ELSEVIER

Journal of Chromatography A, 905 (2001) 309–318

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Applications of a copper microparticle-modified carbon fiber microdisk array electrode for the simultaneous determination of aminoglycoside antibiotics by capillary electrophoresis

Wen-Chu Yang, Ai-Min Yu, Hong-Yuan Chen*

Department of Chemistry, State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing 210093, China

Received 26 May 2000; received in revised form 23 August 2000; accepted 21 September 2000

Abstract

A copper microparticle-modified carbon fiber microdisk array electrode was fabricated and employed in capillary electrophoresis for the simultaneous determination of the five aminoglycoside antibiotics (AGs) including netilmicin, tobramycin, lincomycin, kanamycin and amikacin. The array electrode exhibited high catalytic activity for AGs, good reproducibility and stability. Under the optimum separation conditions (separation voltage of 6.2 kV, electrophoretic medium of 125 mM NaOH), the five AGs above were baseline separated within 20 min. At a working electrode potential of 0.7 V (versus saturated calomel electrode), the calibration curves were linear over two orders of magnitude of concentration, and the detection limits ($S/N=3$) were below 2 μM except for lincomycin (6.7 μM). The developed method was successfully employed for the simultaneous determination of the five AGs studied in pharmaceutical injections. The feasibility of this method for the simultaneous determination of lincomycin, kanamycin and amikacin in urine sample was also demonstrated.

Keywords: Electrochemical detection; Detection, electrophoresis; Aminoglycosides; Glycosides; Antibiotics

1. Introduction

Aminoglycoside antibiotics (AGs), such as netilmicin, tobramycin and so on, are widely used in both human and veterinary medicine against both Gram-positive and -negative bacterial infections. However, it has been well known that they cause damage to the kidneys and cranial nerves, resulting in renal dysfunction and irreversible hearing less. To assure therapeutic serum concentration and to minimize these toxicities, frequently and carefully monitoring of AGs levels in blood or urine is essential. Therefore, it is necessary to develop a simple, efficient, and sensitive method for the determination of AGs in both pharmaceuticals and biological fluids.

Yet, the separation and detection for AGs are difficult for their similarities in structure and having no strong UV chromophore or fluorophore which is necessary for direct detection by conventionally spectrophotometric method. Standard methods employed usually for AGs analysis are microbial assay methods [1], which determine the total antibiotic

*Corresponding author. Tel.: +86-253-324-198; fax: +86-263-317-761.

E-mail address: hychen@nju.edu.cn (H.-Y. Chen).

0021-9673/01/$ – see front matter
PII: S0021-9673(00)00985-7

activity in a sample, that is, neither identify nor quantitate the specific AGs actually present. Consequently, HPLC has been employed for carrying out such identification and quantification of the individual Ags. However, tedious derivation processes with strongly fluorescing [2–4], or chemiluminescing reagents [5] are often needed.

At present, capillary electrophoresis (CE) has become a very important technique in the area of liquid-phase separation. CE with direct UV [6] and indirect UV detection [7] has been exploited for AGs while their detection sensitivities were not so satisfactory. In order to make full use of the powerful separation capacity of CE to determine AGs, more sensitive detection system is required. Electrochemical detection (ED) combined with CE, described firstly by Walliagford and Ewing [8] is a sensitive and selective method with many advantages over the widely used UV–visible detection as reviewed by Buchberger [9]. The principal difficulty for the determination of AGs is that AGs, a carbohydrate, exhibit a large overpotential for oxidation at the carbon electrodes commonly used in conventional CE–ED, which restricts the detection sensitivity and selectivity for them. The first approach to solve this problem is to employ platinum [10] or gold [11] electrodes. But they easily suffer from electrode fouling. The cleaning and regeneration steps of the electrode surface with double- or triple-pulsed potential waveforms are necessary to obtain stable, repetitive response. Unfortunately, most CE–ED units used in the present permit only constant potential operation. Pulsed electrochemical detection for AGs was mainly used in HPLC [10–12]. The second approach is to use an oxidizable metal electrode, such as copper [13] or nickel [14], which have been employed in CE–ED for the determination of some AGs. The third effective approach seems to be the application of chemically modified electrodes (CMEs). Various CMEs have been exploited in CE–ED for the determination of the wide range of biological compounds, such as a CoPc–carbon paste microelectrode for cysteine and glutathione [15], ribonucleoside [16] and thiol compounds [17], a mixed-valent RuCN-modified microelectrodes for thiols and disulfides [18], Pt [19], Pd [20] modified carbon fiber microelectrodes or a 4-pyridylhydroquinone self-assembled platinum electrode [21] for hydrazines, and so on. However, applications of CMEs for the determination of AGs by CE–ED have not been reported to our knowledge.

Here, we have developed a new procedure for electrodepositing Cu microparticles on a carbon fiber microdisk array electrode. The electrode exhibited good stability and highly catalytic activity towards AGs. A mixture of the five AGs including netilmicin, tobramycin, lincomycin, kanamycin and amikacin, whose structures are shown in Fig. 1, were successfully separated within 20 min and detected sensitively with the modified electrode by CE–ED. The applications to the determination of the pharmaceutical injections were carried out and the possibility of detecting them in human urine was also investigated.

2. Experimental

2.1. Reagents

AGs standards were purchased from the National Institute of the Control of Pharmaceutical and Biological products (China, Beijing), which were used as received without further purification. Other chemicals were of analytical reagent grade. AG stock solutions were prepared in deionized water, stored at 4°C. Prior to use, the stock solutions and the pharmaceutical injections were diluted to the desired concentration with 125 mM NaOH, filtered with a 0.25-μm membrane filter and then degassed under ultrasonic wave. NaOH solutions were prepared freshly, filtered and degassed.

2.2. Preparation of working electrode

The microelectrode array was fabricated from a bundle of carbon fibers (ca. a few hundreds of fibers, ca. 8 μm i.d. for single one). One end of a glass capillary (2 mm O.D.×1 mm I.D.) was drawn to obtain about 0.5 mm tip diameter. Its other end was cut to the desired length, ca. 5 cm. A bundle of carbon fibers was introduced into the capillary until it protruded about 1 cm from the tip. Epoxy was

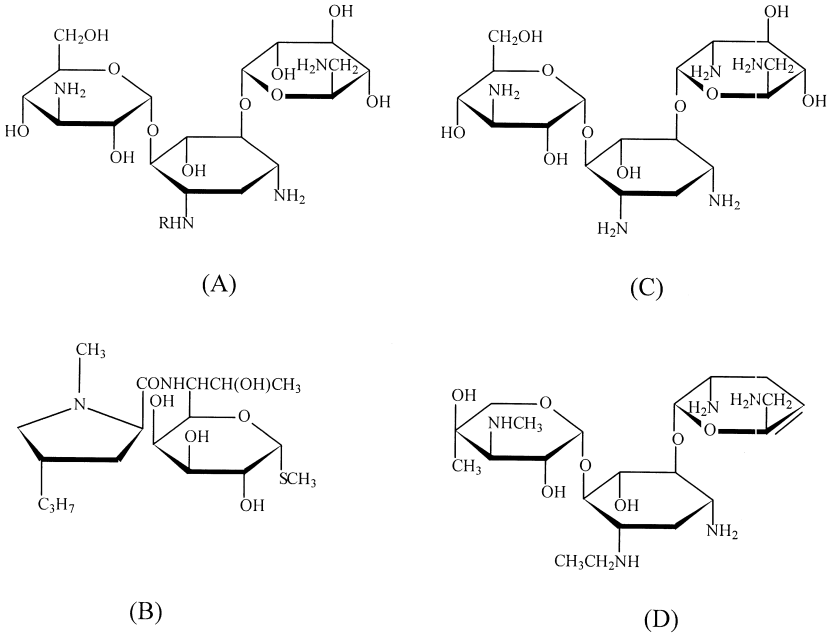

Fig. 1. Aminoglycoside antibiotics structure. (A) R=H, kanamycin; R=$H_2NCH_2CH(OH)CO$, amikacin, (B) lincomycin, (C) tobramycin, (D) netilmicin.

applied at the tip to seal the carbon fiber bundle. The capillary cavity was filled with carbon powder and a copper wire inserted from the other end, and then, this end was sealed with epoxy. The exposed carbon fiber bundle at the tip was cut. Since only one end of the carbon fibers is exposed, this array can be regarded as an ensemble of microdisk [20], and was referred to as CFE. As a comparison, a copper electrode (diameter 200 μm) was also constructed as previously reported [22].

The microdisk array electrode was polished with 400 mesh sandpaper, and washed thoroughly with deionized water under sonication. This electrode surface was electrodeposited at −0.4 V for 100 s in 50 mM $CuSO_4$ solution. Then, the copper modified fiber microdisk array electrode like copper microparticle-modified array electrode was ready to use and referred to as Cu-CFE. Its efficient surface was estimated to be ca. 0.017 cm^2 by comparing its cyclic voltammetric peak current with that of a well-polished metallic copper electrode (diameter 200 μm, roughness was neglected) in 0.1 M phosphate buffer (pH 7.0).

2.3. Apparatus

Electrophoresis experiments were performed with a CE–ED system assembled in the laboratory, similar to that described previously [23]. Electrophoresis was driven by a 30-kV high-voltage power supply (Shanghai Institute of Atomic Energies, Academy of Sciences of China, Shanghai, China). Uncoated fused-silica capillary (45 cm×50 μm I.D.) was obtained from Yongnian Optical Fiber Factory (Hebei, China). The working electrode was vertically aligned with the outlet of the separation capillary in the detection cell based on the wall-jet configuration, whose structure has been described elsewhere [24,25]. The distance between the electrode tip and the capillary outlet was adjusted to obtain the optimal signal response state, and then this optimized position was fixed. The outlet end of the capillary was always maintained at ground. The detection cell was shielded in a copper box to reduce external disturbance. A conventional three-electrode mode was used. A saturated calomel electrode (SCE) and a platinum wire were used as reference counter elec-

trodes, respectively. The electrodes were connected to an amperometric detector (Shanghai Institute of Organic Chemistry, Academy of Sciences of China, Shanghai, China), which applied the constant potential and measured the resulting current.

Cyclic voltammetry (CV) experiments were performed with a CHI660 electrochemistry workstation (CH Instruments, USA) with a three-electrode system as described above. Experimental conditions are described in the corresponding legends for the figures.

2.4. Electrophoretic procedure

Prior to the first use, a new capillary was washed successively with 0.1 M NaOH overnight, deionized water and the electrophoretic medium, each for 5 min, and then equilibrated under the separation voltage about 2 h until the migration times of analytes did not change significantly. After every run, the capillary was washed with the electrophoretic medium for 2 min and equilibrated under the separation conditions for 2 min. The electrophoretic medium in the anodic and cathodic reservoirs was renewed before each run in order to keep the same pH between the two reservoirs. Sample introduction was performed electrokinetically.

3. Results and discussion

3.1. Electrochemistry

Fig. 2 is the stable CV obtained at a Cu-CFE in 0.1 M phosphate buffer (pH 7.0). After the second cyclic scanning, the CVs became stable. Both peak shape and potential are very similar to those at a Cu electrode [26]. All these characters in Fig. 2 showed that copper microparticles were electrodeposited on the CFE surface and the electrodeposited layer was stable. The well-defined cathodic peak at −0.2 V, which corresponded to Cu(II) reduction [26], was used to estimate the quantity of copper loading on a CFE surface.

Investigation of the electrochemical behavior of a Cu-CFE was performed by cyclic voltammetry in 125 mM NaOH solution. During the first scan in the anodic direction, a broad anodic peak with a peak

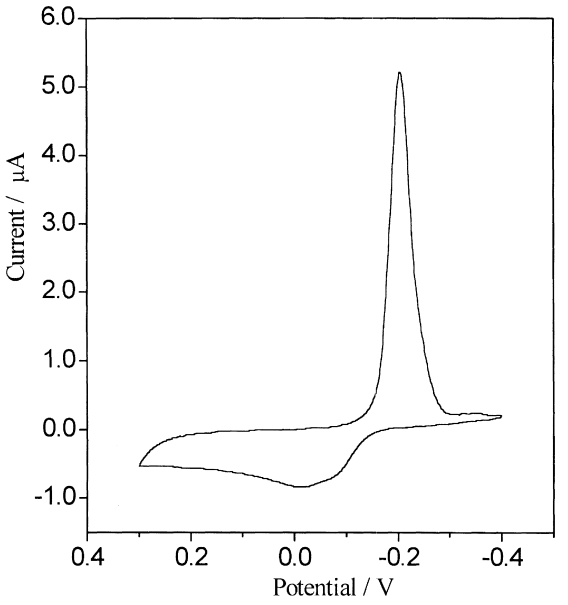

Fig. 2. Cyclic voltammogram obtained at a Cu-CFE in 0.1 M phosphate buffer (pH 7.0). Scan rate, 20 mV/s.

potential of +0.65 V was observed (not shown). Analogous results were also obtained when substituting a Cu electrode for a Cu-CFE. So, like a metallic copper electrode, the anodic peak at +0.65 V corresponds to the Cu(III)/Cu(II) redox couple [27]. However, the peak height decreased and rapidly disappeared on the subsequent scans, and then reached a stable CV after the fourth cyclic scan, as shown in Fig. 3 (dashed line). This phenomenon was also observed by Prabhu and Baldwin at a cupric-modified glassy carbon electrode and was attributed to a very rapid film formation and passive process [28]. The electrocatalytic activity of a Cu-CFE towards netilmicin at is illustrated in Fig. 3 (solid line). A Cu-CFE gave anodic currents, proportional in magnitude to the netilmicin concentration added, at a potential of +0.64 V similar to that given by an initial active Cu-CFE surface by itself. However, unlike the response obtained for the Cu-CFE in the blank solution (Fig. 3, dashed line), this peak was considerably reproducible in the presence of netilmicin after the second scan, showing no surface passivation. The regeneration of the passive surface may be due to chemical redox reaction. The CVs of other AGs were nearly identical to that of netilmicin,

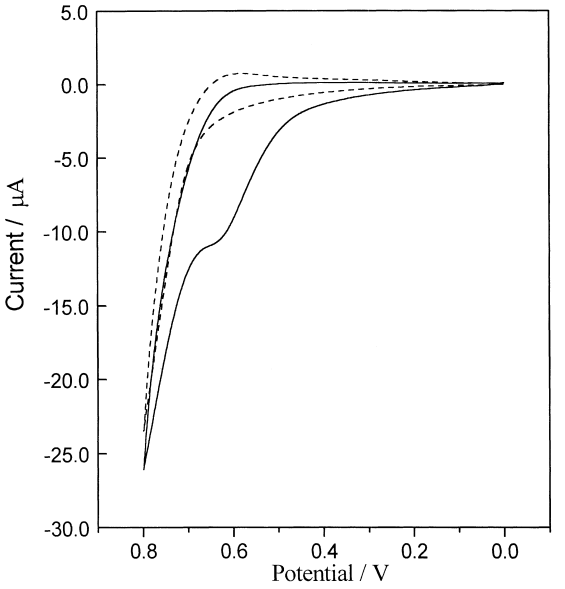

Fig. 3. Cyclic voltammogram obtained at a Cu-CFE in 125 mM NaOH without (dashed line) and with (solid line) 0.5 mM netilmicin. Scan rate, 50 mV/s.

except for peak potentials and shape. These observations illustrated that AG detection at a Cu-CFE in constant potential oxidation mode would seem to be feasible.

The effect of copper loading on a CFE surface on electrocatalytic activity was investigated. The time of electrodeposition was changed from 60 to 160 s at the potential of −0.4 V. The results showed the maximum catalytic current was obtained as the electrodeposited time was 100 s or more. From the steady-state cyclic voltammogram of CFE in $K_3Fe(CN)_6$, its efficient diameter can be calculated (ca. 150 μm). And also the amounts of copper loading was estimated for ca. 12 $\mu g/cm^2$ by the area of stripping voltammetric peak of Cu-CFE. Qualitatively, the electrochemical behavior of a Cu-CFE was similar to that of a metallic Cu electrode; but the oxidation currents of AGs at the former were greatly higher than those at the latter. For example, for 0.5 mM netilmicin in 125 mM NaOH solution, the oxidation current at a Cu-CFE was 110 $\mu A/cm^2$, while only 4.4 $\mu A/cm^2$ was obtained at a Cu electrode under the same experimental conditions. This resulted from the advantage of microelectrode array.

3.2. Effect of the detection potentials

In order to assess the effect of the working potentials on the current response in flowing system, the hydrodynamic voltammograms (HDVs) for the AGs were investigated. The plots of the peak currents versus the working potentials for netilmicin, tobramycin, amikacin at a Cu-CFE are given in Fig. 4. The other AGs showed almost the same trends in response except the peak amplitudes. When the potential was lower than 0.5 V, nearly no evident oxidation currents were obtained. The peak currents of three AGs started to increase after 0.5 V, and increased sharply above 0.55 V. However, the noise level increased drastically due to solvent oxidation when the potential was higher than 0.75 V, and the detection signals completely disappeared in the noise. Therefore, the detection potential of 0.7 V was selected, which gave the best compromise between signal and noise.

3.3. Effect of NaOH concentration and separation voltage

From their structure, one could know that AGs are neutral molecules for most solution conditions. In

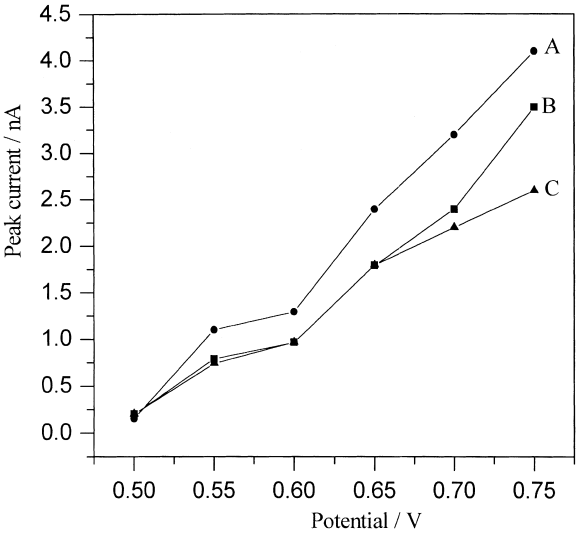

Fig. 4. Hydrodynamic voltammograms of 100 μM tobramycin (A), 120 μM netilmicin (B) and 70 μM amikacin (C). Electrophoretic medium, 125 mM NaOH; separation voltage, 6.2 kV; injection, 6.2 kV, 10 s.

order to separate them by free zone electrophoresis, the ionization for them are necessary. Although amino groups in AGs can be protonized in acid medium and based on which, the separation for them by CE with UV detection was realized [7], a strongly basic condition was needed with electrochemical detection for them. Fortunately, the hydroxyl groups in AGs can be partially deprotonated in a strongly basic medium. Therefore, strongly basic NaOH solutions were selected as electrophoretic medium for meeting the requirements of both detection and separation. The effect of NaOH concentration from 75 mM (pH 12.8) to 125 mM (pH 13.1) on the resolutions (R_s) is shown in Fig. 5. When NaOH concentration was below 75 mM, the separation was rather poor. As the concentration increased, the separation was improved, and a baseline separation (R_s>1.3) was obtained in 125 mM NaOH separation medium at a separation voltage of 6.2 kV. The corresponding electropherograms are shown in Fig. 7A,B,D.

The effect of separation voltage on R_s for AGs is illustrated in Fig. 6. The higher the separation voltage was, the faster the electroosmotic flow

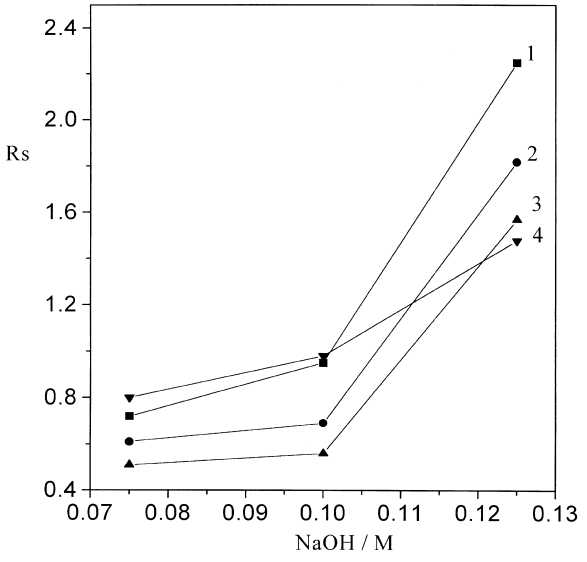

Fig. 5. Effect of NaOH concentration in electrophoretic medium on resolutions of (1) netilmicin and tobramycin, (2) tobramycin and lincomycin, (3) lincomycin and kanamycin, (4) kanamycin and amikacin. Detection potential, 0.7 V versus SCE. Other conditions as in Fig. 4.

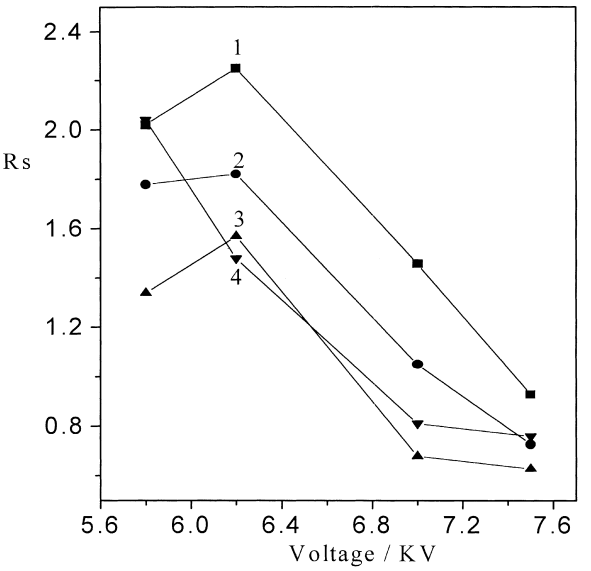

Fig. 6. Effect of separation voltage on resolutions of (1) netilmicin and tobramycin, (2) tobramycin and lincomycin, (3) lincomycin and kanamycin, (4) kanamycin and amikacin. Electrophoretic medium, 125 mM NaOH. Other conditions as in Fig. 5.

became and R_s decreased. However, the higher R_s at the lower separation voltage was at the expense of longer analysis time. Moreover, when separation voltage was too low, such as 5.8 kV, R_s decreased owing to peak broadening. The maximum R_s was obtained at 6.2 kV with 125 mM NaOH. Typical electropherograms are shown in Fig. 7C–E.

3.4. Analytical performance of the CE–ED system

On the basis of above investigations, 125 mM NaOH, 6.2 kV separation voltage and 0.7 V detection potential were chosen as appropriate analytical conditions for the quantitative assessment of the CE–ED system studied here. The analytical performance of the CE–ED for the five AGs was investigated first on linearity and detection limit, and the results are summarized in Table 1. The linearity was evaluated by analyzing the standard solution with respect to the peak current. The detection limit was obtained at the S/N of 3. The present CE–ED exhibited the wide linearity, and the low detection limits for the analytes studied. Additionally, the reproducibility of response was estimated by repetitively injecting the standard mixture of the five AGs under the same experimental

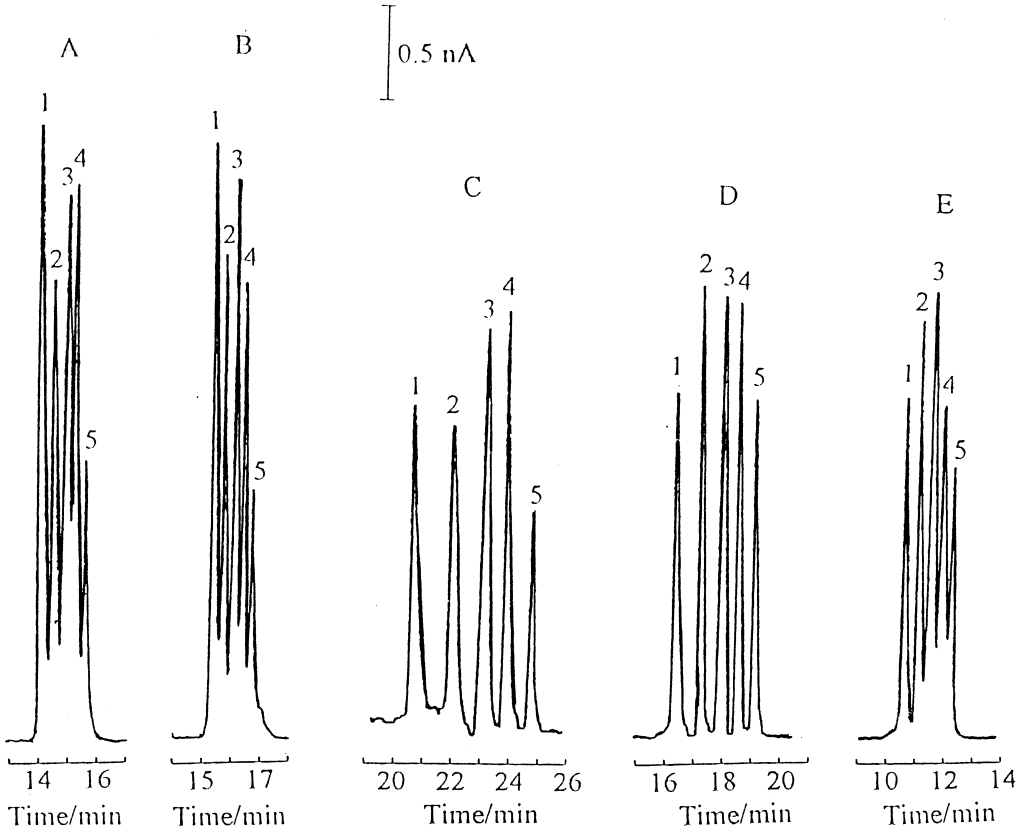

Fig. 7. Electropherograms showing the effects of NaOH concentration and separation voltage on the separation results for the five AGs. The peaks correspond to (1) netilmicin (120 μM), (2) tobramycin (100 μM), (3) lincomycin (400 μM), (4) kanamycin (80 μ M) and (5) amikacin (70 μ M). Separation medium and voltage: (A) 75 mM NaOH, 6.2 kV; (B) 100 mM NaOH, 6.2 kV; (C) 125 mM NaOH, 5.8 kV; (D) 125 mM NaOH, 6.2 kV; (E) 125 mM NaOH, 7.5 kV. Detection potential, 0.7 V versus SCE; injection, 6.2 kV, 10 s.

conditions within a period of 8 h. During this time, the response gradually decreased. After 8 h of injections, the peak current for netilmicin is still maintained about 85% of the initial value. The reasons for the slow decrease in electrode response was likely due to partially dropping of modified copper microparticles from the Cu-CFE surface under flow conditions or the electrode fouling. Nevertheless, this decrease was very slow. If the decrease of the response was evident, the renewing

Table 1
Analytical performance of CE–ED for the determination of aminoglycoside antibiotics[a]

Analyte	Migration time (min)	Linearity (mol/l)	Slope (nA/mM)	Correlation coefficient	Detection limit (μM)	Injection volume (nl)	Detection limit (fmol)
Netilmicin	16.50	1.2×10^{-5}~1.0×10^{-3}	20.07	0.9931	2.0	4.5	9.0
Tobramycin	17.01	1.0×10^{-5}~1.0×10^{-3}	25.38	0.9996	1.7	4.3	7.3
Lincomycin	18.25	4.0×10^{-5}~2.0×10^{-3}	6.34	0.9996	6.7	4.0	26.8
Kanamycin	18.83	8.0×10^{-6}~1.0×10^{-3}	25.22	0.9952	1.3	3.9	5.1
Amikacin	19.50	7.0×10^{-6}~1.0×10^{-3}	25.03	0.9983	1.2	3.8	4.6

[a] Experimental conditions are the same as in Fig. 7D. The injection volume was calculated from migration time and capillary dimensions.

of the surface of a Cu-CFE was easy and reproducible with the relative standard deviation of peak currents for different modifications at the same CFE of about 5.2% ($n=6$, for netilmicin). Therefore, the reproducible response of AGs at a Cu-CFE still seemed to be obtained.

In the past, end-column amperometric detection was assumed to be only applicable in conjunction with capillaries of $\leq$25 μm I.D. owing to the influence of the electric field of separation. But it could been seen from the present investigation and our previous work [23] that a 50-μm I.D. capillary coupled with this detection approach operate well. Similar reports could also be found in the recent literature [29,30]. So, the use of a 50-μm I.D. capillary in end-amperometric detection mode was also acceptable.

3.5. Applications

3.5.1. Simultaneous determination of AGs contents in their pharmaceutical injections

Firstly, the five AGs injections were separately analyzed under above established conditions in order to examine the interference from impurities in an injection to the analyte peak or to other AG peaks when five AG injections were determined simultaneously. The results showed that the impurities peaks in every sample were separated far from the analyte peak; and they did not matched the migration times of any other analytes. Therefore, the simultaneous determination of five AG injections was feasible under the chosen conditions in this work. A mixture of the five AGs injections was prepared, in which the concentration of each analyte was adjusted with 125 mM NaOH to be within the linear range of the calibration curve. The quantitative analyses were carried out on the calibration curve, and the results are listed in Table 2. Additionally, if the five AG injections were determined separately, the experiments showed that a separation medium of 75 mM NaOH and a separation voltage of 10 kV could be used. Under these conditions, the detector noise was minimized and the separation time could be decreased to ca. 8 min without interference from other impurity peaks.

3.5.2. Investigation of the possibility for the determination of AG contents in urine

In order to evaluate the capability of the present CE–ED system for the determination of AG contents in body fluid, a urine sample from a healthy volunteer was assayed. Fig. 8A shows the electropherograms of the urine sample; and Fig. 8B is that of its spiking with the five standard AGs. Comparing Fig. 8A with Fig. 8B, it can be seen that, of the spiked five AGs, lincomycin, kanamycin and amikacin were well resolved from the unknown species in the urine sample while both netilmicin and tobramycin overlapped with their neighboring unknown peaks in the sample. From the literature [31], AGs are eliminated in unchanged form by glomerular filtration, and about 40–90% of an administered dose (0.5 g usually) appears in the urine within 24 h. So, If AGs were injected by intramuscularly, the amount that was excreted in urine was far above the present detection limit. Consequently, it is feasible to determine lincomycin, kanamycin and amikacin in urine samples.

4. Conclusions

The work has demonstrated that a novel copper

Table 2
Determination of aminoglycoside content of pharmaceutical injection[a]

Product (injection)	Analyte	Labeled value (mg/ampoule)	Determined value (mg/ampoule)	RSD (%, $n=5$)
Netilmicin sulfate	Netilmicin	100	91.2	4.2
Tobramycin sulfate	Tobramycin	80	78.5	4.6
Lincomycin hydrochloride	Lincomycin	60	591.0	5.9
Kanamycin sulfate	Kanamycin	500	488.7	5.7
Amikacin sulfate	Amikacin	200	194.9	3.6

[a] Experimental conditions are the same as in Fig. 7D.

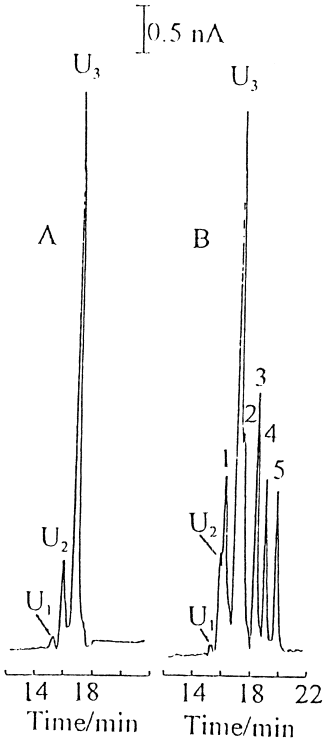

Fig. 8. Electropherograms of (A) urine sample diluted 1:2 and (B) (A)+120 μM netilmicin (1), 100 μM tobramycin (2), 400 μM lincomycin (3), 80 μM kanamycin (4) and 70 μM amikacin (5). Peaks U_1, U_2, U_3 are unknown peaks present in the urine sample. Other conditions as in Fig. 7D.

microparticle-modified carbon fiber microdisk array electrode can be used in CE–ED to sensitively detect AGs. The feature of this electrode is significantly different from that of a metallic copper electrode, e.g., its catalytic activity to the netilmicin is ca. 25 times higher than that of the latter. The troublesome problem for the AG separation has been resolved with the satisfactory R_s by choosing the appropriate separation voltage and electrophoretic medium. Using the developed method, they have been simultaneously and directly determined with wide linear ranges and low detection limits, without any derivation as needed with optical detection. Finally, the CE–ED with a Cu-CFE is shown to provide a convenient, precise determination of AGs in pharmaceutical injections and can be used to determine lincomycin, kanamycin and amikacin in urine samples.

Acknowledgements

This project was supported by the National Natural Science Foundation of China (Grant No. 29835110) and Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry.

References

[1] Pharmacopoeia of the People's Republic of China, Part 2, Chemical Industry Press and People Hygiene Press, Beijing, 1990, Appendix, p. 113.
[2] F.J. Schenck, P.S. Callery, J. Chromatogr. A 812 (1998) 99.
[3] R. Tawa, H. Matsunaga, T. Fujimoto, J. Chromatogr. A 812 (1998) 141.
[4] G. Morovjan, P.P. Csokan, L. Nemeth-Konda, Chromatographia 48 (1998) 32.
[5] M.A. Targove, N.D. Danielson, J. Chromatogr. Sci. 28 (1990) 505.
[6] C.L. Flurer, K.A. Wolnik, J. Chromatogr. A 663 (1994) 259.
[7] M.T. Ackermans, F.M. Everaerts, J.L. Beekers, J. Chromatogr. A 606 (1992) 229.
[8] R.A. Walliagford, A.G. Ewing, Anal. Chem. 59 (1987) 1762.
[9] W. Buchberger, Fresenius J. Anal. Chem. 354 (1996) 797.
[10] J.A. Polta, D.C. Johnson, K.E. Merkel, J. Chromatogr. 324 (1985) 407.
[11] E. Adams, D. Puelings, M. Rafiee, E. Roets, J. Hoogmartens, J. Chromatogr. A 812 (1998) 151.
[12] W.R. LaCouse, C.O. Dasenrock, Adv. Chromatogr. 38 (1998) 189.
[13] P.D. Voegel, R.P. Baldwin, Electroanalysis 9 (1997) 1145.
[14] X. Fang, J. Ye, Y. Fang, Anal. Chim. Acta 329 (1996) 49.
[15] T.J. O'Shea, S.M. Lunte, Anal. Chem. 66 (1994) 307.
[16] J. Zhou, T.J. O'Shea, S.M. Lunte, Anal. Chem. 67 (1995) 13.
[17] X. Huang, W.Th. Kok, J. Chromatogr. A 716 (1995) 347.
[18] J.J. Zhou, T.J. O'Shea, S.M. Lunte, J. Chromatogr. 680 (1994) 271.
[19] W. Zhu, L. Xu, M. Wu, E. Wang, Anal. Chim. Acta 29 (1994) 189.
[20] J. Liu, W. Zhou, T. You, F. Li, E. Wang, S. Dong, Anal. Chem. 68 (1996) 3350.
[21] T. You, L. Niu, X. Yang, S. Dong, E. Wang, J. Pharm. Biomed. Anal. 191 (1999) 231.
[22] L. Hua, D.K. Xu, H.Y. Chen, J. Chromatogr. A 760 (1997) 227.
[23] W.C. Yang, A.M. Yu, Y.Q. Dai, H.Y. Chen, J. Chromatogr. A 867 (2000) 261.
[24] D.K. Xu, L. Hua, H.Y. Chen, Chem. J. Chin. Univ. 17 (1996) 707.
[25] D.K. Xu, L. Hua, H.Y. Chen, Anal. Chim. Acta 375 (1996) 90.

[26] W.C. Yang, A.M. Yu, Y.Q. Dai, H.-Y. Chen, Anal. Chim. Acta 415 (2000) 75.
[27] B. Miller, J. Electrochem. Soc. 116 (1969) 1675.
[28] S.V. Prabhu, R.P. Baldwin, Anal. Chem. 61 (1987) 852.
[29] A. Durgbanshi, W.T. Kok, J. Chromatogr. A 798 (1998) 289.
[30] F.-M. Matysik, J. Chromatogr. A 802 (1998) 349.
[31] J.-Z. Li, in: Clinical Pharmacology, People's Hygiene Press, Beijing, 1991, p. 503.