Double-chained cationic surfactant modification of SU-8/Pyrex® microchips for electrochemical sensing of carboxylic ferrocene after reverse electrophoresis

Rebeca Alonso-Bartolomé

Andrea González-López

M. Teresa Fernández-Abedul*

mtfernandeza@uniovi.es

Departamento de Química Física y Analítica, Universidad de Oviedo, 33006, Oviedo, Spain

*Corresponding author.

Abstract

This paper describes the effect of the modification of microchip microchannels with two different cationic surfactants on the electrochemical behavior of ferrocene carboxylic acid (FCA), common redox-probe in bioanalysis. Cetyltrimethylammonium bromide (CTAB), a single-chain surfactant, and didecyldimethylammonium bromide (DDAB), double-chained, were evaluated. The purpose was to obtain a reversal of the electroosmotic flow for allowing precise determination of FCA, an anionic probe that is employed in electrochemical bioassays. Although this was possible in both cases, modification of the microchannel with a high concentration of CTAB produced a differentiation between the free CTAB fraction and the CTAB-combined FCA. DDAB is presented as a good alternative for this modification because this double-chained cationic surfactant forms a more stable quasi-permanent coating on the microchannel surface, avoiding these surfactant-probe interactions. Linear relationship was found between the analytical signal and the concentration of FCA (evaluated between 10 and 150 µM) for a modification with 0.1 mM of DDAB.

Keywords: Ferrocene carboxylic acid; Microchip electrophoresis; Electrochemical detection; DDAB surfactant; SU-8 photoresist

1 Introduction

Detection of molecules can be approached using an intrinsic property such as e.g., electroactivity, absorbance or fluorescence. In the case of molecules ~~not having~~ without groups providing these properties, derivatization with a marker allows their detection. Procedures for labeling molecules with chromophores and fluorophores are commonly found in the bibliography [1] but when electroactivity is concerned, examples are scarce. Miniaturized electrochemical sensors and platforms are gaining interest for decentralized analysis and innovative approaches, not only ~~on the~~ for analytical tools [2,3] but also ~~in~~for ~~the~~ instrumentation [4], are being continuously developed. Although some electroactive labels have been studied, especially for selective purposes, either biosensors [5] or separation methodologies [6], more research is needed in order to study possible new markers and establish labeling procedures for developing promising analytical methodologies. Ferrocene, in the form of monocarboxylic acid (FCA) has been employed as mediator of electron transfer reactions, usually as modif~~ying~~ier of the working electrode [7] or more scarcely, as covalent electrochemical label of biomolecules such as e.g., antibodies [8], DNA [9], peptide nucleic acids [10,11] or aptamers [12]. In this context, it is of paramount relevance to have techniques that allow monitoring the bioconjugation process. Then, measurement of the label, either free or conjugated to biomolecules, is required in many cases for following the bioconjugation procedure of for indirect determination of analytes [13].

Electrophoresis is a powerful separation technique of biomolecules that is being increasingly adapted to the microchip format (microchip electrophoresis, ME). This is mainly due to recent developments not only in materials [14] but also in surface modifications [15,16] as well as in detection systems and associated instrumentation [17,18]. Different detection principles have been integrated with MEs [19,20]. Electrochemical (EC) detection schemes [21–24] are gaining acceptance and maturity, mainly due to their high sensitivity, ease of application and possibility of electrode integration into the chip during the fabrication process, leading to fully integrated microfluidic systems [25–27].

Currently, polymeric MEs have displaced the more traditional glass devices [17], due to a lower cost of materials and simplicity of fabrication techniques. A wide range of polymeric substrates with different fabrication protocols is reported in the literature [28–30]. The photoresist EPON SU-8 (SU-8), a negative tone epoxy photopatternable resist, mechanically reliable, optically transparent, chemically resistant and hydrophilic, has been used to fabricate ME devices [18,24,31]. The electroosmotic flow (EOF) in SU-8 is very similar to glass and it does not require any modification to be used with proteins and peptides since no adsorption of biomolecules on the microchannels occurs [32]. In the normal electrophoresis mode, where the microchannel wall is negatively charged, anions migrate after cations, with the neutral molecules in between. Then, separation of relatively small anions by capillary

electrophoresis is often a challenging task under cathodic EOF. A high charge-to-size ratio gives some inorganic anions extremely high electrophoretic mobility, which is generally comparable to EOF, or is even higher ~~than EOF~~. As a result and due to the net migration towards the anode [33], many anions can be detected only if reversed polarity is applied. If the wall is positively charged, both electrophoretic and electroosmotic mobility have the same sense for anions and they migrate before EOF and cations. Reversed EOF has been used in capillary and microchip electrophoresis for the separation of small inorganic and organic anions [33], acidic plant hormones [34], basic proteins [35], and peptides [36]. Apart from changing the polarity of the high-voltage electrodes, a positive charge should be generated on the wall and dynamic coating with cationic surfactants has been employed with this aim [37].

Cetyltrimethylammonium bromide (CTAB), a single-chain surfactant, has been extensively studied as dynamic modifier to obtain a reversal of the EOF in CE [38–40] and ME [41–43]. Thus, borosilicate glass microchips were modified with CTAB for the determination of inorganic ions [41], or arsenic species [42] as well as a PMMA microchip for the determination of urine markers [43]. Thereafter, didecyldimethylammonium bromide (DDAB) has been also employed to obtain reversed EOF [44]. In contrast to CTAB, DDAB has two hydrophobic chains that interact in solution forming bilayers or vesicles, creating more stable coatings. Although there are several studies about the effect of DDAB on the EOF in capillary electrophoresis [44–46], ~~the~~its use in MEs is very scarce. Coating with DDAB has been studied in PDMS for the determination of two fluorescent labels [37] or the bioluminescent determination of cellular ATP and ATP-conjugated submetabolome [47].

Since MEs can be fabricated from many different materials (capillar~~y~~ies for electrophoresis are commonly fused silica), the effect on the analytical signal of any modification can ~~be different and~~ vary from one to another [48]. In this work, we evaluate two cationic surfactants (CTAB and DDAB) aimed to obtain a reversal of the EOF for the determination of anionic FCA. This is the first time, to the best of our knowledge, that these coatings are employed in SU-8/Pyrex® electrophoresis microchips, with evaluation of the best conditions for the determination of FCA as the first step towards the determination of ferrocene-labeled biomolecules by ME-EC.

2 Experimental section

2.1 Chemicals

Cetyltrimethylammonium bromide (CTAB), didecyldimethylammonium bromide (DDAB), ferrocene monocarboxylic acid (FCA) and 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich (Madrid, Spain). Sodium hydroxide was obtained from Merck (Darmstad, Germany).

FCA solutions were prepared daily in the running buffer (RB). CTAB and DDAB solutions were prepared in the running buffer and stored at 4 °C. All solutions were filtered through Nylon syringe filters (0.45 µm, 25 mm) from OlimPeak (Technokroma, Barcelona, Spain). Water was purified employing a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA).

2.2 Materials. Electrophoresis microchips

The SU-8/Pyrex® chips used in this study were purchased from MicruX Fluidic (Oviedo, Spain). ~~It consists~~ They consist of 35 and 10-mm long separation and injection channels, respectively. The last one is situated 5 mm from the beginning of the separation channel. Both are 50-µm wide and 20-µm high. Holes of 2 mm diameter that act as reservoirs were situated at the end of the channels: A and B in the separation channel and C and D in the injection channel (Fig. S-1). One of the reservoirs of the injection channel (C) is filled with sample solution meanwhile reservoirs A, B and D are filled with the running buffer. Reservoir B is employed for detection and contains the electrodes of the potentiostatic system. The working electrode was a 50-µm wide platinum film and was located at a distance of 20 µm from the end of the separation channel. The other two electrodes (reference and ~~auxiliary~~counter) were 250-µm wide platinum films and were separated 100 µm one from each other. The distance between working and reference electrodes was also 100 µm.

The platinum thin-film interdigitated microelectrode array used for recording cyclic voltammograms was purchased from Micrux Fluidic (Oviedo, Spain).

Micropipettes employed for preparing and adding solutions to the reservoirs as well as 1.5 mL tubes were obtained from Eppendorf (Hamburg, Germany). The rest of volumetric material (flask, pipettes, vessels...) was of analytical reagent grade.

2.3 Dynamic coating of microchannels

For modifying the microchannel with CTAB, it was rinsed with 0.1 M NaOH for 10 min and then surfactant-containing buffer (different concentrations of CTAB in 25 mM HEPES-NaOH buffer pH 7.0) is flowed for 15 min, time commonly employed in many pretreatments and dynamic modifications [38]. For determination of FCA, CTAB is present in the RB in an optimized concentration. In the case a high concentration of CTAB is employed (e.g., 0.5 mM), washing with 0.1 M NaOH and then with CTAB-containing buffer has to be made between injections.

The concentration of DDAB was chosen according to a previous work [49] and the semipermanent coating was performed following a procedure reported previously [50], with some modifications. Microchannels were rinsed sequentially with 0.1 M NaOH for 10 min and with surfactant-containing buffer (0.1 mM DDAB in 25 mM HEPES-NaOH pH 7.0) for 20 min. Before a final washing with the running buffer for 5 min, the microchannel is let to stabilize in

0.1 mM DDAB (in RB) for 15 min. For the determination of FCA with DDAB semipermanent coating, RB does not contain DDAB.

2.4 Electrophoresis and electrochemical detection

Microchip zone electrophoresis was carried out using HVStat from MicruX Fluidic (Oviedo, Spain), which includes a high-voltage power supply and a bipotentiostat. MicruX Fluidic has developed a chip holder for using electrophoresis microchips with electrochemical detection. The system is controlled by user-friendly PC software MicruX Manager. Before use, the microchannels were preconditioned and coated as previously described. Reservoirs C and D were filled with 50 µL of sample and RB solution respectively, meanwhile A and B reservoirs were both filled with 50 µL of RB. Analyte detection was made amperometrically~~with amperometric detection~~ by application of a constant potential to the working electrode. Before analyte injection was performed, base-line stabilization was made applying a ~~separation potential~~voltage between A and B and recording the electropherogram after applying the detection potential to the working electrode.

Unpinched sample injection was performed applying −700 V between C and B for 30 s, meanwhile a voltage of −900 V was maintained between A and B for separation.

3 Results and discussion

Electroactive labeling requires the employ of highly detectable molecules that could be attached to the analyte or to a molecule related to it. Ferrocene carboxylic acid (FCA) contains a group that can be used for biomolecule labeling [51,52] and the reversibility of the redox process of ferrocene derivatives is well known. Then, labeling with FCA enables the detection of originally nonelectroactive analytes (e.g., proteins) by electrochemical methods [53]. However, its use in microchip electrophoresis assays requires a previous knowledge of its electrophoretic behavior as well as the possibilities of its electrochemical detection. In capillary electrophoresis (CE), normal mode, the EOF goes towards the cathode. Then, anions with an electrophoretic mobility higher than the electroosmotic mobility of the RB cannot reach the detector and the polarity of the voltage applied must be reversed in order to detect them. Therefore, for most anion separations it is also necessary to eliminate the EOF or reverse its direction. The most common method is the addition of a cationic surfactant, which is adsorbed on the capillary wall and produces a net positive charge on the surface. Under these conditions and using a negative power supply, all anions can be detected at the anode [54]. Getting an effective and reproducible dynamic coating for SU-8/Pyrex® MEs, which reverses EOF direction, is then aimed. Thus, the effect of two different cationic surfactants, CTAB and DDAB which are single and double-chained molecules respectively (Fig. S-2), is evaluated.

3.1 Effect of CTAB coating on the analytical signal of FCA

The first parameter that was studied was the concentration of surfactant since it can affect FCA mobility. ~~The~~First modification was made with a 0.05 mM CTAB solution. The electropherogram corresponding to a 200 µM FCA solution in 25 mM HEPES-NaOH buffer pH 7.0 at a detection potential of +0.4 V, where oxidation of ferrocene occurs (see cyclic voltammograms of Fig. S-3) is recorded. ~~As~~It can be seen in Fig. 1A (a), with a~~the~~ migration time (t_m) for FCA ~~is~~of 54 ± 3 s (n = 5).

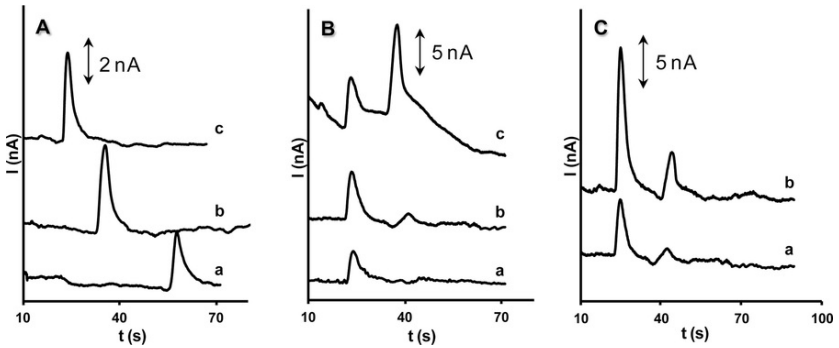

Fig. 1 Electropherograms of 200 µM FCA in RB buffer with (A) (a) 0.05, (b) 0.2 and (c) 0.5 mM CTAB modification (+0.4 V of detection potential); (B) (a) +0.4, (b) +0.6 and (c) +1.0 V of applied potential (0.5 mM CTAB modification). (C) Electropherograms recorded for injections of (a) 200 and (b) 500 µM FCA (0.5 mM CTAB modification, +0.6 V of applied potential). E_{INJ} = −700 V, t_{INJ} = 3 s, E_{SEP} = −900 V.

alt-text: Fig. 1

Electropherograms were also recorded after modification with different CTAB concentrations (0.2 and 0.5 mM) (Fig. 1A (b) and (c)). Increasing CTAB concentration produced a decrease in the migration time and ~~in the~~ peak width as well as a slight increase in the peak height. Although the intensity of the peak (i_p) when using 0.2 mM CTAB concentration does not differ significantly from this obtained for 0.5 mM, (3.6 ± 0.3 and 3.5 ± 0.3 nA

respectively), t_m was lower for the higher concentration (30.6 ± 0.24 s and 23.7 ± 0.15 s respectively) since a higher number of positive charges are present on the microchannel surface. The width of the peaks moved varied from 3.3 ± 0.2 to 2.8 ± 0.1 and 2.3 ± 0.1 s for increasing CTAB concentration. Therefore, further studies were performed after modification of the micro channel with 0.5 mM CTAB solutions.

The analytical detection potential can be determined directly from hydrodynamic voltammetry (HDV) or alternatively it can be estimated indirectly from e.g., cyclic voltammetry. CV measurements in a separate cell can be made much more rapidly, but the potential at the plateau in HDV will generally have a magnitude greater than the CV peak potential under typical measurement conditions for slow electron transfer reactions [55]. In our case, although initially and according to a cyclic voltammogram recorded in the running buffer (Fig. S-3), a detection potential of +0.4 V was chosen, electropherograms were recorded using increasing detection potentials in order to determine the most adequate. Electropherograms corresponding to injections of 200 µM of FCA in RB for +0.4, +0.6 and +1.0 V are shown in Fig. 1B. A clear increase in the peak height is observed when the potential detection moves from +0.4 to +0.6 V. Cyclic voltammograms were recorded in an electrochemical cell with platinum thin-film microelectrodes in order to use a similar material to the employed in MEs. However, differences in the design (e.g., distances between the electrodes) can produce a slight change in the potential. Surprisingly, a second peak appears in the last case at higher t_m (41.8 ± 0.5 s) and increases when a detection potential of +1.0 V is applied.

However, the peak current for the first peak remains constant. In order to know if this was due also to ferrocene, electropherograms were recorded at +0.6 V adding FCA (to a final concentration of 500 µM). An increase in the height of both peaks was observed, as shown in Fig. 1C, which suggests that both peaks are due to FCA. Single-chained surfactant additives, such as the CTAB, must be present in the RB at relatively high concentrations above the CMC concentration (0.9 mM in water [56] and 0.15 mM ~~[56]~~ in 50 mM phosphate buffer [57]) for producing a stable coating ~~the coating to stay intact~~ and generate a reproducible EOF. This can lead to interactions between anionic FCA and surfactant, ~~producing~~ generating structures of higher size. It results in strong changes (increases) in the t_m of anionic species [58,59]. The increase of the peak height with the potential detection might be ~~caused by~~ related to the need of higher potential values required for oxidising FCA when is associated to CTAB, probably less favoured. Although free FCA attained the maximum intensity, a sharp increase is seen for the second one when varying from +0.6 to +1.0 V.

In order to evaluate the response to the concentration of FCA, a calibration curve was made after modification with 0.5 mM CTAB in the RB applying a potential of +0.6 V, for concentrations ranging between 10 and 80 µM of FCA. The electropherograms obtained are shown in Fig. 2A and the value of sensitivity and limit of detection are reported in Table 1. Even when these values could seem adequate (good linearity between the peak current and the concentration for four points, Fig. 2B), the electropherograms recorded showed again two peaks (with shoulders in some cases), especially visible when the FCA concentration increased. On the other hand, migration time was not reproducible and the regeneration of the coating between successive injections was required to obtain analytical signals. In Fig. S-4 the electropherograms recorded for five successive injections of 100 µM FCA when the coating was not regenerated in between measurements indicated the necessity of this regeneration.

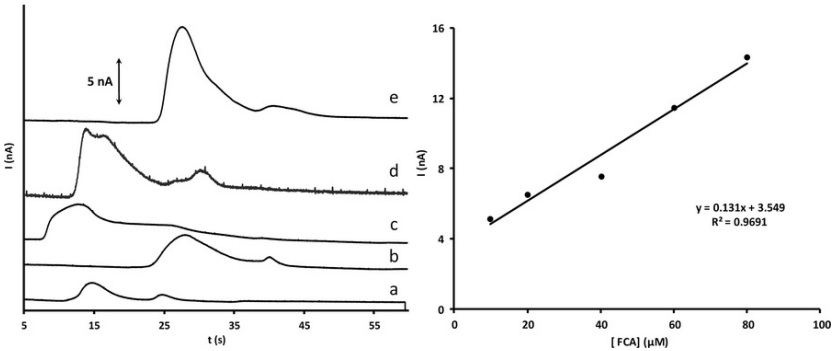

Fig. 2 Electropherograms of successive injections (n = 1 for each point) in the same MEs for a) 10, b) 20, c) 40, d) 60 and e) 80 ~~m~~µM solutions of FCA in 25 mM HEPES-NaOH pH 7.0 buffer with 0.5 mM CTAB and +0.6 V of detection potential, E_{INJ} = −700 V, t_{INJ} = 3 s, E_{SEP} = −900 V.

alt-text: Fig. 2

Table 1 Sensitivity and limit of detection obtained with calibration curves performed in the concentration range of 10–150 µM FCA for a 0.1 mM DDAB coating applying +0.6 V. In the case of the modification with 0.5 mM CTAB, the calibration is performed in the range 10–80 µM FCA applying also +0.6 V. *r* is the regression coefficient and *n* is the number of injections considered for each calibration point.

alt-text: Table 1

	Microchip	Day	m (pA µM⁻¹)	LOD (µM)	r	n

DDAB coating	ME-1	1	8.24	8	0.998	5
		2	12.65	6	0.998	5
	ME-2	1	7.18	10	0.998	5
		2	8.89	12	0.998	5
CTAB coating	ME-1	1	131	3	0.969	1

3.2 Effect of DDAB coating on the analytical signal of FCA

To avoid the effect of the interaction between CTAB and FCA, and with the aim of obtaining a reproducible and stable coating, we investigated the effect of a double-chained surfactant, DDAB, employed also to reverse the EOF. This surfactant forms aggregates at concentrations above a critical vesicle concentration (CVC); its value for DDAB in water at 25 °C is 0.0035 mM [60]. This behavior favors the generation of bilayer structures facilitating the formation of semipermanent coatings [53], attractive for microchannel wall coatings. In this case, the modification includes a stabilization step in such a way that removal of DDAB excess occurs. In this way, ion-pairing effects between the analyte and surfactant are decreased.

For evaluating the stability and precision of the film and therefore of the analytical signal of FCA, a 0.1 mM concentration of DDAB, which has already been shown to produce a reversal of the EOF [44,51] has been chosen. Firstly, stability of the semipermanent coating was studied and data after 24 and 48 h of modification were also acquired for a SU-8/Pyrex® microchip, which was stored in RB at 4 °C. This is important in order to know if microchips can be modified and keep stored until use. Results obtained are shown in Fig. S-5. Meanwhile the intensity of the peak values remains practically unaltered, a variation in the migration times of the peaks as well as a large decrease in the S/N were observed (from 89 in the first day to 8 and 7 in the second and third days respectively). Therefore, the coating was prepared at the beginning of each working day.

The intra and interday stability of the DDAB semipermanent coating was evaluated by measuring the t_m for twenty successive injections of 100 µM FCA solutions prepared in 25 mM HEPES-NaOH buffer pH 7.0 ~~solutions~~. At the end of the day, the microchip was rinsed with RB and kept dried. In Fig. 3A, t_m values obtained for two consecutive days when the dynamic coating was generated daily in the same microchip are presented. In Fig. 3B ~~values obtained~~electropherograms recorded for ~~CTAB~~DDAB modification are included for the sake of comparison. When the coating is performed daily it remains stable over several successive injections, with a mean t_m value of 31 ± 2 s. The second day we generated the semipermanent coating in the same way and t_m values are 30 ± 3 s. RSD values are less than 4% for all the values. However, in the case of CTAB, the value of RSD was as high as 31%, with a high increase in the migration time for the second day.

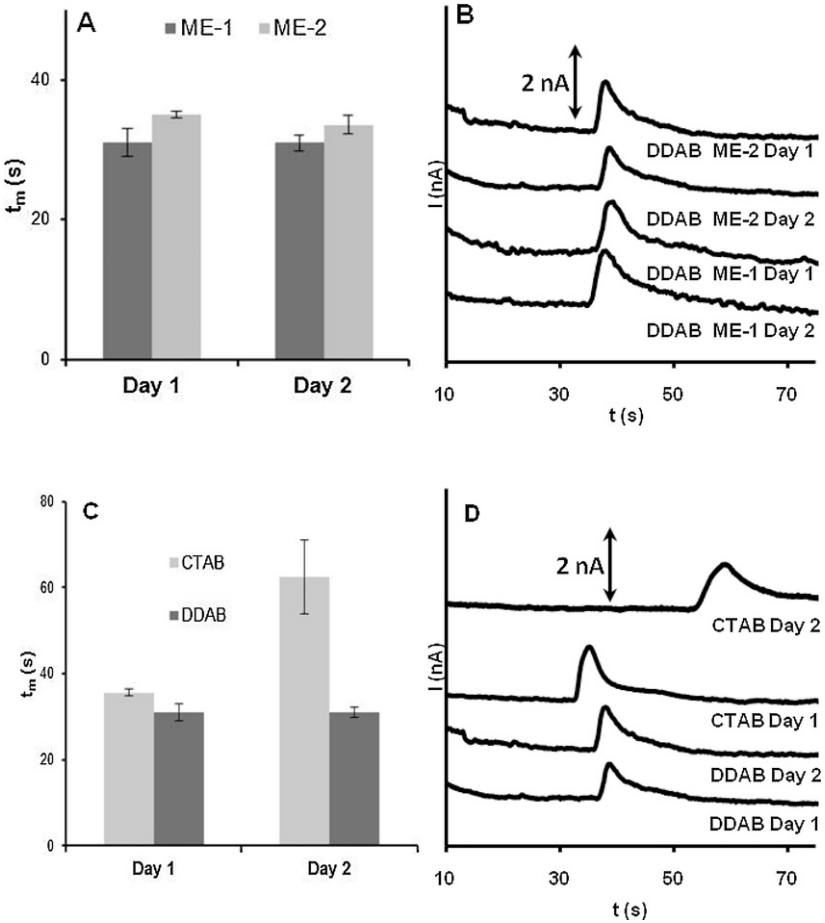

Fig. 3 A) Precision of the migration time and B) corresponding electropherograms for injections of 100 μM FCA solutions, recorded in two microchips modified with 0.1 mM DDAB in two different days. C) Precision of the migration time and D) corresponding electropherograms for injections of 100 μM FCA solutions, recorded in two successive days on a microchip with daily prepared 0.5 mM CTAB or 0.1 mM DDAB coatings. (Other conditions as in Fig. 1).

alt-text: Fig. 3

Electrophoresis microchips are not used as disposable devices but there is a trend to use cheaper materials and manufacturing methodologies in order to make them single-determination or one-day use devices. It is therefore interesting to know the precision of the analytical signal obtained on different MEs. Fig. 3A presents the comparison of the migration times obtained with different MEs in different days, with the corresponding electropherograms in Fig. 3B. It can be observed that similar migration time values are obtained for consecutive days in each microchip. Moreover, differences between both microchips are not significant. The reproducibility (including successive injections in consecutive days and different microchips) in terms of RSD is 5%. Fig. 3C shows the comparison of the electropherograms obtained for injections of 100 μM FCA in DDAB and CTAB modified microchips in two consecutive days, with the corresponding electropherograms in Fig. 3D.

Once a stable coating is obtained, a hydrodynamic curve was made to optimize the detection potential in the running buffer. Fig. 4A shows the curve obtained with injections of 200 μM FCA solutions and applied potentials varying from +0.4 ~~and~~to +0.7 V. A plateau is attained at +0.6 V, potential chosen for the remainder of the work. In Fig. 4B electropherograms corresponding to five successive injections recorded at an applied potential of +0.6 V are presented. The mean value of the peak intensity is 3.7 ± 0.2 nA for a migration time of 30.2 ± 0.5 s.

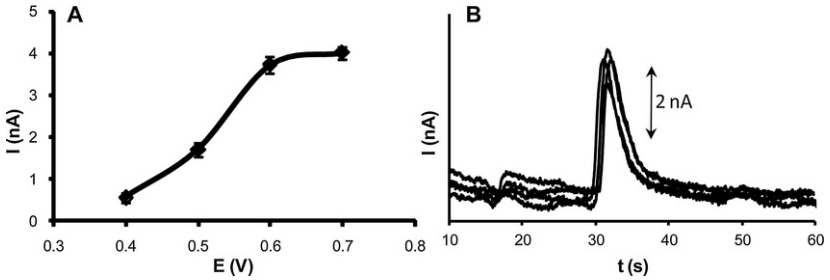

Fig. 4 A) Hydrodynamic curve for injections of 200 µM FCA solutions in RB with 0.1 mM DDAB modified microchannels. B) Electropherograms corresponding to five succesive injections of 200 µM FCA solutions in RB with 0.1 mM DDAB modification and an applied potential of +0.6 V. (Other conditions as in Fig. 1).

alt-text: Fig. 4

Under the optimized conditions mentioned above, 0.1 mM DDAB in 25 mM HEPES-NaOH pH 7.0 buffer, we studied the effect of the modification on the response of the analytical signal to the concentration, evaluating the sensitivity and limit of detection. Calibration curves for FCA using DDAB were performed in two different MEs and two consecutive days (the semipermanent coating was generated daily) in the concentration range comprised between 10 and 150 µM of FCA. The electropherograms obtained are shown in Fig. 5 and the values of sensitivity and limits of detection are reported in Table 1. The average sensitivity is 9.2 ± 2.4 pA $\mu M^{-1}$and the limit of detection, calculated as the concentration corresponding to three times the standard deviation of the intercept is 9 ± 3 µM.

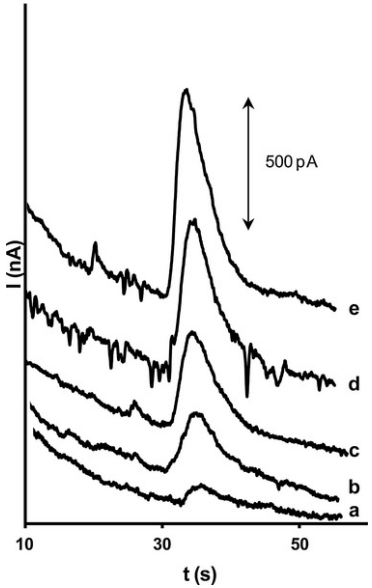

Fig. 5 Electropherograms of: a) 10, b) 20, c) 40, d) 60 and e) 100 µM solutions of FCA in 25 mM HEPES-NaOH pH 7.0 buffer with0.1 mM DDAB modification and +0.6 Vof detection potential. (Other conditions as in Fig. 1).

alt-text: Fig. 5

Calibration performed on different days and different MEs produces very similar slopes and detection limits, confirming the reproducibility and adequacy of this coating.

4 Conclusions

A simple and reproducible methodology has been proposed for determination of anions ($FcCOO^-$ in this case) by reversing the electroosmotic flow in hybrid SU-8/Pyrex® MEs. Two different cationic surfactants, CTAB and DDAB have been evaluated. Although when we used CTAB as dynamic coating, EOF was reversed, interactions between surfactant and analyte produced a second peak due to the interaction between the indicator species and the

surfactant. Moreover, t_m values obtained were not reproducible on different days so that, although it could be useful for the study of this type of interactions, their use as dynamic modifier does not seem to be suitable for the determination of anionic species in SU-8/Pyrex® MEs. However, results indicate that DDAB induces a reproducible and stable semipermanent coating by dynamic modification of the microchannel surface. A simpler procedure is employed in this case, since DDAB coating remains after stabilization for a period of time and addition to the running buffer (as in the case of CTAB) is not required. In view of the results, it can be concluded that using DDAB (0.1 mM) as dynamic modifier, adequate intra- and inter-day precision is obtained for different MEs. The analytical signal responded linearly to the concentration. The results are very promising for the use of the indicator in bioassays and then, work is in progress in order to take advantage of this analytical methodology and use FCA for labeling biomolecules and further ME-EC determination.

Notes

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2017.08.012.

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Rebeca Alonso-Bartolomé received her M.Sc. in Chemistry (University of Oviedo) in 2011 with the work entitled “Microchip electrophoresis with electrochemical detection: modification of microchannels and improvements in the design for the determination of analytes of interest”. Her research interests include electrochemistry, microchip electrophoresis, protein covalent modification and synthesis and applications of different nanoparticles.

Andrea González-López received her B.Sc. in Biology (University of Oviedo) in 2012 and her M.Sc. in Analytical Chemistry (University of Oviedo) in 2015 with the work entitled “Design of a paper-based electrophoresis microchip with integrated electrochemical detection”. She is nowadays a Ph.D student and her research interests include immunology, biosensors, electrochemistry, microfluidics and LOC.

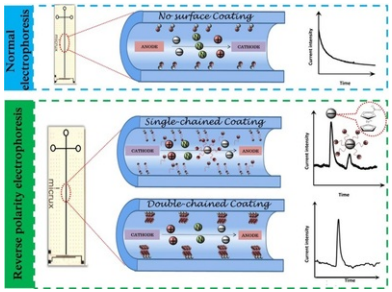
M. Teresa Fernández-Abedul received her Ph.D. in Chemistry in 1995 at the University of Oviedo, Spain. Since 2002 is working as Associate Professor in Analytical Chemistry (Department of Physical and Analytical Chemistry) at the University of Oviedo. Her current research interests are the development of (bio)sensors employing (nanostructured) electrochemical transducers as well as the design and development of electroanalytical methodologies based on miniaturized devices, some of them microfluidic (e.g., microchip electrophoresis, miniaturized electrodes, paper- or thread-based electrochemical cells), employing mass-produced low-cost elements (e.g., pins, staples), using different labels (e.g., silver nanoparticles, ruthenium complexes) or integrated principles of detection (e.g., electrochemiluminescence). At this moment, she is working in the project: “Paper electroanalytical devices: a new generation of transducers and platforms for decentralized and low-cost analysis”.

Appendix A. Supplementary data

The following is Supplementary data to this article:

Multimedia Component 1

Graphical abstract



Highlights

- Dynamic modification of SU-8/Pyrex® electrophoresis microchips with CTAB and DDAB.
- Single and double-chain cationic surfactant modification to obtain reversal electroosmotic flow.
- Formation of stable quasipermanent coating on the microchannel surface with DDAB.
- Electrochemical determination of ferrocene monocarboxylic acid by reverse electrophoresis.
- Linear response between ferrocene monocarboxylic acid concentration and analytical signal.

Queries and Answers

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Answer: I think it should be better if all the columns were centered. On the other hand, I consider this table should be located after section 3.2. The supplementary information is again uploaded with minimum changes, also a change in the font of some figures.