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Effects of Heterogeneous Electron-Transfer Rate on the Resolution of Electrophoretic Separations based on Microfluidics with End-Column Electrochemical Detection

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Abstract

We demonstrate here that the electrode kinetics of an electrochemical detector contributes greatly to the resolution of the analyte bands in microchip electrophoresis systems with amperometric detection. The separation performance in terms of resolution and theoretical plate number can be improved and tailored by selecting or modifying the working electrode and/or by controlling the detection potential. Such improvements in the separation performance reflect the influence of the heterogeneous electron transfer rate of electroactive analytes upon the post-channel band broadening, as illustrated for catechol and hydrazine compounds. The electrode kinetics thus has a profound effect not only upon the sensitivity of electrochemical detectors but upon the separation efficiency and the overall performance of microchip-electrochemistry systems.

Keywords

electron transfer; resolution; surface modification; amperometry; microfluidics

Introduction

We report here an investigation of the effect of heterogeneous electron transfer rate upon the resolution in electrophoresis microchips with end-column amperometric detection. We demonstrate that the electron transfer rate plays an important role in improving the separation efficiency of such microfluidic devices. The electron transfer rate and hence the separation efficiency can thus be controlled by tailoring the electrode material or the detection potential.

Microfluidic devices have experienced tremendous success since their introduction and the advantages of such analytical microsystems have been well documented [1–5]. Microfluidic devices have experienced tremendous success since their introduction and the advantages of such analytical microsystems have been well documented [4–8]. Electrochemical detection (ED) has been widely adopted for microchip systems over the past decade and has been

reviewed recently [6–8]. Since Mathies and coworkers [9] integrated electrochemical detection with electrophoresis microsystems, there has been tremendous progress in this field. Although the Mathies' group illustrated the effect of placement of the reference and counter electrodes upon the detector performance [9], little efforts have been made to understand the role of the detection conditions upon the microchip separation [10]. While one would expect that the geometry of the detector and placement of the electrodes (i.e., channel-electrode gap) will affect the post-channel band broadening, the contribution of the electrode material or the detection potential to such broadening has rarely been considered [10].

Electrochemical reaction at the electrode surface involves heterogeneous transfer of electrons from/to electrode and can therefore be followed by measuring current as a function of time. The current is an instantaneous measure of the rate of an electrochemical process which is dependent on how many electroactive molecules or ions strike the electrode surface per unit time and the proportion of these release or gain electrons in the redox process. When an analyte elutes from the channel and the potential applied on the working electrode is sufficiently positive for its oxidation, as the analyte band passes over the electrode surface, those molecules immediately adjacent to the electrode surface will be oxidized in a heterogeneous transfer of electrons. The current that results from this exchange of electrons with the surface is measured as a function of time. Since the rate of material conversion by the electrochemical reaction is proportional to the solute concentration, the current will be directly related to the amount of compound eluted as a function of time.

If the heterogeneous electron transfer rate is not high enough, then when the analyte band passes over the electrode surface, its electrochemical reaction will be slow, leading to a broadening of the analyte zone within the detector. The reasons for this behavior originate in wall-jet detector design and in its typically significantly larger detector volume than the volume of injected sample [10]. It was shown that microfluidics with electrochemical detection in wall-jet configuration using macroelectrodes operate basically in the coulometric mode [10]. This may severely limit the ultimate resolution obtained during the assay. The response fidelity of the electrochemical detection is determined by the extent to which extra column factors (e.g., the heterogeneous electron transfer rate) are responsible for spreading of peaks in addition to peak broadening associated with the separation process itself. Ultimately, the limit of the performance of microfluidics with amperometric detection is set by the resolving power of the electrochemical detection, considering that all extra detector effects are the same.

EXPERIMENTAL SECTION

Apparatus

Amperometric detection was performed with an Electrochemical Analyzer 621 (CH Instruments, TX, USA). The high voltage power supply was laboratory-built and had an adjustable voltage range between 0 and +4000V. The glass micro-channel chips, fabricated by means of wet chemical etching and thermal bonding techniques, were purchased from Micalyne (Model MC-BF4-001, Edmonton, Canada). The original waste reservoir had been cut off by AMC, leaving the channel outlet at the end side of the chip. The glass chip consisted of a glass plate, 120 × 87 mm, with a 77 mm long separation channel leading between a reservoir (unused) and the channel outlet at the detection reservoir, and a 10mm long injection channel leading between the sample reservoir and the buffer reservoir. The separation channel had an effective length of 72 mm from the sample and buffer reservoir cross-section and the channel outlet, after leaving out 5 mm of channel length of unused buffer reservoir. The channels had a maximum depth of 20 μm and a width of 50 μm at the top. A pipette tip was cut and placed in each of the reservoirs.

A laboratory built Plexiglass construction served as a holder for the glass chip and was in detail described elsewhere [11]. The holder contained a sample, a buffer and a detection reservoir and a reservoir not used. A platinum wire was inserted into each reservoir and served as contacts for the high voltage power supply. A platinum wire and an Ag/AgCl wire were inserted into the detection reservoir, serving as counter and reference electrodes, respectively, for the amperometric detection. The Ag/AgCl wire had been prepared by electrolytic oxidization of a silver wire in 0.10M hydrochloric acid. Reproducible positioning the SPE was achieved by inserting the strip in a special groove into which the strip fits exactly. The SPE strip is further held in place by a plastic screw pressing the strip against the channel outlet.

Chemicals

Hydrazine sulfate were procured from J. T. Baker Chemical Company; methylhydrazine sulfate from Eastman Kodak Company; Catechol and epinephrine from Sigma; MES hydrate from Lancaster and 1,2-dimethylhydrazine dihydrochloride, palladium atomic absorption standard solution and other chemicals were purchased from Aldrich and were used without any further purification. Stock solutions (10mM) were prepared daily in distilled water and filtered with 0.45 μ m filter (Gelman Acrodisc). Prior to use, all the stock solutions were diluted in electrophoresis buffer. All experiments were carried out at room temperature.

Electrode Preparation

The screen-printed electrodes were printed with a semi-automatic printer (Model TF 100, MPM, Franklin, MA). The Acheson ink Electrodag 440B (49AB90) (Acheson Colloids, Ontario, CA) was used for printing electrode strips. The printing process and exact dimensions were described elsewhere in detail [11]. The final active working electrode area had the dimensions 0.30 $\times$ 2.50 mm. A tape (50 μ m thickness) placed on the SPE served as spacer in order to control the distance between the strip and the channel.

The palladium-modified SPE was prepared by cyclic scanning of the potential from 0.8 to -0.6V (vs. Ag/AgCl wire) for 60 cycles in a solution containing 1000ppm Pd (VI) and 0.5M HCl.

Electrophoresis Procedure

Prior to use, the channels were treated by rinsing with 0.1 M sodium hydroxide and de-ionized water for 20 and 5 min respectively. The buffer and sample reservoirs in the chip holder and the corresponding pipette tips on the micro-channel chip were filled with 200 μ L buffer and sample solutions, respectively. Then, the chip was placed in the chip holder with the pipette tips pointing downwards into the reservoirs and the detection reservoir was filled with buffer solution. Finally, the high voltage power supply was connected to the reservoirs.

The electrophoresis buffers used were phosphate buffer (10mM, pH 7.3) and MES buffer (25mM, pH 6.5) for the separation of hydrazines and catecholamines, respectively. Prior to use, the buffer solutions were filtered through a 0.45 μ m filter (Gelman Acrodisc) and sonicated for 20 min. After initial loading of the sample in the sample arm, the injection was performed electrokinetically by applying +1000V to the sample reservoir for 2s with the detection reservoir grounded and the buffer reservoir floating, while separation of catecholamines and hydrazines was performed by applying +1000V and +1500V respectively. The solutions were not deaerated.

Electrochemical Detection

The electropherograms were recorded with a time resolution of 0.1s while applying the desired detection potential (vs. Ag/AgCl wire). Sample injections were performed after stabilization of the baseline. No software filtration of the signal was used.

Calculations

The number of theoretical plates (N) was calculated according Eq 1 [12]:

$$N=5.56\left(\frac{t_m^2}{w_{1/2}^2}\right) \quad (\text{Eqn. 1})$$

where t_m is migration time of the analyte and $w_{1/2}$ is the peak width at half of height.

The resolution (R) was calculated using Eq. 2 [12]:

$$R=\frac{2 \times (t_2 - t_1)}{w_1 + w_2} \quad (\text{Eqn. 2})$$

where t_1 and t_2 are migration times of corresponding analytes and w_1 and w_2 is the corresponding peak width at the peak base of two adjacent analyte bands.

Safety Considerations—To avoid electrical shock the high voltage power supply should be handled with extreme care. Hydrazines are toxic compounds and should be handled with care.

RESULTS AND DISCUSSION

We demonstrate here that the separation efficiency observed at an end-column microchip amperometric detector is strongly dependent upon the rate of the electrochemical reaction of the electroactive analytes. To study the effect of the heterogeneous electron transfer rate upon the resolution of solutes in CE microchips, we selected well known model electroactive analytes, such as hydrazines [13,14] and catecholamines [11,15] in connection to different electrode materials and detection potentials. If the electron-transfer reaction is fast enough so that the analyte reacts immediately upon reaching the electrode surface, narrow peaks with less tailing can be achieved. Two factors affecting the reaction rate, the detection potential and the electrode surface, have been examined. For example, we evaluated the effect of the surface modification upon the resolution of hydrazine compounds by coating the carbon surface with an electrocatalytic palladium layer. Figure 1 compares electropherograms obtained at the bare (B) and a palladium-modified (A) screen-printed electrodes detectors using identical electrophoretic conditions and a detection potential of +0.5V. With the bare electrode, the three peaks, hydrazine (a), methylhydrazine (b), and dimethylhydrazine (c), are broad and partially overlapped (because of large tailing), leading to a poor resolution. In contrast, sharper peaks with a nearly complete baseline resolution are observed at the palladium-modified electrode. As expected, the migration times are independent of the nature of the working electrode.

In order to evaluate in detail the influence of the heterogeneous electron transfer rate upon the separation parameters, the effect of the detection potential on the microchip performance has been evaluated in connection to different groups of analytes. For example, Figure 2 shows the effect of the detection potential on the separation of epinephrine (a) and catechol

(b) over the +0.2 to +0.8 V range (A-G). The catecholamine peaks (appearing above +0.3 V) become sharper upon increasing the detection potential due to decreased tailing (observed at lower potentials). This behavior is due to the fact that the analyte band entering the detector zone is being exhausted according to chronoamperometric curve, where current is indirectly proportional to $\sqrt{t}$. As expected, the current observed when the analyte band reaches the detector is smaller for a slow heterogeneous electron transfer rate, compared to a fast electron transfer reaction [16]. The tailing resulting from the slow heterogeneous electron transfer rate (at low detection potentials) reflects the fact that the wall-jet (flow-onto) detector works practically in the coulometric regime [10]. Higher detection potentials thus lead to faster electrochemical reactions and to sharper peaks. Namely, once the analyte band elutes out of the channel, it immediately reaches the working electrode and it is oxidized instantaneously there because of the favorably high detection potential and the very fast electron-transfer rate.

In order to evaluate how much the tailing associated with such changes in the electron transfer rate affect the analytical separation, the separation efficiency parameters (theoretical plates and resolution) were estimated as a function of the detector potential. Figure 3 displays the influence of the detection potential upon the plate number of reversible (A, catechol (a) and epinephrine (b)) and irreversible (B, dimethylhydrazine (a) and hydrazine (b)) electrochemical reactions. Since the elution time is independent of the detection potential, the different separation efficiencies reflect the variation of the peak widths (Eqn 1). For the catechol compounds, with their faster electron-transfer rates, a narrow “transient” potential range (potential difference $E_{pa}-E_{pc}$ in cyclic voltammetry) is expected. The nearly sigmoidal dependence, with a fast increase of the separation efficiency upon raising the detection potential, is related to the narrow “transient” potential range of such fast reactions. The theoretical plate number for catechol and epinephrine increased from 800 to 7000 and from 50 to 2600, respectively, upon raising the detection potential from 0.5 to 0.7 V, with a nearly leveling off thereafter. In contrast, a more gradual (yet ~30-fold) change in the plate number with the detector potential is observed for the hydrazine compounds (B).

Figure 4 illustrates the effect of the detection potential upon the resolution of catechol (A) and hydrazine (B) compounds. The resolution between epinephrine and catechol (R) increased dramatically from 3.6 to 13.0 upon raising the detection potential by just 0.1 V (between 0.5 to 0.6V) and reaches a plateau after +0.7 V (A). In contrast, the increase in the resolution between hydrazine and dimethylhydrazine (B) is gradual, yet it triples at the highest potential of +1.0 V compared to detection at the lowest potential of +0.1 V.

Conclusions

We have demonstrated the profound effect of the heterogeneous electron transfer kinetics of an amperometric detector upon the separation efficiency of electrophoretic microchips. Different electrode materials and detection potentials thus lead to a different resolution. The influence of reaction kinetics is an important consideration for microchip applications because it can strongly influence the ultimate analytical performance of microfluidic devices. Apparently, fast electron-transfer reactions are essential not only for attaining high sensitivity but also for minimizing post-channel band broadening and to ensure high separation efficiency. It is clear that the suitability of electrochemical detection to a given problem ultimately depends on the voltammetric characteristics of the target molecules at a suitable electrode surface. It is important to bear in mind that the faster electrode kinetics under one set of conditions with a particular compound at a given electrode material may not infer generally faster kinetics for that material. Therefore, each electrode material and electrochemical reaction must be considered and interpreted on its merits.

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Abbreviations

MES	2-(N-morpholino)ethanesulfonic acid
SPE	screen-printed electrode

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Figure 1. Comparison of the separation of 100 μ M (a) hydrazine, (b) methylhydrazine and (c) 1,2-dimethylhydrazine with palladium-modified (A) and bare (B) screen-printed carbon electrodes. Conditions: 10mM phosphate buffer (pH 7.3) containing 1mM KCl. Detection potential, +0.5 V. Separation potential, +1000V; sample injection at +1000V for 2s.

Figure 2. Influence of the detection potential on the resolution of 100 μM (a) epinephrine and (b) catechol at a bare screen-printed carbon electrode. Detection potential, increasing from +0.2 to +0.8 V in 0.1V increments (A to G). Other conditions, are as in Figure 1.

Figure 3.

Influence of the detection potential on the number of theoretical plates. (A) 100 μ M catechol (A,a), and epinephrine (A,b), separation +1500 V, injection +1000 V for 2s. (B) 100 μ M 1,2-dimethylhydrazine (B,a), and hydrazine (B,b) in 10 mM phosphate buffer (pH 7.3), separation +1000 V, injection +1000 V for 2s.

Figure 4. Influence of the detection potential on the resolution of (A) epinephrine and catechol, and of (B) hydrazine and dimethylhydrazine. Other conditions, as in Figure 3.