Portable microfluidic chip electrophoresis device with integrated Pt electrodes for the analysis of AgNPs

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A portable electrophoresis device based on the SU-8/Pyrex microfluidic chip with integrated three 100 μm platinum (Pt) thin-film electrodes (working, reference, and auxiliary) was adapted for the analysis of silver nanoparticles (AgNPs). The microfluidic platform includes the main electrophoresis instrument, reusable holder, and microfluidic chip with integrated Pt electrodes. The electrochemical oxidation behaviours of AgNPs on Pt thin-film electrode were investigated by using cyclic voltammetry, while the electrophoresis behaviours of AgNPs were studied on the 'Lab-on-a-chip' platform. The influences of parameters including running buffer, separation voltage, and detection potential on the electrophoresis behaviours of AgNPs were all investigated. The results showed that AgNPs could be oxidised at 0.2–0.4 V [phosphate buffer solution buffer, pH 7.0] on the Pt thin-film electrode. The peak current enhanced with the increase of separation voltage, while the retention time decreased. The peak current varied with the detection potential and reached the highest value at 0.4 V. This microfluidic platform could be a fast and portable detection system for the analysis of total AgNPs.

1. Introduction: Capillary electrophoresis as a powerful analytical tool has been used for more than 30 years [1]. After the 'miniaturised total chemical analysis system' (μ-TAS) was introduced by Manz *et al.* [2] in the early 1990s, there has been an ever-increasing need for the development of 'Lab-on-a-chip' (LOC) devices, in which all the steps of the analytical process was integrated [3]. Microfluidic chip electrophoresis (MCE) which miniaturises the entire electrophoretic process with the advances of simpler, low cost, faster, and more efficient has been considered as the first stage of the true LOC device [4, 5]. Moreover, with the development of MCE, it has been widely applied in clinic [6], food [7], pharmaceutical [8], and environmental [9] fields.

As an important part of the LOC devices, detection system coupled to MCE requires a miniaturised and sensitive sensor. Although laser-induced fluorescence detection is still widely used, the requirement of derivatisation procedures may be its main drawback [5]. On the other hand, electrochemical detection (ED) has proven to be a very effective detection method for MCE. The inherent advantages of ED such as low cost, high sensitivity, and miniaturisation make it an ideal detection system coupled to MCE. It allows the integration of the detection system in the microfluidic chip using a low-cost process compatible with the microfabrication technologies [5]. Amperometry [10], conductimetry [11], and potentiometry [12] are the three main electrochemical methods commonly used in combination with MCE [5].

Traditional MCE-ED system has been widely employed due to its advantages [13–16]. Moreover nowadays, thanks to the improvements of related instruments, commercial portable MCE-ED devices have been developed and applied in the analysis of serotonin [17], phenolic compounds [18], iodide and ascorbate [19], and urine [20].

Research on NPs has always been a major interest in the scientific community. AgNPs have attracted continuous research efforts because of their superior properties, especially the exceptional antimicrobial effects [21]. Owing to their excellent properties, AgNPs have been widely used in common goods, for example, cosmetics, apparels, medical, and electrical devices [22]. Moreover, due to the large commercial usage of AgNPs which is also harmful to the mammalian organs, there is an urgent need for the analysis of AgNPs and monitor of their risk to the environment and general public health [22, 23]. Considering that AgNPs can be detected via electrochemical oxidation technique [24, 25], it is possible to analyse AgNPs using MCE-ED method. Moreover, Pumera *et al.* have investigated the electrophoresis behaviours of AgNPs using MCE coupled with traditional glassy carbon electrode [22].

To the best of our knowledge, though AgNPs were detected by the electrochemical method, there were no publications about the behaviours of them on platinum (Pt) electrodes. The portable MCE device (with Pt electrodes) had been used in the analytical field; it had never been used for the analysis of AgNPs. So in this work, not only the electrochemical oxidation properties of AgNPs on Pt electrodes were investigated, but also the electrophoresis behaviours of them on the portable MCE device coupled with integrated Pt electrodes were demonstrated. This microfluidic LOC platform could be a fast and portable detection system for the analysis of AgNPs.

2. Experimental section

2.1. Chemicals: AgNPs (with different diameters, citrate capping agent) were provided by Nanjing XFNANO Materials Tech Co., Ltd. (Nanjing, China). Trisodium citrate and sodium chloride (NaCl) were obtained from Sinopharm Chemical Reagent (Shanghai, China). All other chemicals were analytical reagents and used without further purification. Deionised water (specific resistance 18.2 MΩ cm) obtained from a Pall Cascada laboratory water system was used throughout the experiments. Phosphate

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buffer solution (PBS, pH 7.0) was used to disperse the AgNPs. All the solutions injected into the microchip channels were filtrated with the film of 0.22 µm.

2.2. Instrumentation: Electrochemical experiments were carried out in a drop cell (MicruX Technologies, Asturias, Spain) controlled by the CHI 660E Electrochemical Work Station (Chenhua Instruments, Shanghai, China). The drop cell with Pt thin-film electrodes was shown in Fig. 1*a*. Three Pt thin-film electrodes were used as the working, reference, and counter electrode.

The MCE instrument (HVStat) was provided by MicruX Technologies (Asturias, Spain), which included the main instrument, reusable holder, and microfluidic chip with integrated electrodes. The main instrument unit integrated a high-voltage power supply with a maximum voltage of ±3000 V and a potentiostat for amperometric measurements. The system was connected to the computer by Bluetooth and controlled by MicruX Manager Software to allow the automatic control of the experiments, which simplifies the works with microchips. The instrument was complemented with a reusable microfluidic chip holder (MicruX Technologies, Asturias, Spain) inside which the microfluidic chip was placed provides an easy way for the usage of the MCE. Fig. 1*b* shows the photograph of the holder. All the electric contacts for the high-voltage, detection electrodes, and the reservoirs for buffer and samples were integrated in the holder.

2.3. Microfluidic chip design: The cross-shaped single-channel SU-8/Pyrex microfluidic chip with integrated electrochemical detector based on three 100 µm Pt thin-film electrodes with a 100 µm gap among them (MicruX Technologies, Asturias, Spain) was employed. The design of the microfluidic chip with integrated electrochemical detector is shown in Fig. 2. The Pyrex substrate (38×13 mm^2) was used with SU-8 microchannel structure patterned and detection electrodes integrated on it. The 38×13 mm^2 microfluidic chip consisted of a four-way injection cross, with a 35 mm separation channel between the running buffer reservoir (A) and the waste/detection reservoir (B) and 10 mm long injection channel between the sample reservoir (C) and the sample waste reservoir (D). The two channels crossed each other with three side arms of 5 mm each in length. The channels had 50 mm width and 20 mm depth with the holes of 2 mm diameter acting as inlets/outlets, which situated at the end of the channels.

2.4. Amperometric detector: The electrochemical detector in the SU-8/Pyrex microfluidic chip was integrated at the end of the separation channel (Fig. 2). The detector consisted of three Pt thin-film electrodes (working, reference, and auxiliary). The working electrode (WE) was placed at a distance of 20 µm from the end of the separation channel. The distance between the working and reference electrodes (REs), reference, and auxiliary electrodes (AEs) was all of 100 µm. Moreover, the width of all the three Pt thin-film electrodes was also of 100 µm.

2.5. Electrophoresis procedure: Prior to use, the microchannels of the microfluidic chip were rinsed with 0.1 M sodium hydroxide, deionised water and running buffer for 10 min each with the help of a simple water-jet vacuum system. Trisodium citrate (10 mM) with addition of NaCl (20 mM) was chosen as the electrophoretic buffer for the analysis of AgNPs. After washing, all the reservoirs and microchannels were filled with the running buffer solution. After the baseline was stabilised, the sample reservoir (C) was filled with the sample solution. The injection was performed by applying +750 V at this reservoir with the waste reservoir (D) grounded. The separation was performed by applying the corresponding voltage to the running buffer reservoir (A) with the detection reservoir (B) grounded with the appropriate detection potential applied simultaneously. Moreover, all the electropherograms were recorded at room temperature.

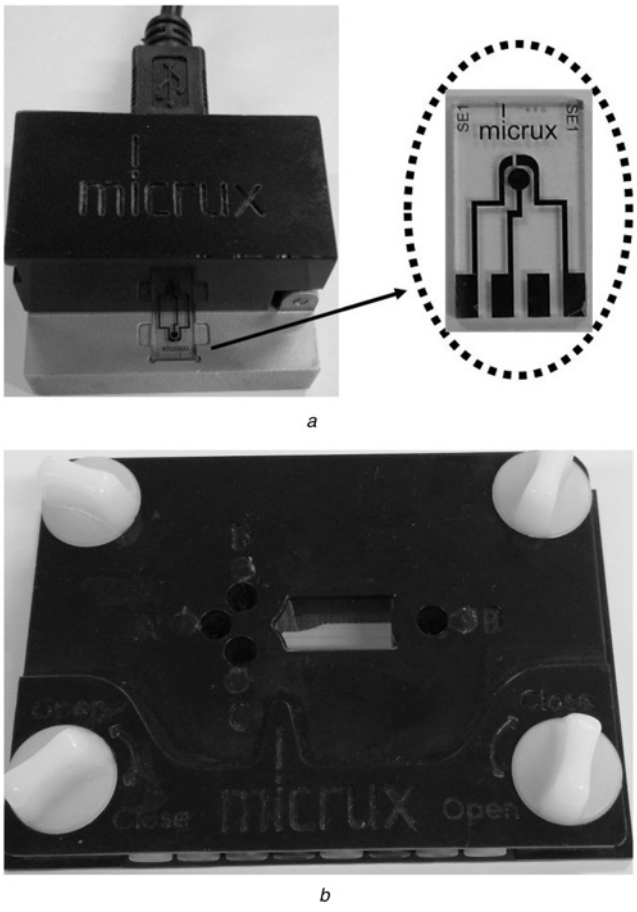

Fig. 1 *Photographs of the commercial drop cell with Pt thin-film electrodes and microfluidic chip holder*
a Drop cell and Pt thin-film electrodes
b Microfluidic chip holder
All the instruments were provided by MicruX Technologies

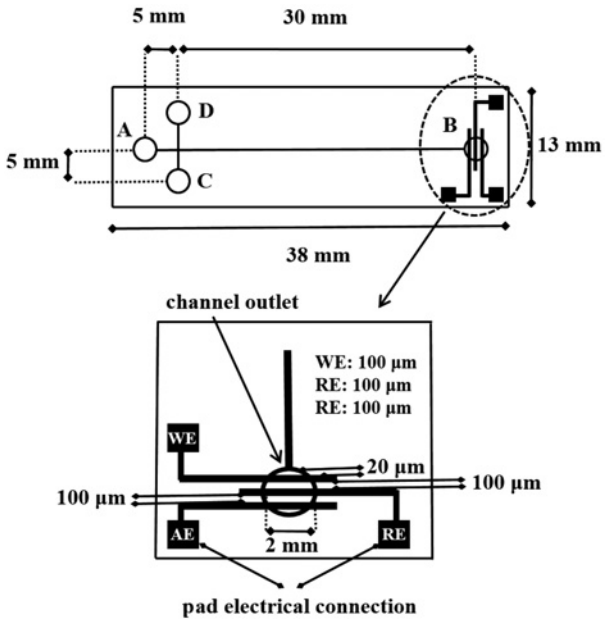

Fig. 2 *Schematic representation of the SU-8/Pyrex microfluidic chip with integrated Pt thin-film end-channel detector. WE: working electrode; RE: reference electrode; and AE: auxiliary electrode*

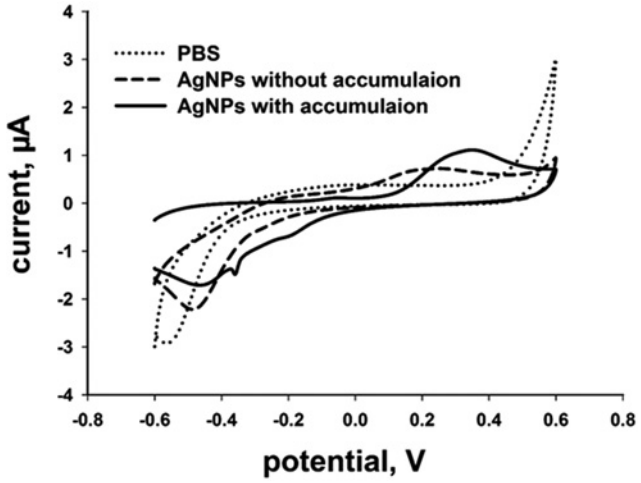

Fig. 3 *Cyclic voltammograms recorded at Pt thin-film electrode in PBS, PBS with AgNPs without and with accumulation. Scan rate, 50 mV/s*

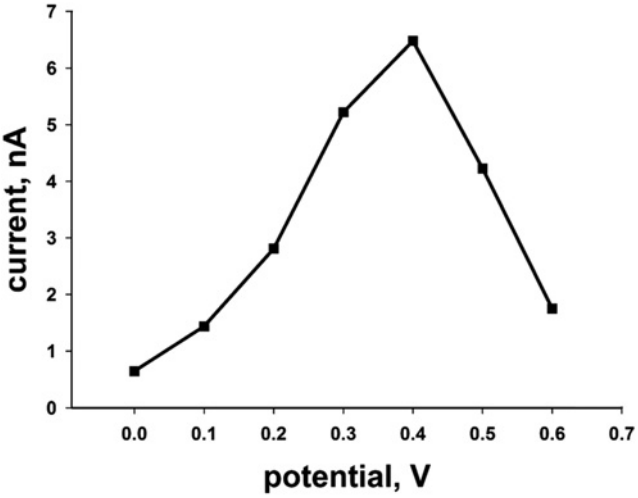

Fig. 4 *Hydrodynamic voltammograms of AgNPs recorded using Pt thin-film electrode. Running buffer: 10 mM citrate with 20 mM NaCl; separation voltage: +1800 V; injection potential: +750 V; and injection time: 3 s*

3. Results and discussion: To investigate the electrochemical oxidation properties of AgNPs on Pt electrode, cyclic voltammetry (CV) was performed in a drop cell with three Pt thin-film electrodes (working, reference, and auxiliary). Fig. 3 shows the typical CV curves for the Pt thin-film electrode recorded between −0.6 and 0.6 V in PBS (pH 7.0) at a scan rate of 50 mV/s. It can be seen that there was no any redox peak obtained in PBS (dotted line). When AgNPs (0.05 mg/ml) were added, an oxidation peak at about 0.25 V was observed in the CV curve (dashed line). Considering that there was no accumulation potential applied in this process, Ag^{+} as the most likely interference could not be reduced to Ag^{0}. The oxidative current response could only be attributed to the AgNPs. When an accumulation potential of −0.4 V was applied before the CV was conducted, the oxidation peak of AgNPs increased significantly and the peak potential shifted to about 0.35 V (solid line). Moreover, this might be caused by more AgNPs adsorbed on the electrode surface. On the basis of the results above, it can be concluded that AgNPs could be detected by the electrochemical method due to its electrochemical oxidation properties.

To achieve the best performance for the electrophoretic analysis of AgNPs, different solutions as the running buffer were investigated and the optimum performance was recorded using 10 mM citrate with 20 mM NaCl, which adopted for the study. Moreover, this was in accordance with the previously reported results in the literature [22]. To evaluate the effects of applied detection potentials on the peak current of the oxidation of AgNPs, hydrodynamic study was conducted and the results were presented in Fig. 4. The curves were recorded at the range from 0 to 0.6 V (versus Pt electrode) by applying the detection potential by stepwise 200 mV increase. As it can be observed in Fig. 4, the peak current rised with the increase of detection potential and achieved the highest current response at 0.4 V for the Pt thin-film electrode. When the applied potential exceeded 0.4 V, the peak current of AgNPs decreased significantly. Similar phenomenon had also been observed in the previous study [22]. The possible reason for the drop of the oxidative current was the aggregation of AgNPs, which might be induced by the exceeded potential. The potential response obtained was consistent with the electrochemical oxidation properties of AgNPs on Pt electrode discussed above.

Fig. 5 shows the typical electropherograms for AgNPs obtained by using the portable MCE-ED device. When AgNPs reached the detect reservoir after the separation channel, they were oxidised by the positive potential (0.4 V) to create the oxidative current which led to the sudden drop of the current recorded using the portable MCE-ED device. As discussed in the part of the electrochemical oxidation properties of AgNPs on Pt electrodes, the

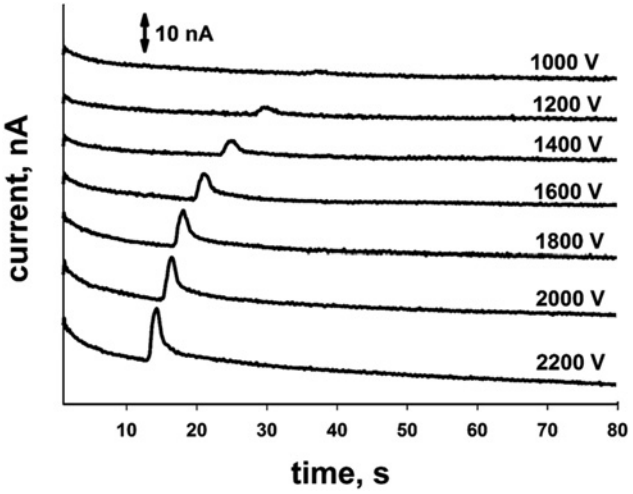

Fig. 5 *Typical electropherograms of AgNPs obtained on the portable MCE device with different separation voltages. Running buffer: 10 mM citrate with 20 mM NaCl; injection potential: +750 V; and injection time: 3 s*

interference of Ag^{+} could be excluded. Furthermore, compared to AgNPs, Ag^{+} had shorter retention time due to its much smaller size. For other NPs, different species had different electrophoretic behaviours and retention times. It is well known that different NPs have different oxidative potentials. AgNPs could be selectively detected by selecting the proper detection potentials. The influence of the separation voltage on the current response and retention time is shown in Fig. 6. As expected, the oxidation peak current of the AgNPs increased dramatically with the increase of separation voltages between +1000 and +2200 V. However, by increasing the separation voltage from +1000 to +2200 V, the migration time of AgNPs decreased from about 37 to 13 s, and the AgNPs peak width (at half height) decreased from about 3.6 to about 1.5 s.

It should be noted that though the current increased with the concentration of AgNPs (10 nm), there was no obvious effect of the variation of diameter (0.05 mg/ml) on the current responses. Moreover, there was no obvious difference of the retention times for the same concentration (0.05 mg/ml) of AgNPs with different diameters (10, 30, 60, and 100 nm), which indicated that AgNPs with different diameters were hardly separated. When compared to the previous study in the literature [22], the difference resulted

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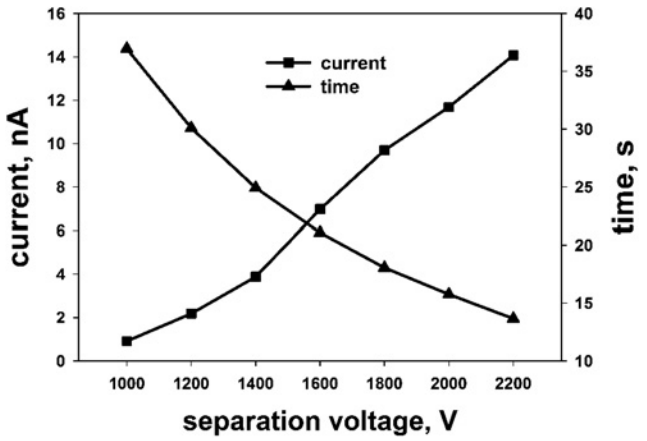

Fig. 6 *Influence of separation voltage on the current response and retention time of AgNPs. Conditions were in accordance with that shown in Fig. 5*

from that AgNPs with different diameters could not be separated on the portable MCE-ED device presented here. The reason might be that the separation channel of this portable MCE-ED device was largely shorter than that used in the literature.

The analytical performance of the portable MCE-ED device for AgNPs in terms of repeatability over multiple measurements was investigated by performing four consecutive runs with the solution of AgNPs (0.05 mg/ml). Consistent current responses among the four measurements were obtained and the repeatability was calculated from the relative standard deviation as 2.16%. So, this portable MCE-ED device could be used for the analysis of total AgNPs.

4. Conclusion: In this Letter, a portable MCE-ED instrument with integrated Pt thin-film electrodes was adapted for the analysis of AgNPs. AgNPs could be oxidised at about 0.2–0.4 V in PBS buffer on the Pt thin-film electrode. The peak current increased with the increase of separation voltage, while the retention time decreased. The peak current reached the highest value with the detection potential of 0.4 V. The detection performance of this portable device could be potentially improved by the usage of modified electrochemical detector in future. This microfluidic platform could be a fast and portable detection system for the analysis of total AgNPs.

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