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Research Article

Interinstrumental transfer of a fast short-end injection capillary electrophoresis method: Application to the separation of niobium, tantalum, and their substituted ions

The interinstrumental transfer of a short-end CE method was studied. A model separation of the hexameric forms of niobium, tantalum, and their substituted ions ($\text{Nb}_{6-x}\text{Ta}_x$ with $0 \leq x \leq 6$) was selected as test case. The method was first optimized on a Beckman instrument and in a second step transferred to an Agilent instrument. The transfer needed updated guidelines that tackled differences in effective capillary length, 8.5 (Agilent) versus 10 cm (Beckman), because of instrumental different capillary cartridges. Differences in effective length lead to migration time and separation efficiency inequalities, illustrated by a decrease in resolution between the substituted ions. The difference in effective length was overcome by adapting the lift offset parameter of the Agilent instrument. The lift offset default setting is 4 mm and by increasing this parameter both the inlet and outlet lifts are lowered and thus the detection window can be displaced and consequently the effective length was increased. The decrease in effective length difference and the effect on the separation efficiency was investigated and led finally to a restored separation of the substituted ions. The adaptation of the lift offset parameter during short-end injection methods was added to earlier developed guidelines to facilitate interinstrumental method transfer of CE methods.

Keywords:

Interinstrumental method transfer / Lift offset / Niobium and tantalum / Short-end injection
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1 Introduction

This study is part of a larger project in which guidelines are developed to facilitate analytical method transfer (AMT) of CE methods. The increased application of (CE) methods therefore entails more frequent AMT, both between laboratories and instruments of different brands. The AMT is an inevitable part of the method life cycle and is a well-documented process that qualifies the receiving laboratory or instrument to successfully execute a method that was developed and validated in another laboratory or on another instrument [1]. A successful AMT guarantees that the receiving laboratory/instrument is able to obtain similar results, with preferably a similar experimental error/variation, compared with the developing laboratory/instrument.

Cornerstones for a successful AMT are well-designed pretransfer studies, such as robustness tests and precision studies [2–6]. The AMT of CE methods is more complex than that of HPLC methods due to several reasons, such as the lower precision and robustness of CE methods and instruments, the lack of uniformity between CE instruments and the greater impact of instrumental settings on the CE separation. Guidelines, based on performed robustness test and precision optimization studies, concerning the interinstrumental method transfer of CE methods, were developed earlier in our laboratory to improve the achievement of similar results and to overcome instrumental differences and detector- and data-handling settings differences [4–6]. In the actual study, an interinstrumental transfer of a particular CE application, namely a short-end injection (SEI) method, was used as test case to develop specific guidelines needed during AMT of fast SEI CE methods. Because of an imposed minimal capillary total length (typically ~35 cm), SEI methods are often used to further reduce the effective capillary length and thus the analysis time. Furthermore, advantages of SEI methods include a potentially reduced peak broadening due to the lower migration length and the use of higher ionic strengths due to a lower applied voltage [7, 8]. Drawbacks of using SEI methods are the potential loss of resolution due to the lower separation length,

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Abbreviations: AMT, analytical method transfer; SEI, short-end injection

and the increased complexity of an interinstrumental AMT due to the discrepancy in effective lengths. Instrumental requirements for SEI methods are a dual polarity power supply and the ability to apply pressure or vacuum on both capillary ends, present on both current leading CE models, being the Beckman P/ACE and the Agilent 7100 [http://www.agilent.com/cs/library/technicaloverviews/public/5990-3409EN.pdf]. SEI methods are often used in high-throughput screening studies in both chiral and achiral drug analysis, in kinetic studies or to follow enzymatic reactions, and in clinical chemistry [9].

In order to study the interinstrumental transfer of an SEI method, a test case based on a SEI separation of the hexameric forms, and their substituted ions, of Niobium and Tantalum, being $\text{Na}_7\text{HfNb}_6\text{O}_{19}\cdot 15\text{H}_2\text{O}$ and $\text{Na}_8\text{Ta}_6\text{O}_{19}\cdot 24.5\text{H}_2\text{O}$, further denoted as Nb_6 and Ta_6 ions, was selected. These hexameric forms are formed at a pH above 9.0, absorbed in the UV spectrum, and possess different electrophoretic mobilities. The method was first developed in [10] and further optimized in [11] on an Agilent G7100 instrument.

The SEI method was further adapted to perform a kinetic study of Nb_6 , Ta_6 plus their substituted ions ($\text{Nb}_{6-x}\text{Ta}_x$ with $0 \leq x \leq 6$) with a Beckman P/ACE CE instrument [12]. This paper deals with the transfer of this method optimized with a Beckman CE system to an Agilent setup. As stated before in De Cock et al. [4], when transferring “long-end injection” methods, the effective lengths are easily interinstrumentally transferred and adaptations needed to be made to overcome the differences in total capillary lengths. This was not the case for the SEI performed in this study. Here the total length was kept constant but reducing the effective length led to a reduction in the separation efficiency (N) as illustrated in Eq. (1), which was also used by the data analysis software of both instruments [13].

$$N = \frac{\mu_e \times V \times l}{2 \times D \times L}, \quad (1)$$

where N is the efficiency, μ_e the electrophoretic mobility, V the applied voltage, l the effective length, D the diffusion coefficient, and L is the total capillary length.

The instrumental difference between the effective lengths of an SEI on the Beckman and Agilent instruments is due to the difference in construction of the capillary cartridges. On the Beckman instrument, the short-end effective length is 10 cm and is not thermostated, while on the Agilent instrument the minimum effective length is 8.5 cm, of which a part, that is, 3.5 of 8.5 cm, is thermostated [5]. In this study an interinstrumental AMT from a Beckman system to an Agilent instrument was performed to analyze this interinstrumental difference. The earlier developed guidelines for method transfer were applied, such as achievement of identical electrical resistances, injection, and rinse volumes and application of as many similar detector and data handling parameters as possible, but the difference in effective length was not encountered earlier and thus no guidelines were included yet to counter this interinstrumental difference. In this study, the increase in the lift offset on the effective length

and separation efficiency was analyzed applying as case study the separation of Ta_6 , Nb_6 , and their substituted ions.

2 Materials and methods

2.1 Chemicals used

Ultrapure water was prepared in house by a Direct-Q3 UV system (Millipore, Molsheim, France) and used for all stock solutions and the preparation of the BGE. $\text{LiOH}\cdot\text{H}_2\text{O}$ and $\text{LiCH}_3\text{COO}\cdot 2\text{H}_2\text{O}$ were both purchased from Alfa Aesar (ThermoFisher Scientific, Waltham, MA). The BGE consisted of 35 mM $\text{LiCH}_3\text{COO}\cdot 2\text{H}_2\text{O}$ and 10 mM $\text{LiOH}\cdot\text{H}_2\text{O}$ dissolved at a pH of 12.0. The BGE was prepared daily by dissolving appropriate amounts of $\text{LiOH}\cdot\text{H}_2\text{O}$ and $\text{LiCH}_3\text{COO}\cdot 2\text{H}_2\text{O}$ in ultrapure water. $\text{Na}_7\text{HfNb}_6\text{O}_{19}\cdot 15\text{H}_2\text{O}$ and $\text{Na}_8\text{Ta}_6\text{O}_{19}\cdot 24.5\text{H}_2\text{O}$ were synthesized as previously reported in Deblonde et al. [14] and stored at -20°C . Stock solutions were freshly prepared every week by dissolving $\text{Na}_7\text{HfNb}_6\text{O}_{19}\cdot 15\text{H}_2\text{O}$ (2 mg/mL) and $\text{Na}_8\text{Ta}_6\text{O}_{19}\cdot 24.5\text{H}_2\text{O}$ (4 mg/mL) in ultrapure water, followed by stirring for 2 h and stored at 4°C . The BGE and all stock solutions were filtered through $0.25\text{ }\mu\text{m}$ syringe filters (Minisart® RC25, Sartorius, Goettingen, Germany) and placed on an ultrasonic bath for 10 min prior to the CE experiments.

2.2 Instrumental setup

The method development and the first kinetic study were performed on a P/ACE Capillary Electrophoresis system from Beckman Coulter (Fullerton, California, USA) equipped with a DAD. The detection wavelength was set at $211 \pm 6\text{ nm}$, the reference wavelength at $325 \pm 50\text{ nm}$, and a data acquisition rate of 4.00 Hz was applied. A fused silica capillary (Photonlines, Marly-le-Roi, France) with an internal diameter of $50\text{ }\mu\text{m}$, an effective length of 10 cm, and a total length of 60 cm was installed in the capillary cartridge. The capillary temperature was kept at 31.0°C and the electropherograms were processed with the 32 Karat™ software version 5.0 (Beckman Coulter). The applied electrical field was -0.375 kV/cm total capillary length and generated a current of approximately $-49.0\text{ }\mu\text{A}$, resulting in a cathodic electroosmotic flow. Capillary preconditioning was performed by rinsing the capillary with consecutively 1.0 and 0.1 M $\text{LiOH}\cdot\text{H}_2\text{O}$; ultrapure water and the BGE at a pressure of 60 psi/4.13 bar for 10 min each, corresponding to a preconditioning volume of $81.16\text{ }\mu\text{L}$ per step at 31.0°C [15]. Between runs, the capillary was rinsed for 2 min with BGE at 60 psi/4.13 bar, corresponding to a rinse volume of $16.14\text{ }\mu\text{L}$ per step at 31.0°C [15]. The samples were hydrodynamically injected at 0.1 psi/6.9 mbar during 9.0 s at the anodic end, generating an injection volume of 1.99 nL or a plug length of 1.01% to the effective length at 31.0°C [15].

The method was transferred to an Agilent G1600 instrument (Agilent Technologies, Santa Clara, CA) likewise equipped with a DAD module. The detection parameters were

kept as identical as possible, as advised in De Cock et al. [6]. The detection wavelength was set at 211 ± 6 nm and the reference wavelength at 325 ± 50 nm. The data acquisition rate was adapted due to different parameter ranges between instruments, 3.33 Hz was applied instead of 4.00 Hz. A fused silica capillary, from the same batch as on the Beckman instrument, with an internal diameter of 50 μ m, an effective length of initially 8.5 cm and after variation of the lift offset and consequentially the capillary window position 9.5 cm, and a total length of 60 cm was installed in the capillary cartridge. Due to Joule heating, the electrical field was reduced to -0.350 kV/cm total capillary length and a current of approximately -46.5 μ A was obtained. The capillary temperature was kept identical at 31.0°C and the electropherograms were processed by means of the 3D-CE Chemstation software (Agilent Technologies). Capillary preconditioning and rinsing steps were performed with identical volumes and parameters as on the Beckman instrument. An identical injection volume was obtained by applying an injection pressure of 0.1 psi/6.9 mbar during 9.0 s, leading to a plug length of 1.07% to the effective length at 31°C .

3 Results and discussion

3.1 Preliminary work

In first investigations performed by Deblonde et al. [10, 11], the separation of the two Nb_6 and Ta_6 anions in a basic medium, using a 45 mM $\text{LiOH}/\text{LiCH}_3\text{COOH}$ BGE, an application of 10 kV, a temperature of 25.0°C , and an effective capillary length of 8.5 cm was achieved on an Agilent 7100 CE system. Subsequently, the SEI method was transferred in De Cock et al. [12] to a Beckman P/ACE CE system, due to the unavailability of the Agilent system, and in a next step adapted in order to obtain a quantitative, instead of the earlier informative, separation of not only Nb_6 and Ta_6 anions, but also all their substituted $\text{Nb}_{6-x}\text{Ta}_x$ with $0 < x < 6$ ions. This paper deals with the interinstrumental transfer of the new SEI method that was developed with a Beckman system to an Agilent G1600 instrument in the same laboratory. The older Agilent G1600 differs from the initially used 7100 instrument in terms of sensitivity, cassette type, and pump system.

Application of a Joule test, a prerequisite during interinstrumental AMT, due to the differences in capillary cooling, revealed that the application of -22.5 kV generated an exponential increase of the current and an increase in current fluctuations and baseline noise, and had to be reduced to -21.0 kV. The reduction of the applied voltage led furthermore to similar electrical resistances, being 0.45Ω on both instruments, therefore the capillary temperature was allowed to be kept identical at 31.0°C and was not reduced as recommended in De Cock et al. [16]. Although the applied voltage was reduced, thus potentially leading to a lower separation efficiency and longer migration times as shown in Eq. (1), the migration times were reduced in this study, potentially also due to the reduced effective length. On the other hand, the

separation efficiency did reduce. The application of earlier defined guidelines [4–6, 16], such as adapting injection parameters, ensuring identical capillary electrical resistances, and keeping the detector- and data-handling parameters as constant as possible, did not result in a baseline separation of all substituted ions after transfer to the Agilent instrument as illustrated in Fig. 1. Since no earlier work exists to our knowledge to overcome this SEI interinstrumental difference, it was decided to set up a study to increase the effective capillary length of an SEI method by increasing the lift offset on the Agilent CE system from 4 to 14 mm. The effect of the variation of the lift offset parameter on the separation efficiency and resolution of Ta_6 , Nb_6 , and their substituted ions was evaluated.

3.2 Lift offset

The lift offset value, an Agilent specific parameter, is determined by the heights of the inlet and outlet lifts and has a default value set at 4 mm. The lift offset value is variable between 3 and 18 mm and defines the distance between the capillary tip and the bottom of the vial according to the Agilent manual [http://www.agilent.com/cs/library/usermanuals/public/G7100-90000_CE_System_User_ebook.pdf]. Increasing the lift offset decreased the dip/ascent of the electrodes in the vials and avoided that, while increasing the effective length of the capillary, the capillary ends touch the bottom of the vials as illustrated in Fig. 2. In this study, the lift offset was increased from 4 to 14 mm stepwise by decreasing the lift height per 2 mm while maintaining the total capillary length constant at 60 cm. Hence, the effective length increased with 2 mm per step by moving the location of a very large capillary window. The tested sample was a mixture of Ta_6 and Nb_6 stock solutions containing a 0.05 M concentration of both hexameric forms. The sample was placed 24 h in an oven at 60°C , resulting in a mixture of the two main ions, Ta_6 and Nb_6 , and the five substituted ions being Ta_5Nb_1 , Ta_4Nb_2 , Ta_3Nb_3 , Ta_2Nb_4 , and Ta_1Nb_5 . The mixture was analyzed three times at each lift offset value. The tested lift offset values and the effect on the separation efficiencies of Ta_6 and Ta_5Nb_1 and resolution between Ta_6 and Ta_5Nb_1 , both calculated according to the European pharmacopoeia [13], are shown in Table 1. The variation of the lift offset did not alter the hydrodynamic injection volume, which is solely determined by the total capillary length, as shown in Eq. (2) [15].

$$V_{\text{injection}} = \frac{\Delta p \times d^4 \times \pi \times t}{128 \times \eta \times L}, \quad (2)$$

where Δp is the applied injection pressure, d the inner diameter of the capillary, π the constant π , t the time the injection pressure is applied, η the viscosity of the solution, and L is the total capillary length.

Although an equal injection volume is necessary to maintain a similar separation efficiency, resolution, peak areas, and widths during AMT as illustrated in De Cock et al. [5],

Figure 1. Electropherograms corresponding to the CE analysis of Ta_6 , Nb_6 , and their substituted $Nb_{6-x}Ta_x$ with $0 < x < 6$ ions obtained on both the Beckman and the Agilent instrument with the initial and adapted lift offset values. Conditions: see Section 2.2.

it was found in this study that an equal injection volume not necessarily involves an equal percentage of injection plug length to the effective length. Both parameters are only equal when the capillary dimensions, such as inner diameter, total

and effective lengths, are identical. The consideration that an equal injection volume or percentage of plug length should be chosen is needed. By increasing the lift offset to 14 mm, the percentage of injection plug length to the effective length

Figure 2. Illustration of a lifting device in an Agilent capillary electrophoresis system showing the lift offset range. Adapted from [17].

decreased from 1.19 to 1.07%. Although still higher than the percentage of plug length on the Beckman instrument, that is, 1.01%, the increase of the lift offset made the percentage of plug length more similar on both instruments and thus leading to less band broadening and a higher separation efficiency as shown in Table 1. The electrical field intensity, not dependent on the effective capillary length, remained constant, despite the variation in the effective length, since the applied voltage and total capillary length remained constant during

this study. Furthermore, the increase of the effective length, while maintaining an identical electrical field intensity, being the applied voltage per centimeter total capillary length, will lead to an increased migration time since the electrophoretic mobility of a compound is considered to be independent of the capillary dimensions. To overcome differences in effective length, we suggest an extra parameter to be considered, namely the electrical field intensity per centimeter effective length expressed as V/cm^2 . During previous AMT, the effective length was always kept constant and thus the differences in electrical field intensity per centimeter effective length did not occur, but when performing an SEI interinstrumental method transfer, this is nonetheless an interesting parameter to evaluate and to keep analogous as much as possible by either varying the effective length, the total length, or the applied voltage. On the Agilent instrument, the electrical field intensity per centimeter effective length of the capillary decreased from -0.04116 to -0.03683 kV/cm^2 , while increasing the lift offset as shown in Table 1. The lower applied voltage on the Agilent instrument, even though initially not intended but needed due to the joule heating effect, combined with the longer effective length after lift offset adjustment, led to a more analogous electrical field intensity per cm effective capillary length (-0.03666 kV/cm^2) on both instruments and more similar electropherograms. As can be predicted from Eq. (1), the separation efficiency of Ta_6 and Ta_5Nb_1 increased when increasing the lift offset values. A significant increase was noticed between 10 and 12 mm lift offset values for both Ta_6 and Ta_5Nb_1 . The increase in separation efficiency was also noticed as an increase in resolution between all peaks and in particular between the peaks of Ta_6 and Ta_5Nb_1 . The baseline resolution was restored at a lift offset value of 12 mm or 9.3 cm effective capillary length, as shown in Fig. 1. After this adaptation, similar electropherograms, migration times, and experimental errors (RSDs below 2.5% on both systems) were achieved. Furthermore, increasing the lift offset value to 18 mm was not performed in this study since the baseline separation was restored at 12 mm and even the maximum lift offset value of 18 mm would not allow an exact effective

Table 1. The evaluated lift offset values and the corresponding effective length, the injection volume plug length percentage to the window, the applied electrical field, the effective electrical field, the separation efficiencies of Ta_6 and Ta_5Nb_1 and the resolution between Ta_6 and Ta_5Nb_1 obtained on the Agilent and Beckman instrument for a given mixture of Ta_6 , Nb_6 and their substituted $Nb_{6-x}Ta_x$ with $0 < x < 6$ ions

Instrument	Lift offset ($n = 3$)	Effective length	Injection plug length to the window	Electrical field intensity	Electrical field intensity per centimeter effective length	Separation efficiency Ta_6	Separation efficiency Ta_5Nb_1	Resolution Ta_6 - Ta_5Nb_1
Agilent	4 mm	8.5 cm	1.19%	-0.35 kV/cm	-0.04116 kV/cm^2	13428 ± 140	10141 ± 120	1.28 ± 0.02
Agilent	6 mm	8.7 cm	1.16%	-0.35 kV/cm	-0.04016 kV/cm^2	13559 ± 142	10854 ± 120	1.33 ± 0.02
Agilent	8 mm	8.9 cm	1.14%	-0.35 kV/cm	-0.03933 kV/cm^2	14785 ± 145	11058 ± 125	1.42 ± 0.02
Agilent	10 mm	9.1 cm	1.11%	-0.35 kV/cm	-0.03850 kV/cm^2	15091 ± 142	11107 ± 130	1.46 ± 0.02
Agilent	12 mm	9.3 cm	1.09%	-0.35 kV/cm	-0.03766 kV/cm^2	16003 ± 145	14119 ± 131	1.52 ± 0.02
Agilent	14 mm	9.5 cm	1.07%	-0.35 kV/cm	-0.03683 kV/cm^2	17590 ± 146	16109 ± 134	1.58 ± 0.03
Beckman	/	10.0 cm	1.01%	-0.35 kV/cm	-0.03666 kV/cm^2	22647 ± 180	27949 ± 200	1.75 ± 0.05

length of 10 cm, nonetheless this possibility should be considered for more critical separations. Performing analyses at the higher lift offset values requires high liquid levels in the vials to maintain contact with the electrodes. Furthermore, the levels in both BGE vials should also be equal during analysis to avoid a siphoning effect. Since both in- and outlet lifts are varied equally while adapting the lift offset parameter, the liquid levels in both vials should therefore remain equal. It should be noted that after interinstrumental transfer an increase in baseline noise on the Agilent system was observed, in particular complicating the separation between Ta_3Nb_3 and Ta_2Nb_4 . This difference in sensitivity, especially when applying an increased lift offset, can be related to the use of the older G1600 system or is potentially related to the decreased dip of the electrodes in the BGE or due to the use of a broader detection window.

4 Concluding remarks

During the AMT from a Beckman to an Agilent instrument of the SEI separation of Nb_6 , Ta_6 , and their substituted ions, we encountered for the first time an interinstrumental difference in effective capillary length, 8.5 versus 10 cm, due to the different construction of capillary cartridges. The earlier defined guidelines for the transfer of a CE method were applied but did not result in similar results on both instruments. The shorter effective length on the Agilent instrument led to a loss of baseline separation, especially between Ta_6 and Ta_5Nb_1 , and thus the inability to perform a correct quantitation. The separation efficiency was restored by adapting an Agilent-specific parameter, that is, the lift offset. The lift offset values in this study were increased from 4 (=default value) to 14 mm and allowed using a separation capillary with an identical total length and an effective length of 9.5 cm on the Agilent instrument. The incremented and more similar effective length led to an increase in the separation efficiency and thus resolution, and consecutively a restored baseline separation of all peaks. The increase of the lift offset furthermore led on both instruments to a more equal percentage of injection plug length to the effective length, making this a parameter to consider during interinstrumental transfer of SEI by either adapting the effective length or the injection parameters. The use of an equal electrical field intensity per centimeter effective length, being the amount of voltage applied per centimeter total capillary length divided by the effective capillary length, is also encouraged during AMT with differences in effective capillary lengths. Therefore, when transferring an SEI method from a Beckman instrument to an Agilent system, the achievement of an increased capillary length by adjusting

the lift offset value on the Agilent CE system was added to the earlier defined guidelines.

The authors have declared no conflict of interest.

5 References

- [1] Swarts, M. E., Krull, I., *LC GC N Am.* 2006, 24, 1–5.
- [2] Naik, G. M., Yeligar, V., *Int. J. Pharmtech. Res.* 2012, 4, 599–601.
- [3] Rozet, E., Dewe, W., Ziemons, E., Bouklouze, A., Boulanger, B., Hubert, P. *J. Chromatogr. B* 2009, 877, 2179–2418.
- [4] De Cock, B., Dejaegher, B., Stiens, J., Mangelings, D., Vander Heyden, Y., *J. Chromatogr. A* 2014, 1353, 140–147.
- [5] De Cock, B., Dejaegher, B., Stiens, J., Mangelings, D., Vander Heyden, Y., *J. Chromatogr. A* 2014, 1353, 148–159.
- [6] De Cock, B., Hamza, N., Mangelings, D., Vander Heyden, Y., Interinstrumental transfer of a chiral capillary electrophoretic method: The use of robustness test information to overcome differences in detector and data-handling specifications. In preparation.
- [7] Altria, K. D., Kelly, M. A., Clark, B. J., *Chromatographia* 1996, 43, 153–158.
- [8] Perrin, C., Vander Heyden, Y., Maftouh, M., Massart, D. L., *Electrophoresis* 2001, 22, 3203–3215.
- [9] Glatz, Z., *Electrophoresis* 2013, 34, 631–642.
- [10] Deblonde, G., Delaunay, N., Lee, D., Chagnes, A., Cote, G., Gareil, P., *RSC Adv.* 2015, 5, 64119–64124.
- [11] Deblonde, G., Chagnes, A., Cote, G., Vial, J., Rivals, I., Delaunay, N., *J. Chromatogr. A* 2016, 1437, 210–218.
- [12] De Cock, B., Delaunay, N., Stiens, J., Mangelings, D., Vander Heyden, Y., Kinetic study of niobium and tantalum substitutions by means of a capillary electrophoretic separation in alkaline media. In preparation.
- [13] Heiger, D., *An Introduction High Performance Capillary Electrophoresis*, Agilent Technologies, Germany 2000, pp. 16–46.
- [14] Deblonde, G., Moncomble, A., Cote, G., Bélair, S., Chagnes, A., *RSC Adv.* 2015, 5, 7619–7627.
- [15] CE Expert Tool, Beckman Coulter, Fullerton, CA. <https://www.beckmancoulter.com/wsrportal/wsr/research-and-discovery/products-and-services/capillary-electrophoresis/ce-expert-lite/index.htm>. Accessed on May 22, 2016.
- [16] De Cock, B., Van Eeckhaut, A., Stiens, J., Mangelings, D., Vander Heyden, Y., *Electrophoresis* 2015, 36, 2658–2664.
- [17] Zimmermann, H. P., Apparatus for performing capillary electrophoresis, US 5364521 A, 1994.