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A two-electrode configuration for simplified amperometric detection in a microfabricated electrophoretic separation device

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The simplified amperometric detection scheme demonstrated is based on the amperometric working and electrophoretic ground electrodes only. The latter serves as counter and pseudo-reference as well. It is shown *via* the successful determination of neurotransmitters, ascorbic acid and phenols on gold or platinum working electrodes that this approach is feasible for detection on a channel based electrophoretic separation device. Also presented is the detection of carbohydrates and amino acids with copper electrodes. The results were found to be similar to those obtained with conventional capillary systems with amperometric detection, albeit at much reduced analysis times.

Introduction

It has been demonstrated amply over the last decade that electrophoretic separations can be carried out with high efficiency and fast analysis times in micromachined channels.[1–5] Sample injection is generally effected by employing a cross or double-T configuration as part of miniature manifolds, often referred to as μTAS (for micro-Total-Analysis-Systems) or chips. Most commonly, detection has been carried out by fluorescence which inherently has good sensitivity and is therefore well suited for the small channel cross-sections employed. However, as most analyte species are not native fluorophores, these have to be derivatized with a suitable reagent, or indirect detection *via* displacement of a fluorescent ion has to be used.[6] Detection by optical absorption, which is the most commonly employed method for conventional capillary electrophoresis, has rarely been employed,[7] presumably because of the short optical path lengths available.

An interesting alternative is the use of electrochemical detection methods. These techniques generally suffer much less from a reduction in the detection volume than optical methods. It is known from conventional capillary electrophoresis that very good detection limits, although not quite matching those obtainable by laser fluorescence, can indeed be achieved in particular by amperometric detection. An advantage of electrochemical methods is the fact that, in contrast to optical detection methods, the information is directly transformed into the electronic domain. This leads to a much simpler overall arrangement. However, even for conventional capillary electrophoresis, the adoption of electrochemical detection methods has been slow, presumably because the combination of separation by the application of a high voltage and the measurement of small electrical signals was often deemed impossible. Indeed, for the early arrangements reported, the electrical field for separation was decoupled by placing the electrophoretic ground electrode ahead of the detector electrode. This is not readily implemented though, and most workers now prefer the non-decoupled wall-jet approach which was found to be possible when capillaries with diameters of 25 μm or smaller were used. The relatively narrow diameters give rise to only small electrophoretic currents and this causes a steep decay of the electrophoretic field outside the end of the separation channel where the cross-section of the fluid conduit is usually very much enlarged and therefore the resistance drastically reduced.

The few prior reports on amperometric detection in microchannel-based electrophoretic separation systems have indeed all been based on the non-decoupled approach.[8–13] However, conventional potentiostats were used for detection. This necessitates the use of a counter and a reference electrode besides the working electrode. Including the electrophoretic ground electrode, this leads to the use of a total of four electrodes at the detector end of the capillary. This requires the construction of special detector cells on the separation device or the coupling with external electrodes, and is not well suited to the small dimensions in capillary electrophoresis. In particular, it is difficult to construct conventional reference electrodes employing flowing liquid junctions small enough for this application and these cannot be integrated on the device. Such electrodes also require careful attention for reliable operation. On the other hand, for conventional capillary electrophoresis, it is possible to employ the electrophoretic ground electrode as pseudo-reference and as amperometric counter electrode as well.[14,15] This leads to a simplification necessitating only the working electrode and the electrophoretic ground electrode, without the use of a conventional reference electrode. This approach was found to be feasible because, at the electrophoretic ground electrode, water electrolysis takes place (leading either to hydrogen or oxygen evolution depending on the polarity of the applied separation voltage) yielding a potential which is stable for a given set of conditions. It should also be borne in mind that the concept of a conventional potentiostat is based on sampling the potential of the solution in close vicinity to the working electrode with the reference electrode in order to avoid any errors due to an $i \cdot R$-drop. When using a normal reference electrode in an electrophoretic system without decoupling, this proper mode of operation cannot be achieved because there is always a small electric field of unknown value (effectively an $i \cdot R$-drop) in the solution due to the application of the separation voltage. The small dimensions do not allow the placement of the reference electrode close enough to the working electrode to effectively eliminate this bias.[16] For this reason, and the fact that the electrophoretic counter electrode provides a sufficiently stable reference potential, the loss of the normal three-electrode potentiostat function is not a shortcoming in this case.

The electrophoretic ground electrode has been used as reference electrode in our group for a number of years for amperometric as well as potentiometric detection without

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problems.[14,15,17–21] The adaptation of the non-decoupled and simplified electrode arrangement has now also been demonstrated for a miniature planar separation system, where the simplification is even more relevant than in conventional capillary electrophoresis. It is shown that this approach works well and a number of possible applications are presented, most notably the detection of amino acids and carbohydrates with the copper electrode, which has not previously been demonstrated with miniaturized electrophoresis systems.

Experimental section

Apparatus

The microfluidic device has the typical pattern of an elongated cross and was purchased from Alberta Microelectronic Corp. (AMC, model MC-BF4-TT100, Edmonton, Canada). The total length of the separation channel is 85 mm with a semicircular cross-section of 50 μm width and 20 μm depth. The injection channel has a length of 4 mm on each side of the separation channel and the joint has the shape of a double-T. At the detection end the channel was widened by us to a diameter of about 130 μm by etching with 40% hydrofluoric acid in order to allow better access of the detector electrode. Pipette tips, serving as buffer and sample reservoirs, were glued on top of the device with epoxy (Epo-Tek OG 116, Polyscience AG, Zug, Switzerland). The modified device was then mounted on an inverse microscope (model DM IL, Leica, Basel, Switzerland) equipped with an *XYZ*-micromanipulator as a working electrode holder. The working electrode was connected to a laboratory-built amperometric detector circuitry specially designed for this purpose.[14] The assembly was placed inside a Faraday cage, except for the high voltage power supplies. Three supplies with switchable polarity from Spellman HV Electronics Corp. (model CZE1000R, Spellman Europe, Haarlem, The Netherlands) were used. Two of these served for injection, one for the separation. Platinum wire electrodes placed into the buffer reservoirs were used for the application of voltages to the channels. Injection and separation parameters were controlled and data acquisition was performed with a program written in LabVIEW and a multifunction I/O-card (both from National Instruments, Austin, TX, USA) running in a Power Macintosh Computer (Apple, Cupertino, CA, USA). Three high voltage relays were used for switching of the voltages for injection and separation.

Electrodes

Teflon-coated platinum wires (diameter of the platinum core, 50 μm; diameter of the wire with Teflon coating, 75 μm; available from Advent Research Materials Ltd., Oxford, UK), attached to the micromanipulator, were used as working electrodes. The ends of the wires were bent into an L-shape in order to facilitate insertion into the separation device. Copper and gold electrodes were prepared by plating the tip of the platinum wire as insulated gold and copper wires with the desired diameter are not readily available. Plating with gold was carried out in a solution containing 23×10^{-3} mol dm^{-3} KCN, 20×10^{-3} mol dm^{-3} AuCN, 115×10^{-3} mol dm^{-3} K_2HPO_4 and 110×10^{-3} mol dm^{-3} KH_2PO_4 at 1.5 μA for 5 min.[22] For copper plating, the insulated platinum wire was dipped for 10 min into a solution containing 0.752 mol dm^{-3} $CuSO_4$, 0.01 mol dm^{-3} HCl and 0.71 mol dm^{-3} H_2SO_4 under application of a deposition current of 1.5 μA. The electrodes were freshly prepared before each series of measurements. A photograph of a copper plated electrode inserted into the conically etched end of the separation channel is given in Fig. 1. The Teflon insulation of the wire is only barely visible because of its transparency and the copper layer extends axially and laterally from the platinum core. Note also that the tip of the electrode is positioned exactly at the point where the channel opens up into the well in the cover plate of the device. The left segment of the circular line visible represents the hole in the cover plate, while the second concentric line represents a small depression etched into the bottom plate at the same depth as the channel.

Reagents and methods

3-Hydroxytyramine hydrochloride (dopamine hydrochloride), L-epinephrine (adrenaline), 2-(4-morpholino)ethanesulfonic acid hydrate (MES), 3-(cyclohexylamino)-1-propane-sulfonic acid (CAPS), 2-chlorophenol, 2,4-dichlorophenol, ascorbic acid, L-tryptophan, L-histidine, glycine, D-sucrose, D-galactose, D-fructose, gold(I) cyanide, sodium hydroxide, potassium cyanide, copper sulfate pentahydrate, potassium dihydrogen phosphate and dipotassium hydrogen phosphate were purchased from Fluka (Buchs, Switzerland). Hydrochloric acid (36–38%) and sulfuric acid (95–97%) were obtained from Baker (Deventer, The Netherlands). All reagents were of analytical reagent grade and water purified with a Milli-Q system (Millipore, Bedford, MA, USA) was used.

The channels were preconditioned daily with a 1 mol dm^{-3} NaOH solution and before every run with the appropriate running buffer. Fresh standard and buffer solutions were prepared daily. The standards were always dissolved in the buffer solution used for the particular experiment. Injection of standards was performed by placing the solution into one of the two openings at the ends of the pair of short arms of the cross and then applying a bipolar voltage across the double-T junction while maintaining the electrophoretic counter electrode connected to ground at all times (one platinum electrode at +1 kV on one side of the channel and the second platinum electrode at –1 kV on the opposite side referenced to ground). This method of injection avoids exposure of the working electrode to high voltages and minimizes charging currents such as those caused by rearrangement of the electrical double layer at the working electrode. The bipolar voltage was applied for 2 s. Note that this does not lead to a complete flushing of the double-T with the sample. The separation voltage was varied between +1 kV and +3.5 kV. Working electrode potentials were determined from hydrodynamic voltammograms.

Results and discussion

The use of the electrophoretic ground electrode as pseudo-reference requires that the amperometric detector current is negligible compared to the electrophoretic current. The reason for this is the fact that the amperometric counter current is also passed through the electrophoretic ground electrode. If the current through this electrode changes significantly during a separation run, then an error in the reference potential, and consequently a shift in the applied detection potential, may

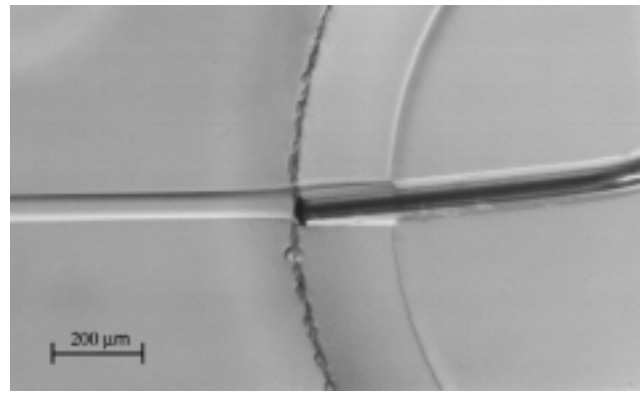

Fig. 1 The conically etched end of the separation channel with the detector electrode. The separation channel and the detector electrode are visible on the left- and right-hand sides, respectively.

result. For normal capillary electrophoresis this prerequisite is fulfilled.[14] In Fig. 2, the detection of the neurotransmitters dopamine and adrenaline as model substances on the present device is illustrated for a range of separation voltages. The peak currents were of the order of 2 nA, while for these separations the electrophoretic currents ranged from about 5 μA to 15 μA. As the electrophoretic currents are much more pronounced than the amperometric detector currents, the resulting amperometric counter currents can be expected to cause only a negligible effect. It can therefore be anticipated that, at least for the present planar arrangement, the simplified detection scheme is also feasible. It was also found that with regard to stability and reproducibility the system performed as well as for the conventional set-up with capillaries.[14] For a given set of conditions (*i.e.* buffer composition and applied voltage), the reference voltage was stable and reproducible.

Also illustrated in Fig. 2 is the effect of altering the separation voltage. As can be seen, faster separation is obtained for higher applied voltages, as would be expected. The peak shape is affected by the analysis time and the plate numbers were found to be 16 500, 20 000, 18 500, 16 500 and 14 000 plates m^{-1} for the separation voltages of 3500, 2500, 2000, 1500 and 1000 V, respectively. These values also compare well with those obtained by others using micromachined devices with amperometric detection[8–13] and match some of the results achieved with fluorescence detection.[1–5]

Short-term peak-to-peak noise on the baseline was determined to be typically of the order of 15 pA, which is comparable to the noise level reported by Woolley *et al.*[9] and better than the values quoted by Wang *et al.*[11,12] for planar systems employing conventional reference electrodes and commercial three-electrode potentiostats. Overall, the baseline is somewhat less stable for the higher separation voltages, possibly because of electrical interference from the applied field, which can be expected to affect the precision of peak measurements. For practical applications, compromise conditions may thus have to be sought. Woolley *et al.*[9] and Wang *et al.*[11,12] have also carried out studies on the effects of the applied separation voltage for dopamine and other species using micromachined electrophoretic devices with conventional three-electrode detectors. These authors have noted similar or, in some cases, even more pronounced effects on the baseline stability as in our case, which indicates that the simplified approach reported here does not lead to a deterioration of the performance in this regard.

Also evident from Fig. 2 is the effect of the applied voltage on the appearance of the peaks. The peak heights increase with the applied voltage up to 2500 V, and decrease again at 3500 V. However, the peak areas are identical, except for the highest applied value of 3500 V, indicating that the electrode efficiency is constant. The peak height differences for voltages of 2500 V and below are indeed only due to diffusional band broadening, as the same pattern was observed when the retention times were altered by adjustment of the electro-osmotic flow (by changing the pH value of the buffer solution). In two of the previous reports,[9,12] a much more marked dependence of the peaks heights on the applied separation voltage was shown, while in the third[11] the peaks were less affected by the applied voltage and the performance was similar to the results of Fig. 2 presented herein. It is thought that, in cases where a strong dependence on the separation voltage is found, this is caused by the fact that the applied separation voltage is not decoupled ahead of the detector.[9,11,12] The working electrode by necessity is placed between the two high voltage electrodes (and therefore exposed to the electric field) and thus a small voltage gradient is present between detector and reference electrodes. The value of this '*i*·*R*-drop' is critically dependent on the exact geometry of the overall detector cell and the applied separation voltage, and must be corrected for when setting the working potential of the detector electrode. If no adjustment is made, it is possible that the applied effective working electrode potential may be shifted away from the plateau region when altering the separation voltage, thus imparting a loss of sensitivity. It is important to realize that this bias voltage in the solution cannot be compensated for in any such device (including the previously reported ones) by the use of a conventional reference electrode because of the unavoidable physical separation between the two electrodes. The magnitude of the bias voltage is of the order of several hundred millivolts for amperometric detection in capillary electrophoresis, but its exact value is not predictable.[14,16,23] For this reason, it is not possible to adopt reported detector potentials for different arrangements, which is true for systems with and without conventional reference electrodes, and these have to be determined in each case. The fact that we found less of a dependence of the peak heights or areas on the applied separation voltage in this case does not imply that our electronic arrangement is less susceptible. The explanation for the reduced effect in this instance, in comparison with some of the previous reports on the neurotransmitters, must lie either in a better optimization of the applied electrode potential or, more likely, in a less steep potential gradient in the solution between working electrode and the reference because of a different cell geometry. The use of the conically etched channel end may be beneficial. In any case, the data in Fig. 2 provide evidence that the simplified system certainly does not perform any worse in this regard than the previously reported arrangements. It appears that, for the robustness of the electrochemistry, a careful design of the cell geometry is more critical than the electronic configuration of the detector. The sensitivities in terms of peak height for Fig. 2 are comparable to the values obtained for the neurotransmitters by Woolley *et al.*[9] and Wang *et al.*[11,12]

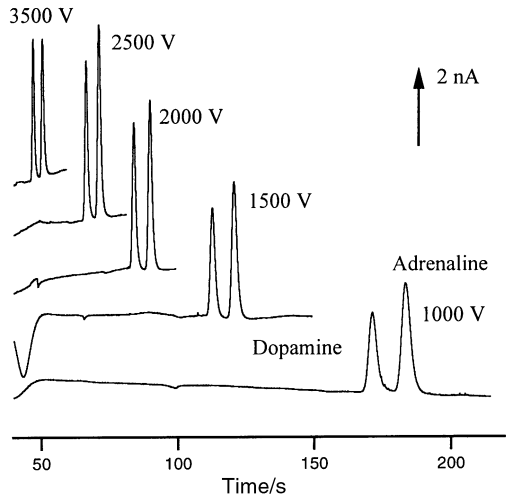

Fig. 2 Electropherograms for the two neurotransmitters dopamine and adrenaline (both approximately 10^{-4} mol dm^{-3}) for different applied separation voltages. Electrode: Au-coated Pt wire. Detection potential: 1100 mV. Buffer: 50×10^{-3} mol dm^{-3} MES–20×10^{-3} mol dm^{-3} phosphate, pH 6.5. Note that the electropherograms are offset against each other for clarity.

The separation of the two neurotransmitters is illustrated again in Fig. 3 in comparison with the separation of the same species in standard capillary electrophoresis with amperometric detection. Identical concentrations were used and the channel cross-sections were similar in both cases. The dimensions of the semicircular channel on the planar device are approximately 50 μm width and 20 μm depth, while the round capillary had a diameter of 25 μm. The resolution (R_s) was calculated as 1.66 and 1.98 for the planar and conventional systems, respectively (for peaks 1 and 3 in both cases), so that the performance is very similar in this regard. However, on the planar arrangement, the separation could be achieved in a period of time approximately six times shorter.

Detection sensitivity is a critical issue when miniaturizing analytical systems, and this is part of the reason why

fluorescence has commonly been employed with such electrophoretic separation devices. A comparison of the peak heights in Fig. 3 indicates that similar sensitivities were obtained. Note that the comparison is made for the overall systems without taking into account differences in injected amount of sample (approximately 20 times more for the microfabricated device as estimated for the electrokinetic injection mode employed in both cases), electrode materials and cell geometries. Rodriguez *et al.*[24] have also carried out a comparison of a microfabricated device with a regular capillary for fluorescence detection. In their work, a loss of sensitivity was found for the micromachined device, which was attributed to the fact that the detection volume on the chip was smaller than that on the capillary.

Calibration curves were acquired for dopamine and adrenaline on the micromachined system. The detection limits were determined as 2.0×10^{-6} mol dm^{-3} and 1.1×10^{-6} mol dm^{-3} for dopamine and adrenaline, respectively (3 × standard deviation). Linear graphs were obtained from the detection limit up to the highest concentration tested (10^{-4} mol dm^{-3}) and the correlation coefficients were determined as 0.9993 and 0.9994 for dopamine and adrenaline, respectively.

Two potential applications are illustrated in Fig. 4. Ascorbic acid, which is of interest because of its role as vitamin C and its use as a food preservative, was determined on a platinum electrode. The sensitivity, 3 nA for a concentration of 10^{-4} mol dm^{-3}, is higher than that obtained in ordinary capillary electrophoresis (0.75 nA/10^{-4} mol dm^{-3}).[14] The analysis time was about 1 min as opposed to 4.5 min by the more traditional method.[14] Chlorophenols, which are of environmental concern, were determined by oxidation on a gold electrode. The sensitivities for these compounds are also comparable to the results reported for regular capillary electrophoresis, and again the analysis could be achieved in a much shorter period of time.[25] However, the separation of the two compounds is not as good as that reported for capillary electrophoresis[25] or for the other compounds reported herein, which presumably is due to a lack of optimization of the background buffer in this case (*i.e.* a poor match of mobilities).

The determination of the carbohydrates sucrose, galactose and fructose is illustrated in Fig. 5. This was carried out on a copper electrode as the use of this material allows direct amperometric determination without the need for potential pulsing as would be the case with other metals. The copper electrode is useful for the detection of a range of species, as has been described mainly for chromatography, but also for capillary electrophoresis.[26–30] The electrochemical oxidation at copper electrodes is not fully understood but, for carbohydrates, has been attributed to an indirect mechanism in which the analytes are catalytically oxidized in the presence of Cu(III) ions.[27] As can also be seen from Fig. 5, the addition of 5×10^{-4} mol dm^{-3} Cu(II) ions to the background buffer solution led to a nearly tenfold increase of the detection signal for the carbohydrates. This effect has not been reported previously, but Stitz and Buchberger[31] found that the long-term stability of a copper electrode used in chromatography could be improved by adding copper hydroxide to the solution. It is possible that the provision of Cu(II) ions leads to an increase in the level of Cu(III) ions in the vicinity of the electrode, which is not achieved otherwise by oxidation of the electrode material itself. It is possible that the formation of insoluble hydroxides and oxides plays a role as well. The addition of the Cu(II) ions to the background buffer enabled the detection of carbohydrates down to a concentration of about 10^{-6} mol dm^{-3}. Also evident from the two electropherograms in Fig. 5 is a small change in the migration times. The baseline dip following the sucrose peak is indicative of the electro-osmotic flow. As the difference in the absolute migration times between sucrose, galactose and fructose is negligible, it can be assumed that no interaction between the carbohydrates and the Cu(II) ions took place, but that these changes were caused solely by the effect on the electro-osmotic flow. The peak resolution is close to the separation achieved in conventional capillary electrophoresis with similar conditions, but the peak sensitivity, after addition of the Cu(II) ions to the buffer, is about 60 times higher.[14]

Also possible with a copper electrode is the detection of amino acids in capillary electrophoresis.[15,26,28,30] The optical

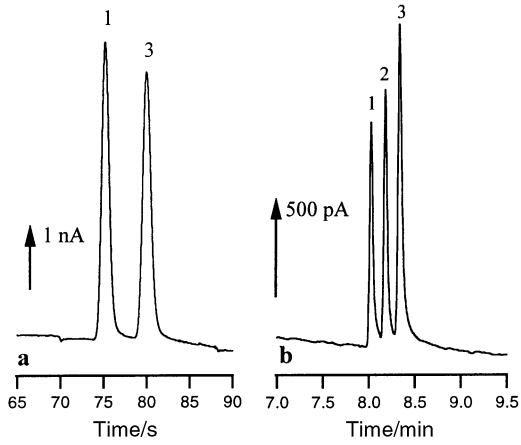

Fig. 3 Separation of neurotransmitters (all 10^{-5} mol dm^{-3}) on (a) the micromachined device and (b) with a standard fused silica capillary: 1, dopamine; 2, noradrenaline; 3, adrenaline. (a) Electrode: Au-coated Pt wire. Detection potential: +1100 mV. Buffer: 50×10^{-3} mol dm^{-3} MES–20×10^{-3} mol dm^{-3} phosphate, pH 6.5. Separation voltage: 2 kV. (b) Capillary: fused silica of 104 cm length, 25 μm internal diameter. Sample injection: electrokinetic, 5 kV, 7 s. Electrode: graphite disc of 250 μm diameter at approximately 25 μm distance from the capillary outlet. Detection potential: +800 mV. Buffer: 50×10^{-3} mol dm^{-3} MES, pH 6.1. Separation voltage: 30 kV. Part (b) was adapted from ref. 14 (reproduced with permission of the Royal Society of Chemistry).

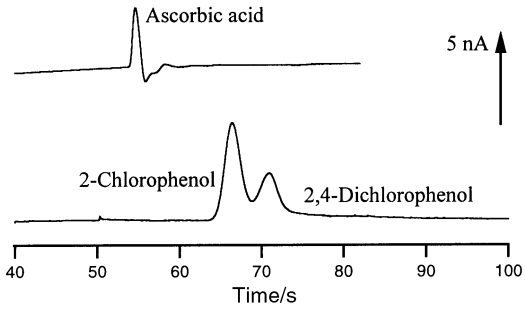

Fig. 4 Detection of ascorbic acid and chlorinated phenols (all 10^{-4} mol dm^{-3}) at platinum and gold-coated platinum electrodes, respectively. Detection potential: 1000 mV (ascorbic acid) and 1400 mV (chlorophenols). Buffer: 10×10^{-3} mol dm^{-3} disodium tetraborate, pH 9.3 (chlorophenols) and 10×10^{-3} mol dm^{-3} MES, pH 6.2 (ascorbic acid). Separation voltage: 2 kV.

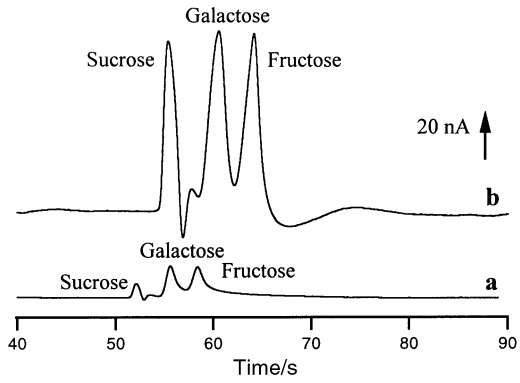

Fig. 5 Detection of carbohydrates (all 10^{-3} mol dm^{-3}) at a copper-coated Pt electrode without (a) and with (b) the addition of Cu(II) ions to the buffer. Detection potential: 1100 mV. Buffer: 10×10^{-3} mol dm^{-3} NaOH with and without 5×10^{-5} mol dm^{-3} copper(II) sulfate, pH 12. Separation voltage: 2 kV.

detection of these species has frequently been reported on microfabricated devices, but has always necessitated derivatization in order to render the analytes fluorescent. For the detection of amino acids with copper electrodes, two different possible mechanisms have been stipulated. In a neutral to basic environment, amino acids form chelate-like stable complexes. It is assumed that the complexation of free Cu(II) ions leads to an anodic current due to the re-formation of Cu(II) ions at the copper surface.[26,28] This mechanism is corroborated by the fact that potentiometric detection is also possible for identical conditions.[15] For highly basic solutions and high applied potentials, a catalytic oxidation similar to that for the carbohydrates has been postulated.[26,28] For our measurements with a copper working electrode, the best sensitivities and highest separation efficiencies could be obtained with 50×10^{-3} mol dm^{-3} CAPS buffer at pH 10.5. The separation and detection of amino acids is illustrated in Fig. 6. Here, the addition of Cu(II) ions to the buffer solution did not lead to an increase but to a decrease in the detection signal and a disproportionate increase in migration times for the three species. Both of these effects are in agreement with the complexation mechanism. According to our results, tryptophan and histidine have a stronger affinity to Cu(II) ions and hence a higher complexation constant than glycine. The theoretical plate numbers for tryptophan, histidine and glycine are 12 000, 10 000 and 8200 plates m^{-1}, respectively. In this case, the effect of the applied separation voltage was also investigated and a similar pattern in terms of separation efficiency and analysis times to that reported in Fig. 2 for the neurotransmitters was observed. However, the peak heights were more profoundly affected by the separation voltage. This is thought to be due to the relatively narrow plateau region in the voltammogram (approximately 100 mV), which is probably a result of the special mechanism on the copper electrode. In practical applications, a careful adjustment of the detection voltage will be required for best sensitivity.

Conclusions

The use of the simplified two-electrode detection scheme for amperometric detection on a micromachined electrophoretic separation device has been demonstrated successfully using different metals for the working electrode. In terms of baseline noise, interference by the high voltage field and separation efficiency, the arrangement performed as well as, if not better than, systems previously reported which were based on the conventional three- electrode approach. A commercial potentiostat was not needed and the detector circuitry used can be easily constructed at a cost of approximately GBP 100. It was found to be readily possible to adapt methods with amperometric detection from normal capillary electrophoresis. The separation efficiencies were close to those obtained in normal capillary electrophoresis. The sensitivities were at least as good as those of the more conventional approach, while the analysis times on the miniature device were always considerably shorter. The added effort in the construction of the device and the somewhat more elaborate periphery compared to normal capillary electrophoresis therefore appears to be justified when short analysis times are mandatory. To our knowledge, the electrochemical detection of carbohydrates and, in particular, of amino acids on such a miniature separation system has not previously been reported. In contrast to the detection of the amino acids by fluorescence, derivatization is not required for detection on copper electrodes, but the detection limits of the amperometric mode are higher. Voltammetric pulsing, which may be desirable for certain species which form residues on working electrodes, has not been implemented, but is compatible with the proposed simplified detector arrangement.

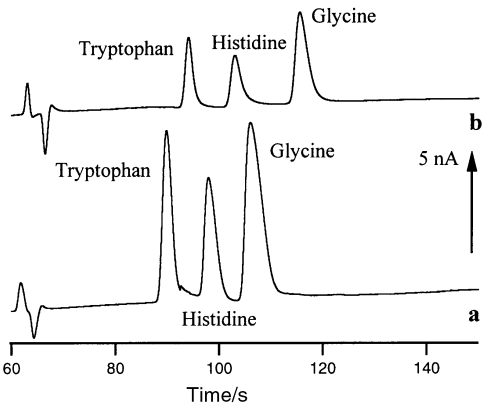

Fig. 6 Detection of amino acids (all 10^{-3} mol dm^{-3}) at a copper-coated Pt electrode without (a) and with (b) the addition of Cu(II) ions to the buffer. Detection potential: 600 mV. Buffer: 50×10^{-3} mol dm^{-3} CAPS–NaOH with and without 1×10^{-4} mol dm^{-3} copper(II) sulfate, pH 10. Separation voltage: 2 kV.

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