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Sample enrichment in a single levitated droplet for capillary electrophoresis

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Abstract

This paper describes sample enrichment in a single levitated droplet for capillary electrophoresis (CE) analysis. The droplet was trapped in an acoustical field. The minute sample volumes needed for the enrichment procedure were precisely handled using a piezoelectric flow-through liquid microdispenser. Droplets with a volume of 65 pl were ejected from the device at a repetition rate ranging from one single droplet up to several hundreds per second. By counting the number of droplets ejected and accumulated in the levitated drop the sample volume was controlled. Through solvent evaporation the analytes were enriched in the diminishing droplet. The droplet was then injected into a CE capillary and the analytes, dansyl-Gly and dansyl-Val dissolved in ethanol, were separated in a 100 m*M* borate buffer (pH 9.0) utilising UV-absorption detection at 200 nm near the capillary outlet. Enrichment of 36 000 sample droplets (2.3 μl) through solvent evaporation in the levitated drop resulted in a concentration limit of detection (CLOD) of 15 n*M* for the dansylated amino acids as compared to a CLOD of 2.5 μ*M* which was achieved using standard hydrodynamic injection without preconcentration.

Keywords: Injection; Preconcentration; Flow-through dispenser

1. Introduction

Capillary electrophoretic (CE) technology has advanced rapidly but is considered insensitive in terms of concentration limit of detection (CLOD) as a consequence of the short path-length available using the most popular detection mode, i.e. UV-absorbance detection. A typical CLOD achieved is in the 10^{-6}–10^{-5} *M* range, which is insufficient for many applications.

There are a number of sample enrichment techniques available, but sample handling and preparation steps become more difficult as one moves towards smaller sample volumes. In order to overcome these problems some on-line enrichment approaches have been developed [1].

One widely utilised approach is to inject the sample from a low conductivity medium, either electrokinetically or pneumatically [2,3]. This approach is normally limited to sample volumes less than the total capillary volume, i.e. less than 1 μl for a standard 50-μm I.D. capillary. Preconcentration schemes that rely on field effects require samples made in buffers of low conductivity. This is not

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feasible with many samples from the real world. A second approach is isotachophoretic focusing (ITP). When the mobility of the sample ions is intermediate between those of the leading and the terminating ions, an isotachophoretic train is established, with the concentration of the sample components increased, possibly by several orders of magnitude [4,5]. ITP is however extremely vulnerable to varying ion matrices in the sample. When using a chromatographic enrichment approach in CE, a small amount of adsorbent is placed on-line like a solid-phase extraction (SPE) microcartridge at the inlet end of the capillary [6]. Although useful, the enrichment method in analogue to SPE requires extra components and quantitative release in a small volume can be a problem.

A levitator is a powerful tool which facilitates a variety of investigations on single particles or droplets. Since it maintains the levitated object in a fixed position, the process under investigation is not disturbed by the influence of any other contacting surface than the surrounding medium, commonly air. This is of importance when striving for single molecule detection [7]. Small objects like a liquid droplet can be levitated in the nodal points of a standing ultrasonic wave [8]. In contrast to other levitation techniques like levitation in electrostatic or magnetic fields the acoustical levitation requires no specific physical properties of the sample. The use of acoustical levitation in analytical chemistry is in its initial stage. The possibility to enrich substances in a levitated drop using gas chromatography as analytical method was recently demonstrated [9].

Easy positioning of fluids with reproducible volumes is a basic requirement for experiments with levitated drops. Microdroplet dispensers have been used for several years in ink-jet printing [10,11]. Over the last years applications have emerged where ink-jet droplet dispensers were adapted for sample handling/sample supply in, for example, analytical chemistry [12–16]. The interesting properties of the microdispensers are that they readily handle minute droplet volumes in the picolitre range and supply samples in a noncontacting fashion. Most droplet dispensers presented hitherto are of a single-end type, which means that they have one liquid inlet and one droplet outlet. This makes filling and cleaning of the device relatively laborious and it offers no simple way of inserting the dispensers in a flow-line for on-line sampling.

In the present study the use of an acoustical levitator for analyte enrichment in a single levitated droplet for CE applications is investigated. Precise sample deployment in the nodal point was achieved utilising an in-house developed flow-through liquid microdispenser [17].

2. Experimental

2.1. Reagents

Ethanol (99.5%) was from Kemetyl (Stockholm, Sweden). Sodium hydroxide solution (Combi-Titrisol, 5 *M*) and disodium tetraborate cryst. (analytical reagent grade) were obtained from Merck (Darmstadt, Germany). Boric acid (analytical reagent grade) was from Riedel-de Haën (Seelze, Germany). Dansyl-D,L-glycine (99%) and dansyl-D,L-valine (99%) were purchased from Sigma (St. Louis, MO, USA). The water was prepared using a Milli-Q system (Millipore, Bedford, MA, USA).

Stock solutions (200–260 μ*M*) of the individual dansylated amino acids (Dns-AAs) were dissolved in ethanol. The stock solutions were stored in a freezer for less than a week. The Dns-AAs were diluted daily to the desired concentrations (0.01–10 μ*M*) in ethanol.

The borate buffer used as electrolyte solution was prepared by mixing 100 m*M* H_3BO_3 and 25 m*M* $Na_2B_4O_7$ to pH 9.0. The pH was measured using a PHM82 Standard pH meter equipped with a GK2421 C electrode which was calibrated with pH buffer solutions pH 7.00 and 9.18, all from Radiometer Copenhagen (Bagsvaerd, Denmark). The electrolyte was filtered through a 0.2-μm DynaGard filter tip (Microgon, Laguna Hills, CA, USA) before CE.

2.2. Acoustical levitator

An acoustical levitator APOS BA 10 with an ultrasonic frequency of 100 kHz was obtained from Martinsson Elektronik (Hägersten, Sweden). The sample drop was positioned in a nodal point using a modified GC syringe (the tip consisted of a thin polyimide coated fused-silica capillary) or a flow-

through liquid microdispenser. The distance between transducer and reflector was adjusted with a micrometer screw. The HF power was normally set at 3 W and was slightly raised when a large drop was to be levitated.

2.3. Flow-through liquid microdispenser

The flow-through liquid microdispenser was manufactured in-house by joining two micromachined silicon structures (Fig. 1a). One had a 10-mm long, 2-mm wide and 100-μm deep channel defined by anisotropic etching, the other had through holes for liquid inlet and outlet as well as a 13-μm silicon membrane, PN-etch stop defined, which acted as a cover for the channel. The total volume of the unit was 2.6 μl. A 50×50 μm nozzle, extending 70 μm from the front surface, was etched in the centre of the flow-through channel. On the membrane structure a piezoelectric bimorph element (Philips Components, Stockholm, Sweden) was attached via three cylindrical Plexiglas stands (diameter 1 mm). When a voltage pulse (7–15 V, 10 ms duration) was applied to the bimorph it inherently bent. The bending action was coupled to the membrane generating a pressure pulse in the liquid filled channel and a droplet was ejected from the nozzle (Fig. 1b). This can be repeated up to about 500 Hz, limited by resonances within the device. The ejected droplet volume depends on the size of the nozzle, the properties of the liquid and the pulse amplitude. The voltage pulse was controlled via a MOSFET switch transistor. The E3612A D.C. power supply and the 8111A pulse/frequency generator were from Hewlett-Packard (Palo Alto, CA, USA). The liquid inlet and outlet of the flow-through droplet microdispenser was supplied with short silicone rubber tubes with an I.D. selected to fit 1.6-mm O.D. tubes. The flow-through liquid microdispenser was mounted on an XYZ-micropositioning stage throughout the experiments in order to facilitate alignment.

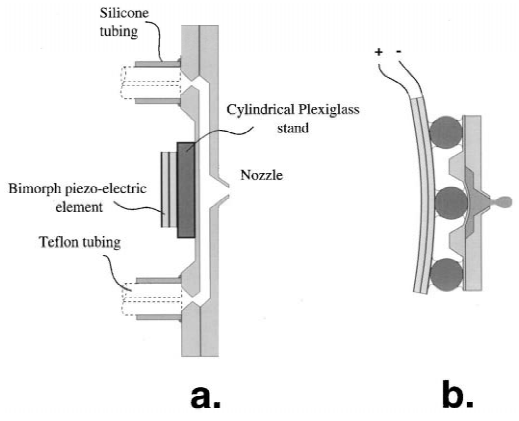

Fig. 1. (a) Lengthwise cross-section view of the flow-through droplet microdispenser. The two silicon structures form the channel and a 50×50 μm nozzle is etched in the centre. (b) A droplet is ejected when a voltage pulse is applied to the piezoelectric bimorph element. The bending of the element is transferred to the thin membrane in the channel wall and a pressure pulse is created in the liquid.

2.4. Image capturing

The experiments were documented with a B/W video camera (C5405, Hamamatsu, Kista, Sweden) attached to a microscope (SMZ-2T, Nikon Corporation, Tokyo, Japan). The images were digitally captured using an image-grabber board (Neotech, Eastleigh, UK) installed in a computer (Macintosh IIfx, Apple, Cupertino, CA, USA). Both the live and captured images were displayed on the computer monitor.

The stroboscopic images of the droplet flight were recorded using a Brüel and Kjaer type 4913 stroboscope (Brüel and Kjaer, Naerum, Denmark) with a type 4915 fibre-optic source.

2.5. Enrichment procedure

Two methods were utilised to deposit sample droplets in the levitator. A GC syringe was used for initial studies and a flow-through liquid microdispenser was used for subsequent experiments.

Starting with a 1-μl sample volume (estimated with the GC syringe) the solvent was allowed to evaporate for 6–9 min before injection. When the flow-through liquid microdispenser was utilised, a burst facility of the pulse generator was employed for ejection of 1500 droplets with a frequency of 100 Hz. This was typically repeated four times (i.e. 6000 droplets were ejected during 1 min). The solvent was allowed to evaporate for 3.5 min and the flow-through liquid microdispenser was rinsed with sam-

ple solution before addition of another 6000 droplets. This procedure was repeated up to six times.

2.6. CE apparatus and methods

CE experiments were carried out using a high voltage supply (0–30 kV) from Zäta Elektronik (Höör, Sweden). The fused-silica capillary (Composite Metals, Hallow, UK) used was 45 cm (30 cm to the detector)×50 μm I.D.×375 μm O.D. At the beginning of each working day the capillary was rinsed with 1 *M* NaOH, water and electrolyte using a syringe. Between injections the capillary was rinsed with electrolyte. A 10 s×13 cm hydrodynamic injection was used for nonenrichment experiments. In order to inject the levitated enriched sample droplet, the CE capillary was manually approached, attaching the droplet to the capillary end. The height difference to the detection end was 6 cm during this droplet pick up. The capillary O.D. was used to gauge the levitated droplet size before injection, the droplet diameter was always smaller than the capillary O.D. The separations were carried out using a constant voltage of 10 kV (222 V/cm), generating a current of 14 μA. The temperature and relative humidity was ambient. A Spectra 100 variable-wavelength detector (Thermo Separation Products, San Jose, CA, USA), equipped with a capillary cell from Grom (Herrenberg-Kayh, Germany), was set at 200 nm. Detection was performed at the cathodic end of the capillary. The output signal was recorded with a dual channel flatbed recorder (BD 112, Kipp and Zonen, Delft, The Netherlands).

3. Results and discussion

3.1. Levitation

The ultrasonic levitator was very easy to use. Stabilisation of the levitated drop was achieved by adjusting the distance between the transducer and the reflector and by setting the ultrasonic power properly (Fig. 2). The drop is spherical with optimal settings. Excessive power results in drop deformation or drop disintegration. With insufficient power the drop falls down. Small droplets are very susceptible to draught and care must be taken to avoid violent movements.

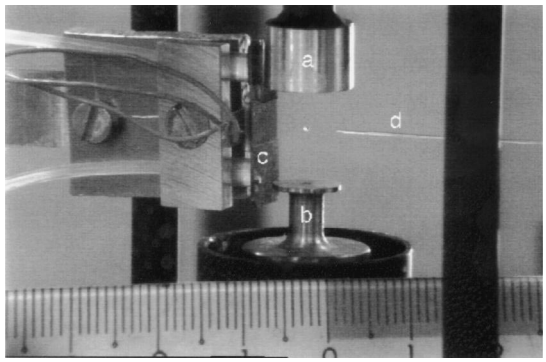

Fig. 2. Droplet levitated in the nodal points of a standing ultrasonic wave. (a) Reflector (b) ultrasound transducer (c) flow-through liquid microdispenser and (d) capillary. The ruler displays the scale in centimetres of the experimental set-up. The levitated droplet is less than 1 mm in diameter.

The optimal droplet diameter, for which minimal ultrasonic power is required, is a function of the ultrasonic wavelength. For water drops in ambient air this corresponds to a drop volume around 1 μl (1.2-mm drop diameter) using an ultrasonic frequency of 100 kHz [8].

3.2. Dispenser

Easy positioning of fluids with reproducible volumes is a basic requirement for experiments with levitated drops. Drops in the microlitre range can easily be positioned using a GC syringe. Accurate free hand positioning required some practice. A small air bubble was often trapped inside the drop when the GC syringe was used. This inevitably led to drop disintegration when the drop diminished in size. In order to overcome the problems associated with the GC syringe a flow-through liquid microdispenser was adopted.

The droplet ejection from the microdispenser has to be adjusted by adapting the voltage pulse amplitude to the liquid properties. With low surface tension solvents like methanol or ethanol the amplitude has to be decreased in order to avoid ejection of smaller droplets, satellites, following the main droplet. In the experiments, satellite free droplet ejection was reached at around 8 V pulse amplitude. The droplet size was then 50 μm corresponding to a volume of 65 pl. Stroboscopic images of a droplet

ejected by the flow-through liquid microdispenser approaching the levitated drop is shown in Fig. 3.

The reproducibility in droplet flight can be seen in Fig. 4 which was captured using a constant light source. The light-track formed by reflections in the ejected droplets shows that the droplets follow the same trajectory on their way to the levitated drop. In the same figure can also be seen that it is not necessary to aim the droplets at the levitated drop with high precision. The force acting on the droplets from the pressure node is strong enough to attract them if they travel sufficiently close to the node.

No splatter could be observed from the droplets hitting the levitated drop which can probably be explained by the fact that the droplets are so small that the surface tension force dominates at the impact. This is of great importance since it ensures that the volume ejected is also delivered to the levitated drop. Another advantage of this effect is that no air bubbles are introduced upon impact.

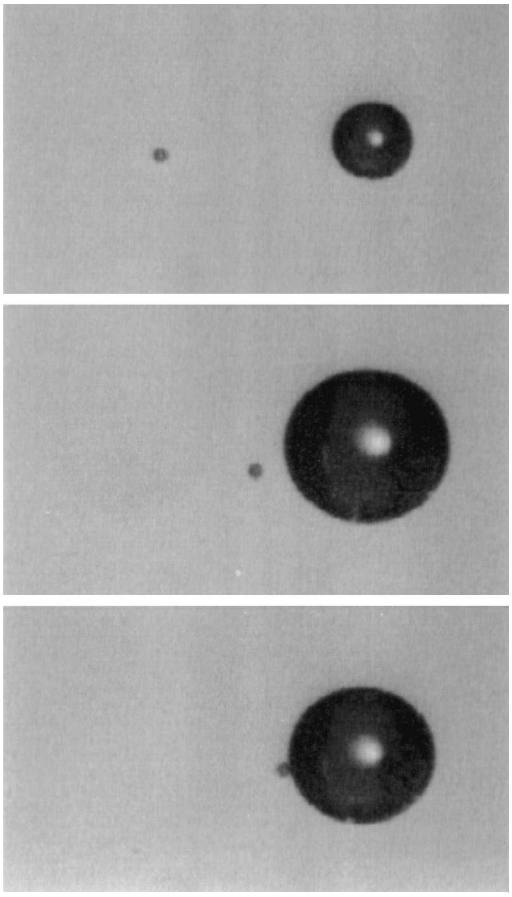

Fig. 3. Stroboscopic images of a 65 pl droplet approaching the levitated drop. The images are from three experiments and therefore the levitated drop size varies.

The two silicon structures in the flow-through liquid microdispenser prototype used in this work were glued together. Because of the high organic solvent contents in the sample, the device had to be rinsed frequently in order to prevent components from the glue being extracted and subsequently also enriched. The next generation of flow-through liquid microdispensers should be silicon direct bonded which requires laborious clean room technology.

3.3. Levitated sample droplet pick up (injection)

The transfer of the levitated droplet into the capillary turned out to be the most crucial step. The capillary volume between the injection end and the detection window was 600 nl in the experimental set-up used. If no stacking technique is employed in CE, a practical limit of injection volume is about 1% of the total volume in order to avoid overloading. A 6-nl droplet has a diameter of 225 μm. Consequently the final droplet diameter should be smaller than the capillary O.D. before injection. There are elegant injection techniques suitable for single droplets. One approach is to keep water droplets under paraffin oil. Injection is then accomplished by connecting a droplet to the capillary tip and by applying a vacuum at the opposite end of the capillary [18]. Here we used a much simpler and rather crude approach by attaching the levitated droplet to the capillary end. The sample droplet diameter was always smaller than the capillary O.D. (375 μm). The pick up procedure was easy since it turned out that the levitated enriched sample droplet was attracted to the capillary end when brought into close proximity. The attraction may be explained by electrostatic forces. The levitated droplet can ‘jump’ more than 1 mm just by slowly moving the capillary, with a steady hand, towards the droplet. In order to observe this phenomena and to check the hit accuracy an injection was video surveyed through a microscope. A hit is demonstrated in Fig. 5. We found that the droplet hit the target properly even if the capillary was approached one capillary diameter laterally or

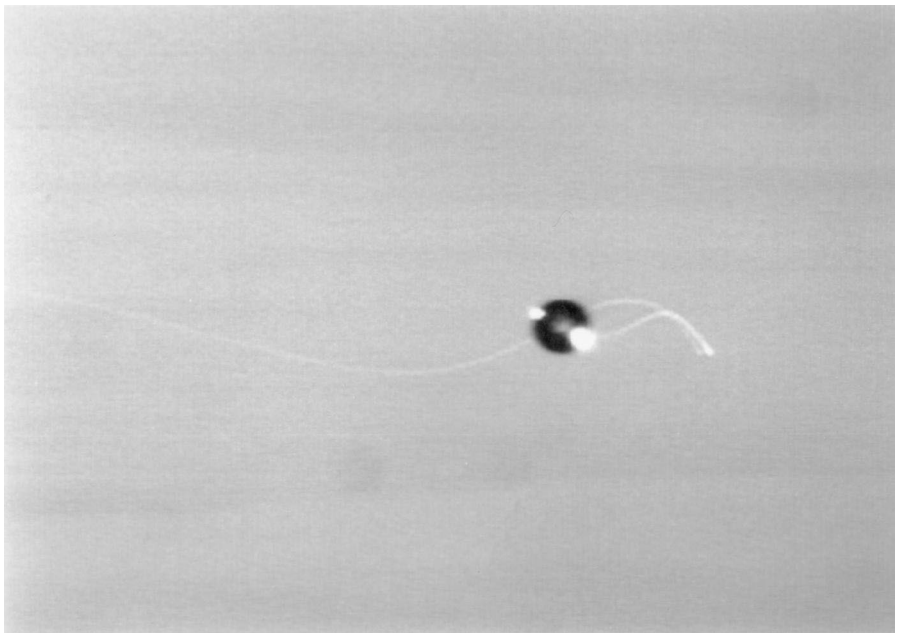

Fig. 4. Flight trajectory of the 65 pl droplets ejected by the flow-through liquid microdispenser on their way to the acoustically levitated drop. The pressure field in the levitator affects the droplet flight.

vertically offset the droplet centre. It was hard to judge from the image whether the whole drop or only a part of it actually entered the capillary. It is thus difficult to determine the exact levitated droplet volume injected. From the levitated sample enrichment data obtained, it can be concluded that the peak heights often varied by a factor two comparing duplicate injections. This indicates that the levitated sample droplet was not injected completely. Approximately 10–20% of the CE runs resulted in current breakdown, probably due to injection of air bubbles. The injection technique can be improved by the use of a micromanipulator instead of the manual procedure. Etching of the capillary inlet creating a funnel or making the capillary end more hydrophobic may also facilitate sample introduction. Laser-induced fluorescence/charge-coupled device real-time imaging of the enrichment process as well as the injection procedure would also increase reliability [19,20].

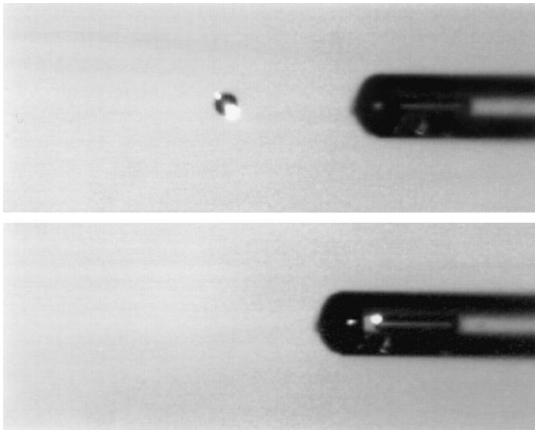

Fig. 5. Levitated sample droplet pick up using the separation capillary (50 μm I.D., 375 μm O.D.). The tip of the capillary was on purpose filled with air (dark) in order to verify that liquid (light) actually entered and forced the air bubble further into the capillary.

3.4. CLOD without enrichment

Dansylated amino acids were used as simple model substances. At pH 9 they are negatively charged. Hydrodynamic injection of 10 μ*M* sample solutions was used for determination of CLOD without enrichment. A rough estimation is that

approximately 4 nl was injected. This resulted in a CLOD (S/N=2) of 2.5 μ*M* for the two analytes, Dns-Val and Dns-Gly. This is a typical value using UV-absorbance detection without any enrichment technique other than the analyte stacking achieved when using ethanol as sample solvent.

3.5. CLOD with enrichment

A sample solution containing 0.1 μ*M* Dns-Val and 0.05 μ*M* Dns-Gly in ethanol was used for enrichment studies. Using the flow-through liquid microdispenser, enrichment of 1500 up to 36 000 drops was investigated. Using the present set-up, it was not feasible to investigate higher droplet numbers due to the excessive time consumed by every experiment. An electropherogram from enrichment of 36 000 droplets is presented in Fig. 6. The separation efficiency obtained is the same as without enrichment. Since ethanol was used as sample solvent, analyte stacking may cover for varying injection volumes. The CLOD (S/N=2) achieved was 0.015 μ*M* for the two analytes. This is almost 200 times lower than without enrichment. Since two different injection techniques were used it is unlikely that exactly the same volume was injected in the experiments with and without enrichment.

The total time consumed, including evaporation and separation was about 35 min. It is thus desirable to increase the evaporation rate. This can be easily achieved through elevated temperatures. The present study was performed at ambient room temperature and relative humidity.

4. Conclusions

The results of the present study show that sample enrichment for CE can be achieved through the use of an ultrasonic levitator and a flow-through droplet microdispenser. The combination of the levitator and the dispenser is very feasible for handling of the minute volumes demanded. It was not practical to manually introduce sample volumes below 1 μl into the levitator. The droplet microdispenser can handle sample volumes down to 65 pl. Larger volumes may be supplied by ejecting several droplets in a row at a rate of up to 500 Hz.

The levitator has the advantage that precise aiming of the droplets is not necessary since the pressure field in the levitator guides the droplets to the correct position. This results in a robust sample supply for containerless enrichment with good volume control since no droplets are lost.

The CLOD was lowered from 2.5 μ*M* to 0.015 μ*M* for the Dns-AAs in the enrichment experiments. The sample enrichment process through solvent (in this case, ethanol) evaporation was relatively slow and it could be even slower when using water as a solvent. It is thus desirable to find methods for

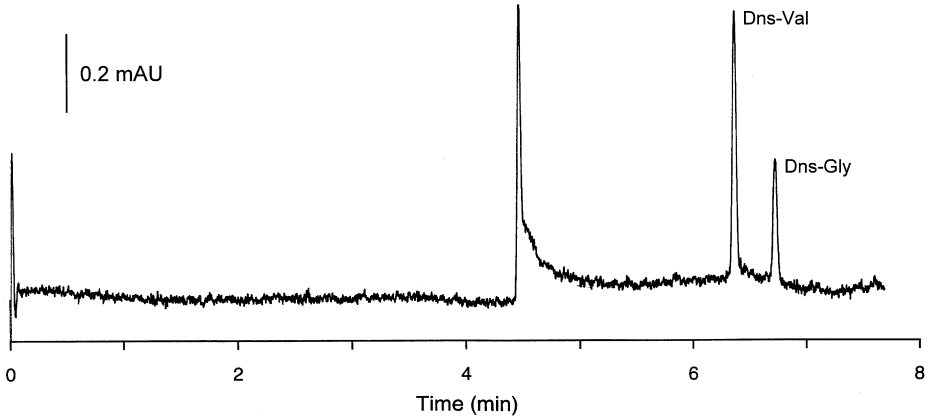

Fig. 6. CE analysis of in all 36 000 sample droplets (2.3 μl) enriched in an acoustically levitated drop for 25 min. Droplets (6000) were added to the diminishing levitated drop every fourth minute. Capillary 45 cm×50 μm I.D., electrolyte 100 m*M* borate buffer (pH 9.0), sample 0.1 μ*M* Dns-Val and 0.05 μ*M* Dns-Gly in ethanol, voltage 10 kV, current 14 μA, detection wavelength 200 nm.

accelerated evaporation, e.g. heating the sample using an IR laser. Not only the analytes of interest will be enriched but also any other nonvolatile component present in the sample. This may lead to high ion concentrations which will impair CE performance.

The most uncertain procedure was the sample transfer from the levitator to the capillary. The capillary was introduced in the levitator and the enriched sample drop was picked up on the tip. As a result, some of the sample may be retained on the capillary end surface.

Although still primitive, the levitator and microdispenser represent novel approaches to sample handling of the minute volumes associated with CE and other microanalytical techniques. Future work will be focused on optimisation of experimental parameters, such as injection technique and evaporation rate, and investigation of the effect of sample matrix in order to reach a practical and highly efficient system for sample enrichment.

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