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Short communication

Miniaturization of sampling for chemical reaction monitoring by capillary electrophoresis

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Abstract

Purpose-made capillary electrophoresis set-ups for reaction kinetics monitoring featuring two automated injectors allowed the easy reduction of the needed reactant amount down to 500 μL. The first set-up is similar to the cross injector used frequently in lab-on-chip designs while the other uses falling droplets for sample/buffer delivery. The versatility of the system was demonstrated by the analysis of oxidation of C-vitamin by hydrogen peroxide. Pseudo first order reaction rates about 10^{-3} s^{-1} were measured with RSD = 1–3% in one experiment and RSD = 20% in interday/person experiment. Plate numbers were typically around 5000–20,000.

Keywords: Capillary electophoresis; Miniaturization; Sampling; Monitoring; Reaction kinetics

1. Introduction

In capillary electrophoresis (CE) there is a sharp contrast between the nanoliter amount of sample, necessary for the separation/detection process inside the capillary and the milliliter amount of chemicals needed for operation in samplers in most of commercially available instruments. In biochemical assays sample amount is frequently limited to microliters and such amount can be usually sampled to a CE instrument manually. However, if monitoring of biochemical reactions by CE is of interest then manual sampling is inconvenient, not economical and imprecise, applicable only to relatively slow processes. On the other hand, because of the speed of analysis, low sample consumption and especially possibility of separating reactants and products makes CE technique very attractive to biochemical reaction monitoring. Thus, development of an automatic sampler for on-line monitoring of reactions, capable to operate with tiny amounts of samples becomes highly desirable.

The same problem exists in microfluidics. During the past decade, much attention has been paid to (CE) microchips [1]. A major obstacle to the realization of real-life applications of microchip CE is the sample introduction. Although several ingenious solutions of this problem have been presented [2–4], the world-to-chip interface currently remains one of the bottle-necks in the development of μTAS [5].

In this paper we describe two simple and economical techniques aimed for computerized on-line sampling of the small amount (couple of hundreds of microliters) reacting media into CE capillary. Although designed for common CE we believe that they might be relevant in lab-on-chip designs also.

The first approach is similar to a “cross” injection device commonly used in lab-on-chip devices where sample and separation channels are located perpendicularly on a chip. However, instead of electrokinetic loading, in this work the sample is loaded into a capillary by a pressure pulse during which an aliquot of the reaction mixture flows through the cross and fills the gap between separation capillaries with the sample. This portion is later carried to the separation capillary by electroosmosis action.

In the second approach sample is delivered as droplets (10 μL volume) into a buffer situated in a pipette tip. The falling droplet sampler was first proposed by Liu and Dasgupta [6] and was late implemented for hyphenating flow injection analysis (FIA) to CE [7–9]. In our design the construction of the falling droplet sampler is simplified further by rejecting a specially designed FI-CE interface body.

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2. Experimental

All experiments were performed using a Spellmann 2000 High Voltage Power Supply (Spellmann, Hauppaugue, NY, USA) and UV detection (Lambda 1000, Prince Technologies, The Netherlands) at 256 nm. The capillary was kept at 24 ± 0.5 °C by ambient forced airflow. Experiments were controlled by PC using software written in house in LabView (National Instruments, Austin, TX, USA) environment via Adam ADC/DAC interface (Advantech, Taipei, Taiwan). Electroperograms were edited by Matlab (MathWorks, Natick, MA, USA) routines written in house. Computer controlled sampling was accomplished by controlling corresponding solenoid valves (Type 6012 Miniature Solenoid Valve, Brükert, Helsinki, Finland) which delivered pressure pulses to the gas displacement pumps.

2.1. Chemicals and procedures

L-Ascorbic acid, sodium tetraborate, sodium hydroxide and hydrogen peroxide were purchased from Merck (Darmstadt, Germany). For the preparation of buffers and solutions of analytes, ultra pure Milli-Q water (Millipore S.A., Molsheim, France) was used throughout the experiments.

The reaction of L-ascorbic acid oxidation by hydrogen peroxide was carried out in either borate buffer or pure water. In the first case reaction medium was composed of 25 mM sodium tetraborate solution (pH 9.4). Solution of L-ascorbic acid was prepared in buffer with initial concentration of 5 mM. The reaction was initiated by adding hydrogen peroxide (35%) to reaction cell with initial concentration of 514 mM. The reaction medium was stirred continuously at 24 ± 0.5 °C. After starting the experiment the aliquots of reaction mixture were periodically injected into the CE capillary by means of either a cross sampler or a falling droplet interface.

2.2. Samplers

2.2.1. Cross sampler

A capillary connector made from Plexiglas and fabricated in the TTU Institute of Chemistry mechanical workshop. The design is patterned from a single "cross" microchip design and is illustrated in Fig. 1A. A 0.4 mm diameter channel was drilled through a 23 mm × 23 mm × 10 mm Plexiglas sampler body. Another 1.5 mm diameter channel was drilled perpendicularly through the sampler body to form a cross. At all four ends threads for 1/8 in. fittings were cut. Two fused silica capillaries (365 μm O.D., 75 μm I.D. capillaries, Polymicro Technologies, Phoenix, AZ, USA) with lengths of 18 and 50 cm correspondingly were inserted into both 0.4 mm channels until their ends were approximately 30 μm apart (estimated visually under a microscope). The longer fused silica capillary passed through the UV detector and served as the separation channel. The both free ends of fused silica capillaries were placed into reservoirs filled with separation buffer. A separation voltage 13 kV was used throughout all experiments. Two 1.5 mm O.D. and 0.3 mm I.D. Teflon capillaries with lengths of 9 and 20 cm were inserted perpendicularly to the fused silica capillaries. The Teflon capillaries served as sampling channels. The outsides of all of the capillaries at the channel entrances were fixed by 1/8 PEEK nuts to prevent leakage.

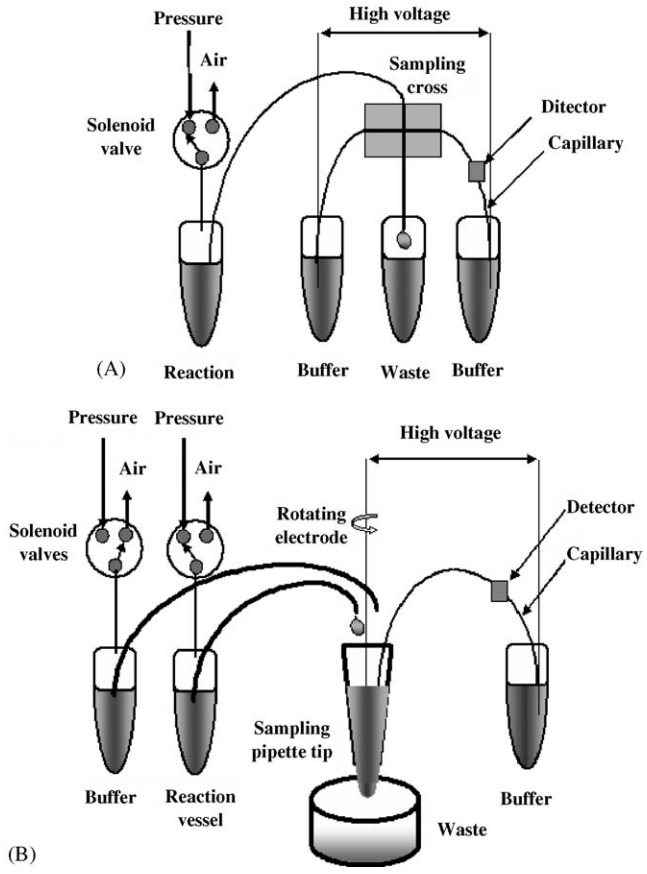

Fig. 1. Schematic of cross sampler A and falling droplet interface B.

One free end of the longer arm of the sample channel was placed into the reaction vessel and the other free end was lead to waste. The reaction vessel situated in the gas displacement pump manufactured from 100 mL DURAN ISO laboratory bottle (Schott UK) with bottleneck attachment (Metrohm AB, Herisau, Switzerland part no. 6.1602.150 (Bottleneck attachment/GL 45–3 × 10/32)). The bottleneck attachment had three threaded connector openings. One of the connectors was used for pressurizing of the bottle via a solenoid valve, the second for passing sample tubing and the third opening was closed.

The falling droplet interface is shown in Fig. 1B. The CE column input vessel was achieved by positioning the inlet of the separation capillary into a 1 mL pipette tip where at the inside a sample/buffer liquid stays by surface tension (ca. 50 μL). A sample/buffer inside the tip can be replaced to a new portion just by delivering liquid droplets into the pipette tip. This action forces the old sample/buffer portion to flow out of the tip and to be displaced by the new sample/buffer portion. A platinum electrode (0.3 mm diameter), serving as the anode/cathode, was inserted through a hole on a 30 mm × 130 mm plastic rod installed above the pipette tip. The electrode was rotated (2 rps) by an isolated DC micro motor (type L149, Elfa, Järfälla, Sweden) to facili-

tate stirring of the liquid inside pipette tip. Also, a separation capillary (365 μm O.D. 75 μm I.D. Polymicro Technologies, Phoenix, AZ, USA) with length of 70 cm (39 cm to detector) was inserted into pipette tip. A separation voltage 25 kV was used throughout all experiments.

Two glass capillaries (5 cm long, 1.6 mm O.D., 0.5 mm I.D.) for the delivery of sample and buffer droplets were inserted through holes on the upper rod. The outlets of the capillaries were coaxially positioned 24 mm above the liquid surface inside the pipette tip. The capillaries were connected via PTFE tubing (20 cm long, 0.7 mm I.D.) to the sample and buffer vessels located inside gas displacement pumps which construction has been described already above.

3. Results and discussion

For the cross sampler the alignment of the separation channel capillaries was critical for proper operation and reproducibility of the sampler. The alignment procedure, however, appeared to be a relatively robust action and could be proceeded routinely (e.g. if replacement of new capillaries was necessary). For the falling droplet case the sample and buffer tubing's must be centered properly to avoid falling of the droplets on the pipette tip wall above the liquid surface stored in the tip. Also this adjustment could easily be performed. The optimal pressure and sampling time was worked out for both samplers. Obviously it is determined by the dimensions of the sample tubing. It was found for a cross sampler that the minimal pressure necessary for the proper functioning was about 98 kPa. Applying pressure pulse for 1 s it was possible to deliver minimum ca. 25 μL of sample (two droplets).

For a falling droplet a 1.0 s long 50 kPa pressure pulse delivered one 25 μL droplet into the pipette tip. Usually up to five droplets were necessary for increasing the weight of the liquid inside the pipette tip and to force sample/buffer from the previous analysis cycle to flow off the pipette tip. Commonly, this was used for the replacement of the sample from the previous cycle by the buffer for the new analysis cycle. Also it was possible to deliver fewer (one/two) droplets of sample into the (e.g. separation) buffer located in the pipette tip. Then a newly delivered sample portion is diluted in the separation buffer. This fact can be advantageously used for matching sample concentration (dictated, e.g. by reaction under study) to the level needed for the separation process.

Summarizing, for both samplers the lower limit of reacting mixture, necessary for monitoring of kinetics could approximately be between 200 and 500 μL depending on the fact how many samplings (measured concentration points) are necessary for reaction rate measurements. However, in this work, in most cases the volume of the reacting media was larger (1 mL and above) for the convenience of performing several kinetic experiments in sequence.

Monitoring of oxidation of L-ascorbic acid by hydrogen peroxide was performed in two media: in water and in separation buffer solution. In the latter case the reaction rate is considerably faster. Initial solutions were prepared by different persons and reactions were repeated in several days (sometimes with an interval of weeks between experiments) to estimate interday reproducibility. Typical results are presented in Fig. 2. Notable difference in the L-ascorbic acid migration times of both samplers is due to the different separation voltages applied. It follows from Fig. 2 that both samplers perform well and indeed on-line computerized monitoring of concentrations in small reaction volumes is possible. Also no carry-over effects between two consecutive measurements were observed.

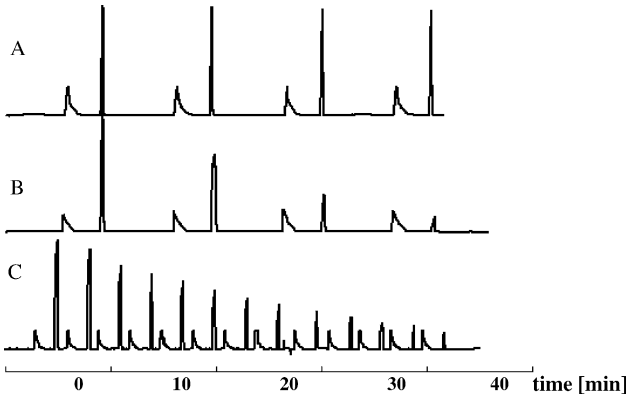

Fig. 2. Electropherograms of the reaction mixture recorded during the monitoring. Experimental conditions: see text. (A) Cross sampler, reaction in water, (B) cross sampler reaction in buffer and (C) falling droplet sampler, reaction in buffer. Peak with constant intensity: H_2O_2, peak with exponentially decreasing intensity: L-ascorbic acid.

Based on the collection of kinetic experiments those data for both samplers are represented in Table 1. It follows from Table 1 that reproducibility of peak migration times can be considered good. However, the efficiency is not very high (except the reaction in water). The low efficiency is probably due to the fact that both samplers contribute much to initial extra-column band broadening which is not surprising taking into account of the construction of the both samplers. Those efficiencies are much larger than accepted in common capillary electrophoresis. However, since both samplers are intended to use for reaction monitoring in which case the sample is a relatively simple solution of a few components it is possible to separate them anyway despite the small efficiency of the separation. Definitely improvements are possible requiring further studies.

From the L-ascorbic acid peak areas it is possible to estimate reaction rate constants. Assuming the simple case of a pseudo first order irreversible A + B → C type reaction, one can consider that the peak shape of either of the reactants is an exponential decay with rate, say k. Rate constants were calculated by a least squares fitting of L-ascorbic acid peak areas to this exponential dependence. Uncertainties in rate constants were calculated according to the procedure proposed in [10] and explained in detail for the case of estimating rate constants in [11].

L-Ascorbic acid oxidation rate constants were found to be $(14.2 \pm 3.0) \times 10^{-4}\,s^{-1}$ ($n = 4$) and $(8.7 \pm 2.4) \times 10^{-4}\,s^{-1}$ ($n = 4$) for cross sampler and falling droplet interface correspondingly, which is in the same order as in published data [12,13]. The relative precision for a particular single experiment is excellent if judged by corresponding RSD values, varying

Table 1
Performance data and their relative standard deviations (RSD) of samplers

Parameter	Cross	Falling droplet
H_2O_2 peak migration time (min)	5.5, RSD = 0.5% (n = 5)	2.8, RSD = 0.2% (n = 7)
RSD of H_2O_2 peak area (%)	4.4 ± 1.6 (n = 3)	7.5 ± 0.5 (n = 3)
L-Ascorbic acid peak migration time (min) (in water)	8.9, RSD = 0.1% (n = 9)	5.8, RSD = 2% (n = 9)
L-Ascorbic acid peak half width (s) (in water)	8.1, RSD = 2.6% (n = 9)	9.3, RSD = 6% (n = 9)
L-Ascorbic acid peak migration time (min) (in buffer)	9.5, RSD = 0.8% (n = 4)	5.1, RSD = 1% (n = 7)
L-Ascorbic acid peak half width (s) (in buffer)	17.1, RSD = 3.4% (n = 4)	13.5, RSD = 4% (n = 7)

from 0.4 to 4.2% for cross sampler and from 1.6 to 5.2% for falling droplet interface. Interday reproducibility is larger as can be expected (about 20%). Evidently, with this precision there is a slight disagreement in the rate constant values between two samplers as the calculated Student t-value is $t = 3.24$ when the critical two-sided t value is $t_{(95\%,4)} = 2.77$. This disagreement and large interday standard deviation values can probably be associated with the 1% variations in temperature control of the reactors. Since the paper is intended as a proof of a principle no further actions for improvement of the temperature control were undertaken.

For correct estimation of the rate constant the reaction must be stopped rapidly which means that the reactants must be rapidly separated after injection. This means that contact time of reactants must be short compared to the reaction characteristic duration. Mathematically this statement can be written as follows:

$$t_c = \frac{d}{v_B - v_A} = \frac{w_A t_B}{t_A - t_B} \ll \frac{1}{k}$$

Here t_c is the contact time of the reactants, d is a sample zone initial width, v_A and v_B are migration velocities of the reactants, say for L-ascorbic acid and H_2O_2 correspondingly. t_A and t_B are migration times of L-ascorbic acid and H_2O_2 and w_A is L-ascorbic acid peak base which can be estimated approximately taken as being 1.7 times wider of corresponding peak half width. k is the reaction rate constant. Velocity values in the denominator are subtracted since the H_2O_2 zone moves with greater velocity than L-ascorbic acid zone.

As follows from above the reaction characteristic time has a value $1/k \approx 10^3$ s. On the other hand using values for migration times and L-ascorbic acid peak half width the contact times are for cross and falling drop samplers 40 and 20 s correspondingly. Thus, indeed, a brief contact of reactants at the beginning of the separation can be neglected when calculating reaction rates.

4. Conclusion

In this paper two simple and economical techniques for automatic and on-line monitoring of the limited amount of reaction media by CE have been described and two samplers of relatively simple construction, which can be manufactured easily in house with good machine shop, were proposed. Neither approach requires advanced technologies for microsystems fabrication. The samplers can perform computerized on-line capillary electrophoretic monitoring of reaction mixtures which amount can be limited above 500 μL. Both samplers can determine rate constants with a precision of about 1–3% on average (in a single experiment) and about 20% on average (interday precision).

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