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Study of post separation pH adjustment by microchip for analysis of aminoglycoside antibiotics

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ABSTRACT

Recently, we have developed a simple microchip to simultaneously accommodate acidic condition

for separation and alkaline condition for electrochemical detection of aminoglycoside antibiotics

[Electrophoresis, DOI: 10.1002/elps.201200309]. With two branch channels connected near to the

end of separation channel, alkaline solution was hydrostatically introduced into a Z-shape mixing

channel and combined with acidic stream from separation channel. As a result, the pH of mixed

solution was adjusted to the desired value for electrochemical detection of aminoglycoside

antibiotics. In this manuscript, the principle and related parameters of the pH adjustment on the

microchip were investigated both in theory and practice. With the guidance of the principle of the

post separation pH adjustment, we applied the functional microchip for analysis of six

aminoglycoside antibiotics in biological sample with the satisfied analytical performances.

Specifically, these compounds were electrophoretically separated under 5 mM sodium acetate (pH

= 4) and 0.6 mM CTAB, and through the post separation pH adjustment, electrochemically

detected in alkaline condition (pH > 12) at a Cu-Sn-Cr alloy electrode. Additionally, this

microchip may provide possible use of post separation reagent addition for enzyme-assisted

electrochemical detection.

Key words: post separation pH adjustment; aminoglycoside antibiotics; alloy electrode; microchip

INTRODUCTION

Aminoglycosides (AMGs) are effective drugs that are widely used in therapy against bacterial

infections, but their clinical use has been restricted due to toxic side effects, especially to the

kidneys and the ear. On the other hand, low cost means that they are often widely used in animal

husbandry leading to potential residues in the food chain, which probably increase the bacterial

resistance against AMG antibiotics in human body. Since these compounds are characterized by

two or more amino sugars linked by glycosidic bonds to an aminocyclitol component, in general,

they are short of chromophores and show high polarity. Due to these reasons, the detection of

AMGs using standard HPLC or GC instrumentation generally requires the pre- or post-column

derivatization[1-5]. Besides these derivatization methods, electrochemical detection is usually

coupled with chromatographic techniques for the determination of AMGs without derivatization.

However, in most cases, the use of electrochemical detection for analysis of carbohydrates

requires strongly alkaline condition (pH > 12), which is unfavorable to the separation of AMGs by

CE or non ionic exchanger column[6-9]. Although the methods based on LC-MS/MS are most

powerful for screening and confirmation of AMGs, they are high cost to operate and require

highly qualified personnel[10-13]. Consequently, the necessity still exists for rapid, facile, and low

cost screening methods both in the laboratory and at onsite locations. Up to now, a few methods of

screening AMGs have been reported with relatively high sensitivity, such as the surface plasmon

resonance (SPR)-based biosensors [14] and aptamer-based sensors[15, 16]. However, most of them

depend on cost antibodies or RNA aptamers without the separation of multiple drugs.

Microfluidic technology has evolved over the past two decades, accompanying a wealth of

inventions and applications which have extensively attracted attentions from a variety of scientific

communities. The recent achievements and applications of microfluidic technology have been

well documented in several review articles[17-20]. Distinctively, the microchip coupled with

electrochemical detection shows several merits, such as miniaturization of analytical systems and

simplicity for use. Recently, we have developed a simple microfluidic chip containing two branch

channels connected to the separation channel nearby the detection point, in which the alkaline

solutions conflux with the acidic stream toward an alloy modified electrode for electrochemical

detection of AMGs [21]. To better take advantage of this microchip, here we did further studies on

the post separation pH adjustment both in theory and practice. In addition, the electroplating

conditions of the alloy electrode were reinvestigated to improve the stability of the electrode. The

experiments show the microchip with re-modified electrode can be kept in use for more than a

week. The applicability of the method has been demonstrated by analyzing six aminoglycoside

antibiotics in bovine serum sample. Such a straightforward approach is a prototype of

"post-column" addition based on microfluidic platform and also properly applied to other post

separation adjustment or reaction with various reagents (e.g. enzymes) to accommodate the

separation and detection in the different conditions.

EXPERIMENTAL SECTION

Chemicals and Solutions

SU-8 2035 photoresist and SU-8 developer were purchased from MicroChem (Newton, MA,

USA). Sylgard 184 silicone elastomer and curing agent were obtained from Dow Corning

(Midland, MI, USA). Cetyltrimethyl ammonium bromide (CTAB) was purchased from

Sigma-Aldrich (Shanghai, China). Analytical grade sodium acetate, copper sulfate, tin chloride,

chromium chloride, acetic acid, sulfuric acid, hydrochloric acid, and sodium hydroxide were

obtained from Beijing Chemical Reagent Company (Beijing, China) and used without further

purification. The six AMG standards, spectinomycin (SPE), streptomycin (STR), amikacin (AMI),

kanamycin A (KAN A), paromomycin (PAR), and neomycin (NEO), were obtained from National

Institutes for Food and Drug Control (China). Running buffers were prepared by diluting the stock

solutions of sodium acetate and CTAB with deionized water to the desired concentrations. The pH

of the buffer solution was adjusted by small additions of either 0.1M NaOH or 0.1M HCl. The

stock solutions of AMG antibiotics were prepared in distilled water and diluted with the running

buffer. All aqueous solutions were prepared using 18.2 MΩ-cm resistance water (Elga Labwater,

UK) and were stored at 4 $^{\circ}$C when not in use.

Fabrication of the Microfluidic Chip

The fabrication of the microfluidic chips was performed as described elsewhere[22, 23]. Briefly, a 100

mm diameter silicon wafer (Tianjin Semiconductor Institute of Technology, Tianjin, China) was

cleaned with piranha solution (3 parts of 98 % H_2SO_4 with 1 part of 30 % H_2O_2; **CAUTION**:

piranha solution is a powerful oxidizing agent that reacts violently with organic compounds and

should be handled with extreme care) and thoroughly rinsed with deionized water. The wafer was

then dried at 200 $^{\circ}$C for 30 min. Next, the wafer was coated with SU-8 2035 negative photoresist

using a spin coater (Laurell Technologies, PA, USA) by dispensing approximate 3 ml of

photoresist onto the wafer. A spread cycle of 500 rpm for 10 s followed by 1500 rpm for 30 s was

performed followed by two pre-exposure baking steps at 65 and 95 $^{\circ}$C for 5 and 30 min,

respectively. A digitally produced mask containing the channel pattern was placed on the coated

wafer, exposed to light via a near-UV flood source (Optical Associates Inc., CA, USA) for 30 s

and then followed by a post exposure baking sequence. The positive relief was developed by

placing the wafer in SU-8 developer (propylene glycol methyl ether acetate) for 15 min, rinsing

with isopropanol and drying under nitrogen stream. The height of the positive patterns on the

molding master is equal to the channel depth created in the PDMS layer. It was measured roughly

by cutting a piece of PDMS with cross section of the channel under a microscope with

microscopic length scale. Two PDMS layers were fabricated by pouring a degassed mixture of

Sylgard 184 silicone elastomer and curing agent (10:1) onto either a molding master or a blank

wafer, followed by curing at 65 $^{\circ}$C for at least 2 h. The cured PDMS was separated from the mold

and reservoirs were made at the end of each channel using a 5-mm circular punch. A platinum

wire coated with Cu-Sn-Cr alloy was then aligned at the end of the separation channel in a

perpendicular channel designed for working electrode. Next, the two PDMS layers were placed in

plasma cleaner (Harrick Plasma, NY, USA), oxidized for 30 s and immediately brought into

conformal contact to form an irreversible seal. The extremities of the electrode channel were

sealed with two drops of super glue. Finally, an electrical connection to the working electrode was

made using silver paint (SPI Supplies, PA, USA) and a copper wire. A schematic drawing of the

microfluidic chip is illustrated in Figure 1.

Figure 1

Instrumentation

A high voltage sequencer (LabSmith Inc., CA, USA), with an adjustable voltage range of – 3000

to + 3000 V, was used for all of the electrophoresis experiments. Before the experiments, the

microchannels were preconditioned by sequentially rinsing them with 0.1 M NaOH, deionized

water and running buffer. The microfluidic chip and the operation were described in detail as

follows. The separation channel was 57 mm long. A double-T injector, with a 500 µm gap between

side channels and defining a 1.2 nL sample plug, was used for all experiments. The amount of

buffer dispensed in each reservoir (RB, S, SW and BW) was 60 µl, except for two alkaline

solution reservoirs (A1 and A2) where 120 µL of 0.3 M NaOH were dispensed in order to

hydrostatically push the alkaline solution into the mixing channel. The solution in each reservoir

was refreshed after 5 to 6 run times in consideration of evaporation. The 2-mm length mixing

channel was designed as Z - shape to aggrandize mix of solution. During the sample injection, the

potentials of – 400 V and + 100 V were applied to the reservoirs S and SW, respectively, while the

reservoir RB was floating. During the separation step, the reservoir RB was applied to – 1000 V, at

mean time, the reservoirs S and SW were floating. During the above proceedings, the waste

reservoir (BW) was always grounded. The present design allows the isolation of the detector from

the separation current through the end-column configuration. Electrical connections were made to

the microfluidic devices with platinum electrodes placed into the reservoirs at the ends of each

channel (except for the reservoirs A1 and A2). Electrochemical experiments were preformed with

a CHI 810 C (Chenghua Instruments Co., Shanghai, China), using a three-electrode setup. An Ag /

AgCl (3.0 M KCl) reference electrode and a platinum wire were used as the reference and

auxiliary electrodes, respectively. A 5-cm length platinum wire (25-µm diameter) was used as the

substrate electrode (effective length = 3 cm), on which the electrodeposization was carried out at –

0.3 V (vs. Ag /AgCl) for 150 s in an electroplating bath containing 50 mM $CuSO_4$, 0.2 mM $SnCl_2$,

1.0 mM $CrCl_3$, 2.0 mM EDTA and 10 mM H_2SO_4. The coated electrodes were also applied for

cyclic voltammetry and amperometric i-t curve experiments.

Sample preparation

0.5 mL bovine serums were spiked with AG antibiotics at three concentration levels (20 μM, 50

μM and 100 μM), vortexed with 0.5 mL TCA (20% (w/v)), ultrasonicated for 10 min, and then

centrifuged at 12,000 rpm for 10 min. After removing the protein sediment, the supernatant was

transferred to another centrifuge tube, and then added EDTA to 10 mM. The extraction of AMGs

in the solution was conducted using a solid phase extraction (SPE) column (Anpelclean MCX, 60

mg, Shanghai, China). The SPE procedures were detailed as follows: loading the solution on the

preconditioned column at a flow rate of 0.2 mL/min, then washing with water until the effluent

was neutral solution, finally eluting with 6 mL of 10% (v/v) ammonia in methanol at a flow rate of

0.2 mL/min. The collected eluent was evaporated under a nitrogen stream in water bath at 60 ℃

and the residue was reconstituted in 0.5 mL of the running buffer for injection.

RESULTS AND DISCUSSION

Theoretical Description of Post Separation Adjustment of pH

To better understand the behavior of the post separation adjustment of pH based on our designed

microchip, the theoretical study was described below. For a laminar flow governed by Poiseuille

equation, the volumetric flow rate is proportional to the pressure difference and the forth power

radius of the channel while inversely proportional to the viscosity and the length of channel. The

equation is given as follow:

$Q = \pi \, \Delta P \, r^4 / (8 \, \mu \, L)$ (1)

where Q is the volumetric flow rate; ΔP is the pressure drop; L is the length of channel; μ is the

viscosity of fluid; r is the radius of channel; π is the mathematical constant Pi. During the

separation step, the pH of the mixed solution arriving at the detection electrode depends on the

amount of alkaline solution flowing into the Z-shape mixing channel, which is controlled by the

pressure drop between the reservoirs A1 / A2 and BW. In this microchip, the pressure drop is

deduced from the difference between the height of the solution in the reservoir A1 / A2 and BW.

$$\Delta P = \rho g \, (h_A - h_{BW}) \qquad (2)$$

where ρ is the density of water, g is gravity constant, h_A and h_{BW} are the heights of solution in the

reservoir A1 / A2 and BW.

For a cylinder-like reservoir of the microfluidic chip,

$$h = V / \pi R^2 \qquad (3)$$

where h is the height of solution in reservoir, V is the volume of solution in reservoir, R is the

radius of reservoir.

Combining the equations (1), (2) and (3), the Poiseuille equation governing the flow rate of the

solution in the branch channels of the microchip can be written as follow:

$$Q_b = \rho g \, (V_A - V_{BW}) \, r^4 / (8\mu \, L \, R^2) \qquad (4)$$

where Q_b is the volumetric flow rate in the branch channel, V_A and V_{BW} are the volume of solution

in the reservoir A1 / A2 and BW, respectively. For the post separation adjustment of pH in this

experiment, the alkaline solutions in the reservoirs A1 and A2 start to flow into the mixing channel

when the pressure drop is formed between the reservoir A and BW ($V_A - V_{BW} > 0$). In order to

sustain an appropriate and steady pH value for the electrochemical detection, a hydrodynamic

equilibrium is required to form in the mixing channel, where an active electroosmotic flow (EOF)

from the upstream separation channel combines two hydrostatically driven flows from the branch

channels toward the detection point. With mixing thoroughly, the pH of the mixed solution at the

detection point can be calculated by the following equation:

$$pH = 14 + \lg [2\ C_{NaOH}\ Q_b / (Q_m + 2\ Q_b)] \qquad (5)$$

where Q_m is the volumetric flow rate of EOF in the upstream separation channel, C_{NaOH} is the

concentration of NaOH applied in the reservoirs A1 and A2. Because the volumetric flow rate

from the separation channel is determined by the EOF, Q_m can be obtained from the following

equation:

$$Q_m = \pi r^2 v_{eof} \qquad (6)$$

where v_{eof} is the velocity of EOF. In this experiment, the actual value of EOF is about 1.0 mm / s

at - 1000V of the separation potential.

Combining the equation (4), (5) and (6), the pH of the mixed solution at the end of separation

channel can be approximately calculated according to the volume difference between the

reservoirs A and BW and the concentration of NaOH in the reservoirs A. Under the certain

velocity of EOF and the given concentration of NaOH, a series of theoretical curves between the

pH and the volume difference (V_A-V_{BW}) are plotted in Figure 2A. All of the plots show the same

tendency that the pH of the mixed solution increases steeply during the volume difference

(V_A-V_{BW}) initially increasing from 2.5 to 25 μL, then the increment of pH becomes relatively flat

when the volume difference is larger than 25 μL. However, when the larger volume difference is

applied, the larger dilution percent formed is likely to decrease the response of analyte. The

dilution percent can be defined as the following formula:

$$Dilu.\ \% = 100 \times Q_m / (Q_m + 2Q_b) \qquad (7)$$

Additionally, we apply Comsol 3.2 (student version) software to simulate the pH distribution in

the mixing channel and virtually estimate the pH of the solution surrounding the detection point.

Figure 2B shows that the designed microchip can generate a high pH nearby the detection point

under the given conditions of $Q_b = 0.1 \times Q_m$ and $C_{NaOH} = 0.1$ M.

<div align="center">

Figure 2

</div>

Experimental Verification of Post Separation Adjustment of pH

With the guidance from the above theoretical descriptions, the effect of the different concentration

of NaOH in the reservoir A (1 / 2) and volume difference of solution (V_{A-BW}) on the response of

AMGs were investigated. Figure 3A shows the electrochemical responses of the analytes under the

different concentrations of NaOH at a constant volume difference (60 µL). Compared to 0.1 M

and 0.5 M NaOH, the peak currents of the analytes were largest under 0.3 M NaOH. It was in part

consistent with the higher concentration of NaOH leading to the higher pH to facilitate the

electrochemical oxidization of AMG. On the other hand, the peak currents of the AMGs under 0.5

M NaOH were smaller than those under 0.3 M NaOH. It can be explained the excessively high pH

over 13 results in the high background current to diminish the peak currents of AMGs. Thus, 0.3

M NaOH was selected in the following experiments. In order to obtain the larger and more stable

peak currents of analytes, the percent of dilution and optimal pH need to be balanced at a certain

concentration of NaOH. For the given concentration of NaOH, the choosing of larger volume

difference means that more alkaline solution flows into the mixing channel resulting larger percent

of dilution and higher pH, whereas applying lower volume difference results lower pH and

dilution. The effect of the volume difference under 0.3 M NaOH on the electrochemical response

of AMGs was given in Figure 3B. At the volume difference of 60 µL, the peak currents were

largest obviously. This phenomenon is well in accordance with larger volume difference resulting

larger dilution percent and smaller volume difference resulting lower pH. Finally, 60 µL was

selected as the optimal volume difference in the following experiments. The above experimental

results show clear evidence for the theoretical descriptions.

<div align="center">**Figure 3**</div>

Electrochemical Characters of the Alloy Working Electrode

Electrochemical detections of carbohydrates have extensively been studied at different metal

electrodes. Conclusively, pulsed electrochemical detection (PED) needs to be applied on noble

metal electrode (e.g. Au and Pt)[24, 25], while constant potential amperometry needs to be applied on

copper or nickel electrode[26, 27]. However, both detection modes need metallic oxide catalysis

under strongly alkaline condition (generally pH > 12). In our preliminary study, both of gold and

copper wires were adopted as the working electrode for PED and constant potential amperometry,

respectively. However, the unavoidable hindrances were incurred during applying detection

potential on these electrodes, such as low sensitivity, bubble formation on gold electrode or severe

corrosion to copper electrode, etc. To overcome these problems, platinum wire was selected as a

substrate, on which the multiple metal elements (Cu-Sn-Cr) were coelectrodeposited to form the

distinctive nanostructures with the large ratio of surface-to-volume (Figure 4 A) and show the

character of corrosion-resistant (at least use for one week). The electrochemical behaviors of the

electrodeposited wire were characterized by conducting cyclic voltammetry (CV) and

amperometric i-t experiments. As can be observed in Figure 4B, the CV data indicate an oxidative

peak of AMI on the modified electrode came out when the pH increased from 11 to 13. The more

details about the catalytically electrochemical oxidation of AMGs at copper based electrode were

discussed in our previous work[21]. On the other hand, amperometric i-t data in Figure 4C exhibit

the alloy modified electrode providing an obviously large sensitivity compared to the copper and

platinum electrode. The improvement of the sensitivity on the modified electrode is mainly due to

the significant nanostructures formed on the alloy electrode.

Figure 4

The Separation of AMGs

The electrophoretic separation of the selected AMGs is likely to be obtained under acidic running

buffer due to the protonation of their amino groups. However, the dissociation of silanols on inner

surface of PDMS microchannel will be suppressed under acidic condition, thus the EOF become

near to zero. To improve the separation, the CTAB is chosen to modify the surface of the

separation channel through forming positively charged layer on the surface to reverse the direction

of EOF. Under anodic mode, the reversed EOF is opposite to the direction of the electrophoretic

mobility of positively charged analytes, leading to the relatively long migration time that is of

benefit to the separation of AMGs. As can be seen in Figure 5A, the reversed EOF became

constant when the CTAB concentrations were larger than 0.6 mM because of the saturation of

CTAB absorption on the surface. The separation order of the selected AMGs depends on the

number of amino groups in molecule. The longer migration time corresponds with the more amino

groups while the shorter migration time is related to the less amino groups (the molecular

structures of the six AMGs shown in Figure S-1 of the supporting information). The effect of the

pH of the buffer on the separation depends on the degree of protonation of amino groups (shown

in Figure 5B), when the pH was 5.0, the three peaks of AMGs (AMI, KAN A, and PAR) merged

into a single peak. In contrast with decreasing the pH to 4.0, the baseline separation of six AMGs

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was achieved within 5 min. Thus pH = 4.0 was selected as the optimal pH of the running buffer in this experiment. Generally, the higher separation voltage produces the larger EOF that makes migration time shorter and peak height larger. It was worth noting in Figure 5C that the peak currents of the AMGs became smaller with increasing the separation voltage; on the other hand, the noise became large obviously at – 800 V of the separation voltage. These phenomena can be explained that the higher separation voltages (e.g.– 1400 V) increased EOF and caused the larger volume of the acidic buffer flowing into the mixing channel to decrease the pH of the mixed solution resulting the smaller peak currents, whereas the lower separation voltage (e.g. – 800 V) decreased EOF and caused the larger volume of the alkaline solution from the branch channels flowing into the mixing channel and resulted in the larger noise at the higher pH condition. Finally, – 1000 V was selected as the optimal separation voltage. The effect of the concentrations of sodium acetate between 2.0 to 10 mM on the separation and detection was not obvious (the data not shown here). However, when the concentration of sodium acetate is larger than 10 mM, the bubbles are formed in the separation channel and the larger buffer capacity requires the more alkaline solution for pH adjustment. Thus, the concentration of sodium acetate was selected as 5 mM.

Figure 5

Analytical Performances

Under the optimal conditions (combining 5.0 mM sodium acetate, pH = 4.0 and 0.6 mM CTAB as the running buffer, – 1000 V as the separation voltage, 60 μL as the volume difference between the reservoir A and BW, 10 s as the injection time, and 0.65 V as the detection potential), the response of the detector was analyzed as function of the concentration for the six AMGs. The linear

relationships between concentration and peak current were obtained for SPE, STR, AMI, KAN A,

PAR, and NEO at the range of 1.0 to 100 μM with R^2 > 0.99 and the limits of detection were

between 0.2 to 2.0 μM at a ratio of signal-to-noise of 3. In order to demonstrate the stability of the

modified electrode, repetitive injections (n = 30) were performed. The RSDs in peak current and

migration time were less than 6.2 % and 3.0 %, respectively. Above analytical parameters (linear

range, slope, correlation coefficient, LODs, and stability) for each analyte were summarized in

Table S-1 of the supporting information. To further evaluate the accuracy and performance of the

method, the recovery experiments under the optimum conditions were conducted with six AMGs

spiked in serum samples at three concentration levels of 20 μM, 50 μM and 100 μM. As can be

seen in Figure 6, a standard mixture of the AMGs (curve a), a spiked serum sample (curve b), and

a blank serum sample extract (curve c) were obtained under the optimum conditions. The average

recoveries (n=3) were 84.2 ± 4.1%, 68.2 ± 5.1%, 73.6 ± 3.9%, 88.9 ± 4.4%, 86.2 ± 4.2% and 79.6

± 4.9% for SPE, STR, AMI, KAN A, PAR, and NEO, respectively.

Figure 6

CONCLUSIONS

This manuscript described in detail the post separation pH adjustment on microchip and its utility

of the analysis of AMGs. Specifically, the present methodology adjusted pH after the separation of

AMGs under the acidic buffer and then applied the electrochemical detection of AMGs under the

alkaline condition. With the optimized conditions, the six AMGs were baseline separated within 5

min and detected at the relatively low micromolar level. The method offers a simple, sensitive,

and portable method for analysis of AMGs in biological sample without derivatization.

Additionally, this type of microchip provides a simple way for use of post separation reagent

addition for other applications.

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The authors declare no competing financial interest

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The list of the figure captions

Figure 1. The scheme and photography of the microfluidic chip. Channels: 50 µm wide, 50 µm deep. Injector (double-T) volume: 1.2 nL, separation channel: 57 mm, total length of Z-shape mixing channel: 2 mm, each double-T arms: 5 mm, each branch-like channel: 8 mm. Solution reservoirs: RB, running buffer reservoir; S, sample reservoir; SW, sample waste reservoir; BW, buffer waste reservoir; A1 / A2, alkaline reservoirs.

Figure 2. The plots (A) of the pH of the mixed solution vs. the volume difference (down x-axis) and the dilution percent of analytes (up x-axis); a computational simulation of the pH distribution in the mixing channel (B).

Figure 3. Electrophoregrams of AMGs under the post separation pH adjustment with the different concentration of NaOH at $V_{(A-BW)}$ = 60 µL (A) and with the different $V_{(A-BW)}$ at 0.3 M NaOH (B). Other conditions: running buffer 5 mM sodium acetate (pH = 4) with 0.6 mM CTAB, separation voltage – 1000 V, injection at -100 V / + 400 V for 10 s, detection potential = 0.65 V. The peak of the analytes (50 µM each): 1 = SPE, 2 = STR, 3 = AMI, 4 = KAN A, 5 = PAR, 6 = NEO.

Figure 4. Characters of the alloy modified electrode: SEM of Cu-Sn-Cr alloy coated Pt wire (A); cyclic voltmetragrams (B) of AMI (50 µM) with scan rate 100 mV s^{-1} under the pH 11 (a), 12 (b), and 13 (c); amperometric i-t curves (C) of AMI (50 µM each step) under pH = 13 at platinum (a), copper (b), and Cu-Sn-Cr alloy modified (c) electrode. Electrolytes: 5 mM sodium acetate with

0.6 mM CTAB.

Figure 5. Electrophoregrams of the six AMGs under the different separation conditions: CTAB (A); pH (B); separation voltage (C). Other conditions were the same as in Figure 3. The peak of the analytes (50 µM each): 1 = SPE, 2 = STR, 3 = AMI, 4 = KAN A, 5 = PAR, 6 = NEO.

Figure 6. Electropherograms corresponding to a mixture of standards (a), a spiking bovine serum sample (b), and the blank bovine serum sample (c). Other conditions were the same as in Figure 3. The peak of the analytes (50 µM each): 1 = SPE, 2 = STR, 3 = AMI, 4 = KAN A, 5 = PAR, 6 = NEO.

Figure 1. The scheme and photography of the microfluidic chip. Channels: 50 µm wide, 50 µm

deep. Injector (double-T) volume: 1.2 nL, separation channel: 57 mm, total length of Z-shape

mixing channel: 2 mm, each double-T arms: 5 mm, each branch-like channel: 8 mm. Solution

reservoirs: RB, running buffer reservoir; S, sample reservoir; SW, sample waste reservoir; BW,
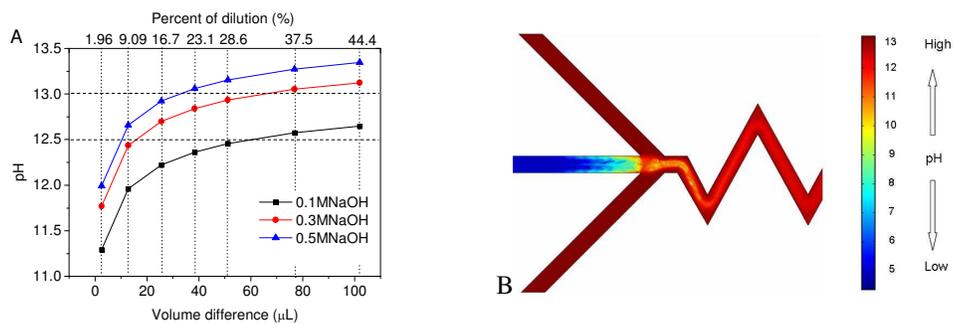
buffer waste reservoir; A1 / A2, alkaline reservoirs.

1

Figure 2. The plots (A) of the pH of the mixed solution vs. the volume difference (down x-axis)

and the dilution percent of analytes (up x-axis); a computational simulation of the pH distribution

in the mixing channel (B).

2

Figure 3. Electrophoregrams of AMGs under the post separation pH adjustment with the different

concentration of NaOH at $V_{(A-BW)}$ = 60 µL (A) and with the different $V_{(A-BW)}$ at 0.3 M NaOH (B).

Other conditions: running buffer 5 mM sodium acetate (pH = 4) with 0.6 mM CTAB, separation

voltage – 1000 V, injection at -100 V / + 400 V for 10 s, detection potential = 0.65 V. The peak of

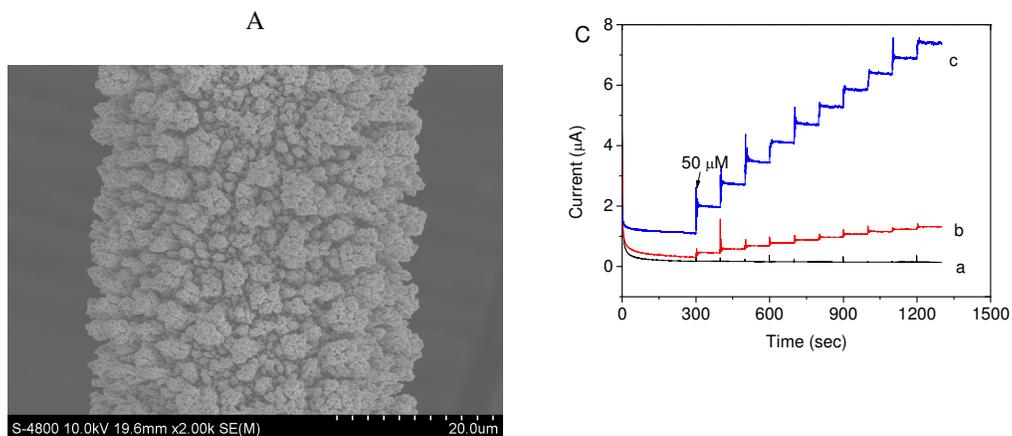the analytes (50 µM each): 1 = SPE, 2 = STR, 3 = AMI, 4 = KAN A, 5 = PAR, 6 = NEO.

A

C

B

Figure 4. Characters of the alloy modified electrode: SEM of Cu-Sn-Cr alloy coated Pt wire (A); cyclic voltmetragrams (B) of AMI (50 µM) with scan rate 100 mV s^{-1} under the pH 11 (a), 12 (b), and 13 (c); amperometric i-t curves (C) of AMI (50 µM each step) under pH = 13 at platinum (a), copper (b), and Cu-Sn-Cr alloy modified (c) electrode. Electrolytes: 5 mM sodium acetate with 0.6 mM CTAB.
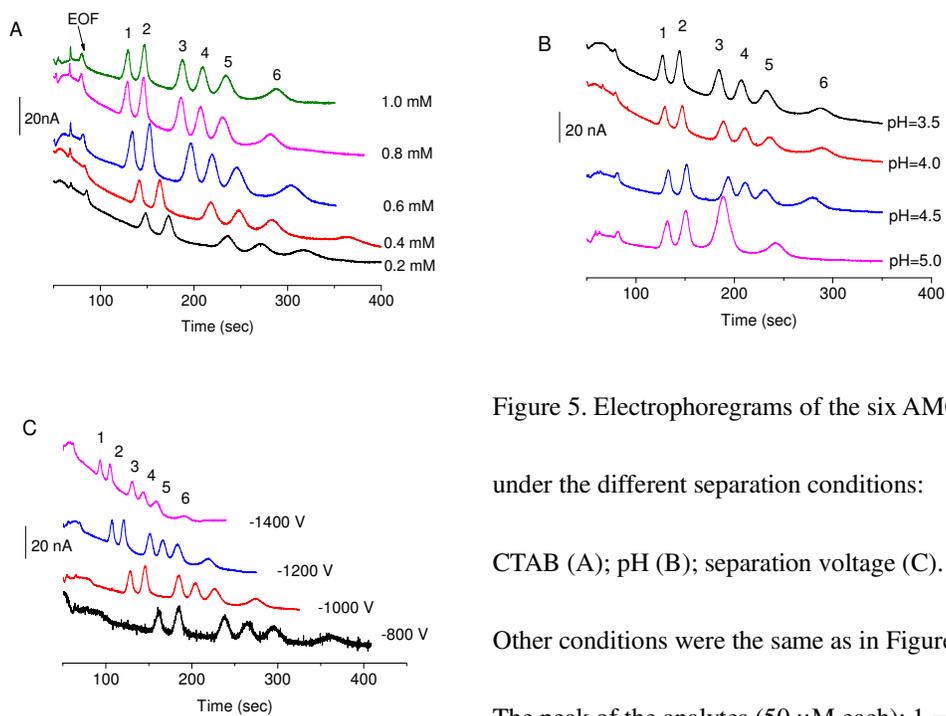
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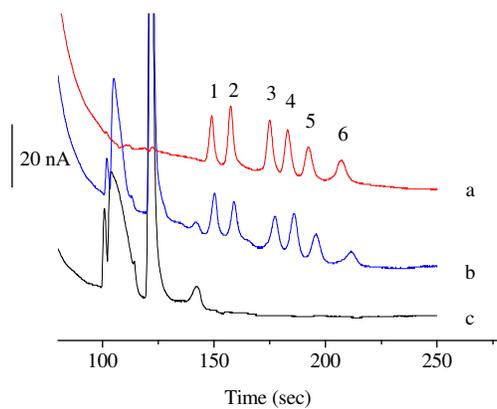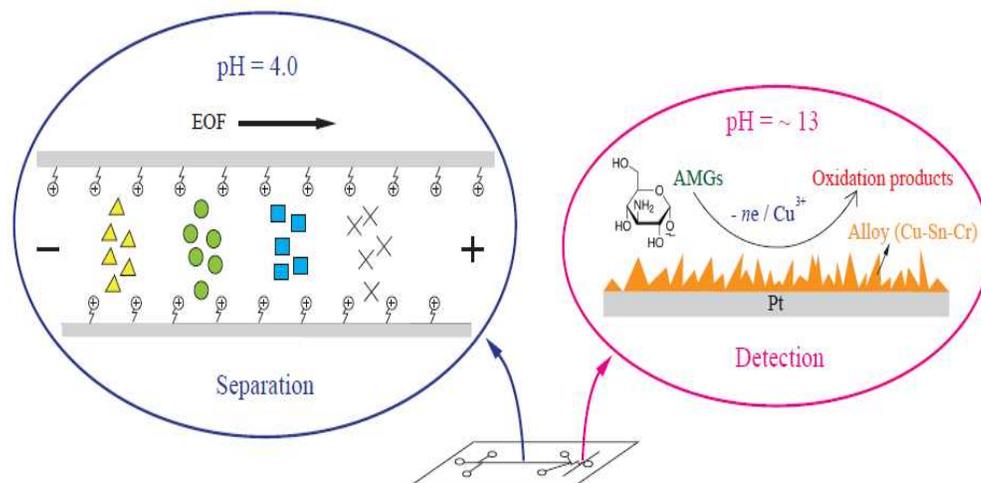
5

Figure 6. Electropherograms corresponding to a mixture of standards (a), a spiking bovine serum

sample (b), and the blank bovine serum sample (c). Other conditions were the same as in Figure 3.

The peak of the analytes (50 µM each): 1 = SPE, 2 = STR, 3 = AMI, 4 = KAN A, 5 = PAR, 6 =

NEO.

6

A simple microfluidic technique was developed with the ability to adjust pH after separation for electrochemical detection of aminoglycoside antibiotics.