study. A micro feeder (Azuma Denki Co., Model MF-2) and a micro syringe (Terumo Co., Model GAN-0.25) were used for feeding the mobile phase and direct injection of samples into the micro separation column. For chromatographic separation, a 15 cm × 0.5 mm I.D. PTFE column was packed with Yanapak ODS by using the technique described previously[3].

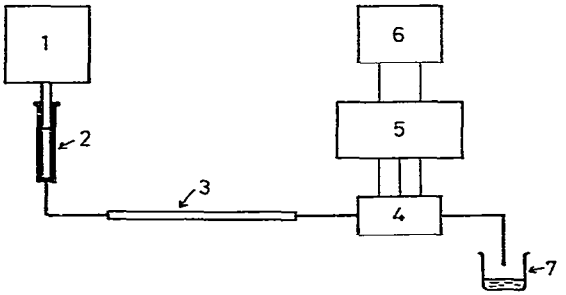

Fig. 1. Schematic diagram of a micro high-performance liquid chromatograph using an electrochemical detector 1 = Micro feeder; 2 = micro syringe; 3 = separation column; 4 = electrolytic cell; 5 = polarograph; 6 = recorder; 7 = waste reservior.

The design[4] of the electrolytic cell detector and the connection with the micro separation column are shown in Fig. 2. The cell blocks were made of a fluorocarbon resin and the working electrode was a glassy carbon disk of diameter 3 mm. An Ag/AgCl electrode was used as the reference electrode. A stainless-steel tube served both as the exit line and the counter electrode. A wide hole was cut around the tip of

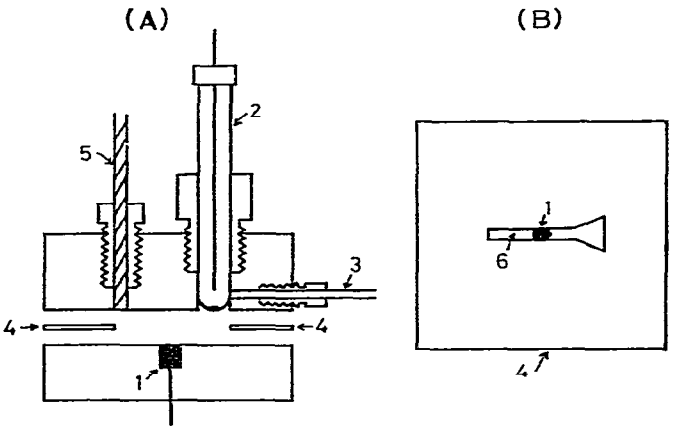

Fig. 2. Construction of thin-layer electrolytic cell and connection with separation column. (A) Side view of cell; (B) top view of spacer. 1 = Working electrode (glassy carbon); 2 = reference electrode (Ag/AgCl); 3 = counter electrode (stainless-steel tube); 4 = spacer (PTFE sheet); 5 = separation column; 6 = hole

the reference electrode in the spacer to lower the cell resistance between the working and reference electrodes.

A polarograph (Yanagimoto Co., Model P8) was employed for potentiostat and current measurements. Chromatograms were recorded on a time recorder (Yanagimoto Co., Model YR-101P).

Reagents

p-, *m*- and *o*-aminophenol for use as standards were obtained commercially and used without further purification. Special-grade *p*-aminosalicylic acid samples from four different suppliers were used for the determination of aminophenol impurities. The mobile phase used for the separation of aminophenol isomers and aminosalicylic acid was 0.1 *N* perchloric acid.

RESULTS AND DISCUSSION

Characterization of electrochemical detector

In order to study the effect of spacer thickness and cell width on the response, the detector was connected directly to a micro syringe containing the mobile phase, in which various concentrations of *o*-aminophenol were present, and the currents were measured at a flow-rate of 8.3 μl/min. Figs. 3 and 4 show the relationships between concentration and current at various spacer thicknesses and cell widths, respectively. The sensitivities for spacer thicknesses of 45, 30 and 20 μm were $105 \cdot 10^3$, $101 \cdot 10^3$ and $94 \cdot 10^3$ A · sec/mol, respectively, at cell width of 2 mm. The sensitivity tended to decrease with decreasing the cell width from 2 to 0.5 mm (see Fig. 4).

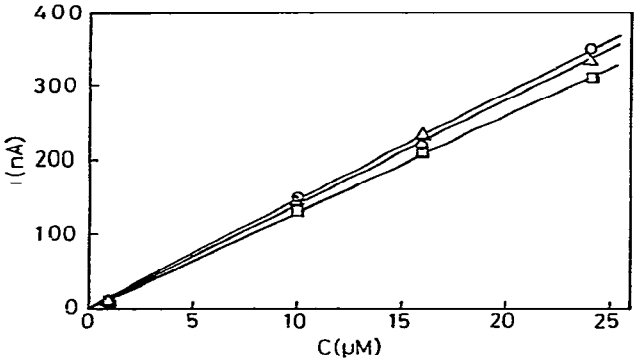

Fig. 3. Dependence of current on *o*-aminophenol concentration at various spacer thicknesses. ○, 45 μm; △, 30 μm; □, 20 μm Cell width, 2 mm; flow-rate, 8 3 μl/min; applied potential, 1.0 V *vs.* Ag/AgCl

On the basis of the results, we decided to use an electrolytic cell with a spacer thickness of 45 μm and a cell width of 2 mm for the chromatographic determination of aminophenol isomers. The cell volume and dead volume in such a cell were about 0.3 and 0.6 μl, respectively. It should be noted that the sensitivity of the cell at a flow-rate of 8.3 μl/min is about 20 times higher than that at a flow-rate of 0.75 ml/min ($4.24 \cdot 10^3$ A · sec/mol for dopamine)[4].

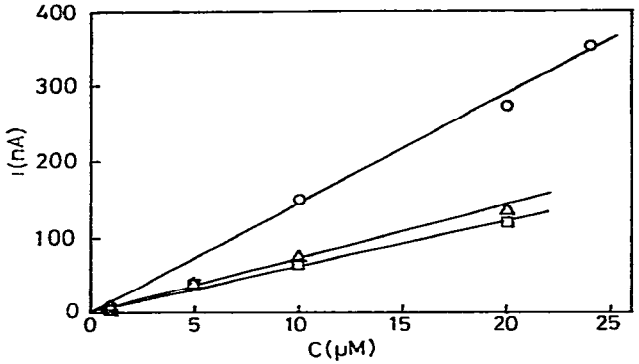

Fig 4. Dependence of current on *o*-aminophenol concentration at various cell widths: ○, 2 mm; △, 1 mm; □, 0.5 mm. Spacer thickness, 45 μm; flow-rate, 8 3 μl/min; applied potential, 1.0 V *vs.* Ag/AgCl.

Chromatogram of aminophenol isomers

To examine the sensitivity of the electrochemical detector, the cell was connected with outlet of the micro ODS separation column and the currents were measured after injection of various amount of aminophenol isomers. Fig. 5 shows the chromatograms of the mixture of aminophenol isomers at different applied potentials. When the applied potential was 1.00 V (*vs.* Ag/AgCl), all three isomers separated on the micro column were detected. At lower applied potentials of 0.75 or 0.50 V, only *p*- and *o*-aminophenol could be selectively detected. Three isomers were completely eluted within 10 min at a flow-rate of 8.3 μl/min with 0.1 *N* perchloric acid. Fig. 6 shows the current–potential curves obtained from the chromatographic peak current of the respective aminophenol isomers at each potential. The peak currents of *o*- and *p*-aminophenol reached a plateaux at about 0.75 V, while that of *m*-aminophenol required about 1.0 V to reach a plateau.

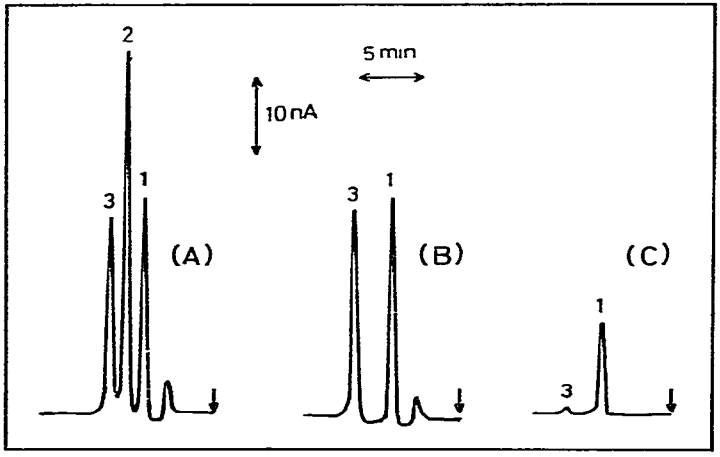

Fig. 5. Typical chromatograms of aminophenol isomers at different applied potentials. Peaks: 1 = *p*-; 2 = *m*-; 3 = *o*-aminophenol. Applied potential (V *vs.* Ag/AgCl), (A) 1.00, (B) 0.75, (C) 0.50; flow-rate, 8.3 μl/min; separation column, Yanapak ODS, 147 × 0.5 mm I.D.; eluent, 0.1 *M* $HClO_4$; sample size, 0.9 μl, each 10 μ*M*; cell width, 2 mm; spacer thickness, 45 μm.

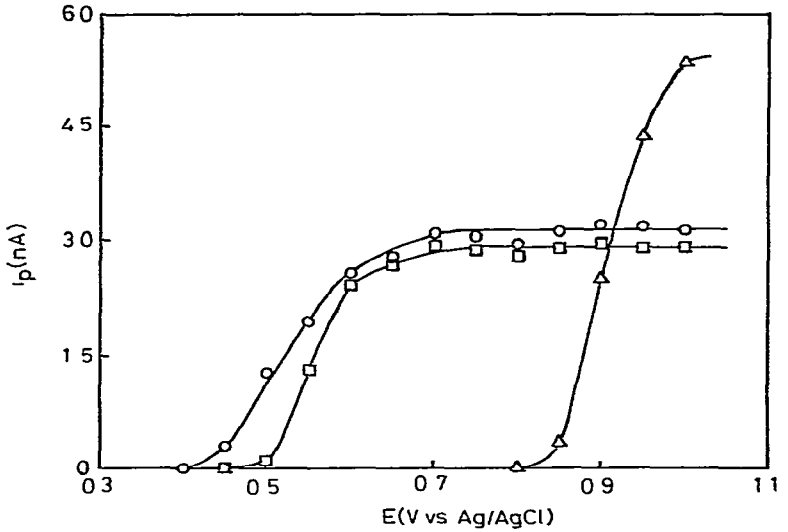

Fig. 6 Relationship between peak current and applied potential for aminophenol isomers □, *o*-; △, *m*-; ○, *p*-aminophenol. Experimental conditions as in Fig 5

The dependence of the sensitivity on the flow-rate was studied by monitoring the chromatographic peak current for aminophenol isomers. As shown in Fig. 7, the peak current tended to increase gradually with increasing flow-rate, which reflects both effects based on diffusion of the sample in the separation column and change of electrolytic efficiency in the electrolytic cell with flow-rate. A flow-rate of 8.3 μl/min was selected for the chromatographic determination of aminophenols, because the separation of the isomers became incomplete at higher flow-rates. The electrolytic efficiencies for *o*-, *m*-, and *p*-aminophenol at a flow-rate of 8.3 μl/min were about 64%, 76% and 52%, respectively. It should be noted that these efficiencies are about 20

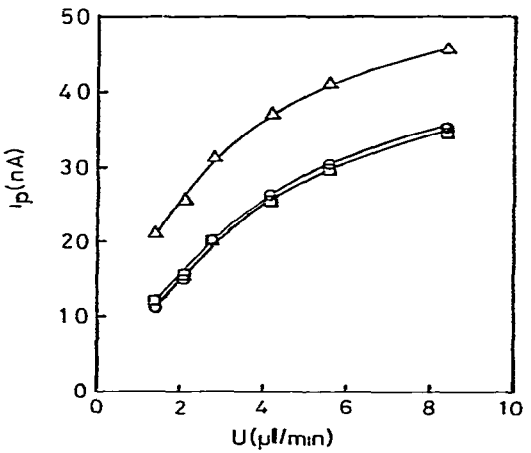

Fig. 7. Relationship between peak current and flow-rate for aminophenol isomers □, *o*-; △, *m*-; ○, *p*-aminophenol. Applied potential, 1.0 V *vs.* Ag/AgCl; separation column, 145 × 0.5 mm I D ; other conditions as in Fig. 5.

times higher than those[4,5] (a few percent) at a flow-rate of about 1 ml/min in HPLC. Fig. 8 shows the relationship between peak current and sample volume injected for aminophenol isomers, each at a concentration of 10 μM. In all instances, the peak current was proportional to the sample volume up to 1.5 μl. This suggests that the maximum possible sample volume in this chromatographic system is 1.5 μl.

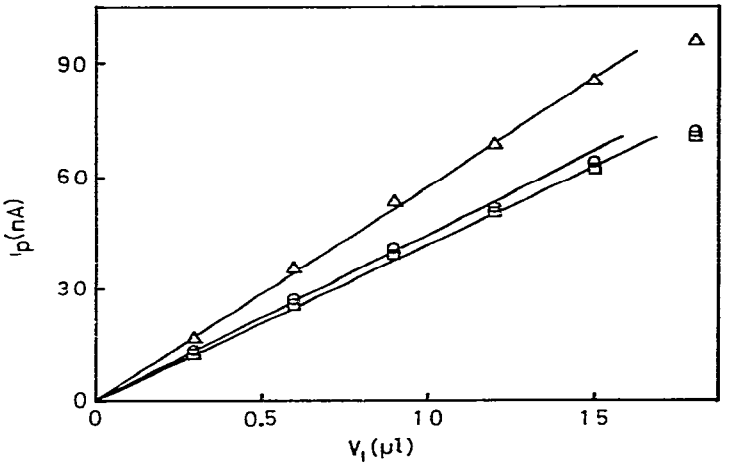

Fig 8. Relationship between peak current and sample volume injected for aminophenol isomers (each 10 μM). □, *o*-; △, *m*-; ○, *p*-aminophenol. Applied potential, 1.0 V *vs.* Ag/AgCl; separation column, 145 × 0 5 mm I.D.; other conditions as in Fig. 5

Fig. 9 shows typical calibration graphs for aminophenol isomers with a flow-rate of 8.3 μl/min, a sample volume of 0.9 μl and an applied potential of 1.0 V. The peak current was proportional to amount up to 10 ng and the linear dynamic range was about 10^3 (10 pg–10 ng). The minimum detectable amount was about 10 pg for

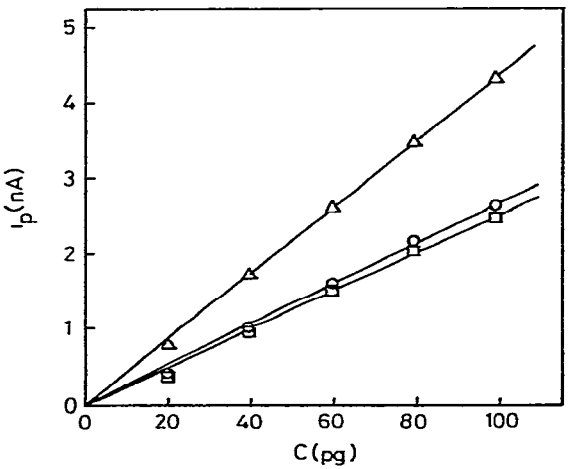

Fig 9. Calibration graphs for aminophenol isomers □, *o*-; △, *m*-; ○, *p*-aminophenol. Sample volume injected, 0.9 μl; applied potential, 1.0 V *vs.* Ag/AgCl; other conditions as in Fig. 5.

each isomer. The relative standard deviations for the determination of *o*-, *m*- and *p*-aminophenol were about 2.1, 2.1 and 2.0%, respectively, for ten measurements. Maruyama and Kakemoto[6] utilized a voltammetric detector with a wall jet electrode for the determination of aminophenol isomers by HPLC and reported the minimum detectable amount to be 0.2 ng for *m*-aminophenol and 0.5 ng for *o*- and *p*-aminophenol. Hence the proposed detector for MHPLC is about 20–50 times more sensitive than the detector using a wall jet electrode for HPLC.

Application to the determination of impurities in aminosalicylic acids

It is expected that small amounts of aminophenol will be present in high-purity aminosalicylic acid reagent, as aminophenol is the starting material in the preparation of the latter. The proposed technique using a thin-layer electrolytic cell was therefore applied to the determination of aminophenol impurity.

Both aminosalicylic acid and aminophenols undergo facile electrochemical oxidation on a stationary glassy carbon electrode. Amperometric monitoring of column effluents can therefore be used to detect both the aminosalicylic acids and aminophenols. Fig. 10 shows typical chromatograms for *m*-aminophenol as an impurity in a special-grade *p*-aminosalicylic acid reagent with and without a standard addition. The chromatographic conditions were the same as above for the separation of aminophenol isomers. It is easy to determine *m*-aminophenol as an impurity, because *p*-aminosalicylic acid elutes behind aminophenol isomers. Table I gives the results for the determination of impurities in special-grade *p*-aminosalicylic acids obtained from four different suppliers. *m*-Aminophenol in the range 0.21–0.91 wt.-% was found as an impurity in the reagents investigated, but *o*- and *p*-aminophenol were not detected on injecting 18 ng of *p*-aminosalicylic acid reagent.

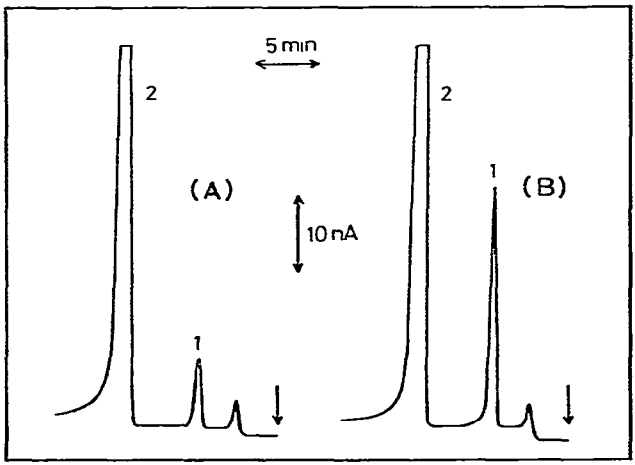

Fig. 10 Typical chromatograms of *m*-aminophenol as impurity in *p*-aminosalicylic acid reagent Peaks 1 = *m*-aminophenol; 2 = *p*-aminosalicylic acid. Sample size. (A) 18 ng of *p*-aminosalicylic acid; (B) 18 ng of *p*-aminosalicylic acid plus 0 25 ng of *m*-aminophenol. Other conditions as in Fig. 5

TABLE I
DETERMINATION OF *m*-AMINOPHENOL AS AN IMPURITY IN SPECIAL-GRADE *p*-AMINOSALICYLIC ACID REAGENTS

Sample No	*Impurity (%, w/w)*
1	0.72
2	0 21
3	0.34
4	0.91

CONCLUSIONS

The electrochemical detector using a flow thin-layer electrolytic cell can be utilized for the determination of 10 pg–10 ng of aminophenol isomers by MHPLC. The sensitivity of the proposed technique is over ten times better than that in ordinary HPLC using electrochemical detectors[4,5]. The electrochemical detector is much more sensitive than the UV detector (254 nm) for aminophenols[6–8]. A small amount of *o*- and *p*-aminophenol in *m*-aminophenol can be selectively determined by means of selection of the applied potential. The technique is applicable to the determination of a small amount of impurity (aminophenols) in high-purity aminosalicylic acids.

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