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Short communication

Post-capillary laser-induced fluorescence detection without sheath-flow ☆

Samuele Bottani[a,b,*], Hans Rebscher[a], Rainer Siebert[a], Luc Valentin[a,b]

[a]*Groupe Interfaces Physique-Biologie, Institut de Physique Nucléaire d'Orsay, 15 Rue Georges Clémenceau, 91406 Orsay Cedex, France*

[b]*Université Paris 7 Denis Diderot, Paris, France*

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Abstract

The sensitivity of laser-induced fluorescence detection in capillary electrophoresis is generally limited by noise due to scattering of the excitation beam on the capillary walls. The highest sensitivities have been obtained with post-capillary detection in a sheath-flow cuvette. In this paper, we report a new method for post-capillary detection, avoiding the hydrodynamic buffer flow at the capillary outlet. In our detection scheme, the analytes coming out of the individual capillaries are kept well separated from their neighbours in the outlet detection chamber because of an adequate electric field surrounding the capillary extremities.

Keywords: Laser-induced fluorescence detection; Detection, electrophoresis; Instrumentation

1. Introduction

In the last decade, numerous laser-induced fluorescence (LIF) detection schemes have been developed for capillary electrophoresis (CE). LIF provides very high sensitivity, which is important for many biological applications and especially for DNA sequencing. However, application of LIF detection to CE is not trivial. A major difficulty is how to avoid the background signal due to light scattering of the exciting laser beam at the capillary surfaces.

For mono-capillary instruments, this problem could be solved in two ways: with an on-capillary confocal system or with detection outside the capillaries. In the on-capillary solution, detection occurs through the capillary walls. Background due to diffusion of the excitation beam on the surfaces is reduced with a confocal system, where the laser is focused on the sample by a microscope objective and the emitted fluorescence is collected by the same objective [1,2]. In this way, the depth of field of the optical system is sufficiently small, so that only the interior of the capillary is probed and stray light contamination from the capillary surfaces is low. Although this detection scheme is very efficient, even better detection limits have been achieved with post-capillary detection in a sheath-flow cuvette [3–6].

In this approach, detection is performed after the analytes have left the capillaries into the outlet reservoir. A sheath buffer flow in the capillaries' axial direction surrounding the analytes coming out

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*Corresponding author.

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of the capillaries prevents them from diffusing. The detection chamber, provided with high quality optical windows for excitation and detection, eliminates the noise due to light scattering. A limit of detection of 10^{-13} mol/l with fluorescein isothiocyanate (FITC) has been reported in a single capillary sheath flow set-up [3]. Chen and Dovichi [7] even reported a limit of detection at the yoctomole level (10^{-24} mol) with fluorescent standards.

In recent years, work on CE–LIF detection has been mainly motivated by the wish for high-throughput DNA sequencing and has focused on capillary array electrophoresis (CAE). Developments of CAE instruments have been slow, one of the main problems being detection. Due to fundamental optics constraints, collection of light from an extended object, such as an array of capillaries, is less efficient than from a compact object. In another approach [8,9], the question is handled by mounting a mono-capillary confocal system on a scanner in order to record fluorescence from each capillary sequentially. High laser power is necessary since, due to the scanning, the excitation intensity for each capillary is inversely proportional to the number of capillaries.

A different approach relies on parallel detection of the signal from all of the capillaries simultaneously. Anazawa et al. [6] and Dovichi [10] used a charged coupled device (CCD) camera to capture images of all of the capillaries in instruments based on the sheath flow technique. The same scheme has also been applied by ABI/Perkin-Elmer for their new 96-capillary DNA sequencer. Thanks to the detection outside the capillaries, stray light problems are eliminated and sensitivity is higher than in on-cap systems. Other instruments using imaging detection with on-capillary detection, e.g. that of Ueno and Yeung [11], do not achieve comparable sensitivities. However, due to the constraints on the regular buffer flow, sheath-flow instruments are known to be less robust than on-cap systems.

In this paper, we present a feasibility study of a high sensitivity post-capillary CAE detector, comparable to sheath-flow instruments, but simpler and more robust. Our method might be attractive for high-throughput DNA sequencing, but also for other electrophoretic separation methods with low concentrated samples.

1.1. Principle

The basic problem of detecting analytes after they have left the capillary is their immediate dilution caused by movements in all directions. Responsible for this are at least two phenomena: thermal diffusion and electrophoretic movement along diverging electric field lines. The latter can be seen in Fig. 1, which shows the equipotential lines, corresponding to a diverging field, resulting from electrostatic potential calculations (see Experimental) in a configuration with a capillary emerging in an outlet reservoir.

In order to avoid dilution of the bands, we propose to prevent the divergence of the electric field lines coming out of the capillary, so that the analyte molecules keep their electrophoretic motion going straight ahead in the detection cell. This is achieved by applying a supplementary electric field in the detection cell, surrounding the capillaries in such a way that it canalizes the electric field coming out of the capillaries. This field actually replaces the hydrodynamic flow of the sheath flow method. By forcing a forward electrophoretic motion of the analytes in an unconstrained volume (no capillary walls), we produce in the detection cell a configuration that can be compared to slab gels: DNA bands migrate

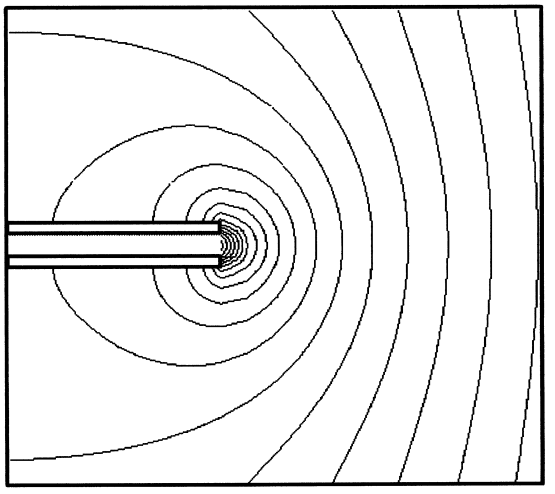

Fig. 1. Equipotential lines at the capillary outlet. The anode is a large distance away from the capillary end compared to the capillary external diameter.

vertically in a lane due to the parallel vertical electric field in the slab. Our method is of course valid only if thermal diffusion perpendicular to the migration lane is low. This is the case at least for large molecules like DNA [12], but also for smaller molecules, as we found with experiments using FITC.

In the following, we propose a solution for the application of these ideas as well as electric field simulations clarifying the conditions of operation.

2. Experimental

We used a laboratory-made capillary array electrophoresis system with 12 capillaries, as can be seen in Fig. 2. Capillary inlets and the cathode were placed in a reservoir filled with polymer solution. The capillary outlets were inserted in the detection chamber, which was filled with a polymer solution. This chamber contained the anode and a supplementary electrode, which is needed to control the electric field configuration. Electrophoretic migration in the capillaries and through the detection chamber was assured by two high-voltage power supplies (Ortec, Oak Ridge, TN, USA and Stanford Instruments, Sunnyvale, CA, USA) connected in series, which allows one to regulate independently the electric field strengths in the capillaries and in the detection chamber. The chamber was mounted at the bottom of a tub, which was formed of Plexiglas plates (dimensions: 1.5 cm width, 10 cm length and 1 cm height). A rectangular cavity with the cross-section adjusted to the dimensions of the capillary array was built using 360-μm-thick PTFE spacers (see Fig. 3) placed between the bottom of the tub and a 2-mm-thick microscope slide. After filling the cavity with the separation matrix polymer solution, the capillary array was inserted on one side of the cavity. As shown in the figure, a platinum anode faces the capillary outlets. The intermediate electrode in this set-up is a platinum wire placed near the capillary array at the entrance of the detection chamber. Electrical contact between the intermediate electrode and the anode is assured by the polymer solution. This way, the intermediate electrode creates an electric field outside the capillaries, with field lines parallel to their axes. Capillaries and the detection chamber were filled with poly(vinylpyrrolidone) (PVP) (Sigma, St. Louis, MO, USA), which was used at a concentration of 5% (w/v), having been diluted in Tris–boric acid (TBE) 0.1× buffer. PVP solutions are known to reduce electro-osmotic flow very efficiently [13]. The sample used was FITC (Sigma) at 100 nmol/l.

The capillaries were uncoated fused-silica tubing (Polymicro Technologies, Phoenix, AZ, USA) with an O.D. of 360 μm, an I.D. of 100 μm and a length of 20 cm. Polyamide coating was removed over 1 cm at the capillary ends by dissolution in hot H_2SO_4. The array of 12 capillaries was held together using adhesive tape. The polymer solution was pumped into the capillaries using a syringe.

Electrophoretic trajectories outside the capillaries were visualized by observing them from the top with a camera (Canon, Tokyo, Japan) and a macro objective. FITC molecules leaving the capillaries

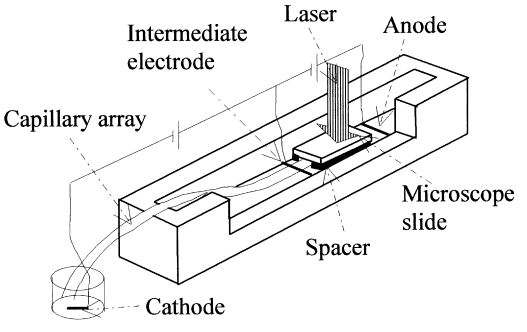

Fig. 2. Set-up for the tests. The outlet reservoir and detection window are in a channel made of Plexiglas. The voltages between the cathode, intermediate electrode and the anode are adjusted with two power supplies using a series connection.

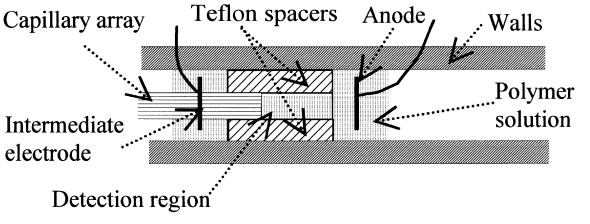

Fig. 3. Details of the detection cavity showing the electrodes and the detection region downstream of the capillaries.

were excited with a laser beam (488 nm, Ar^+, 5 mW; Ion Laser Technologies, Salt lake City, UT, USA) spread over a 1×0.5 cm region of the detection cavity.

Electric field calculations were performed using the package Priam, which is a program developed at LAL (Laboratoire de l'Accélérateur Linéaire, Orsay, France) for solving Maxwell's equations by finite element methods. Boundary value problems modelling different configurations of the capillaries and the electrodes were analyzed using the electrostatics form of the Maxwell equations.

3. Results

In this section, we present first numerical calculations of the electric field configuration in the outlet reservoir, performed in order to find out reasonable geometrical arrangements and to understand the appropriate conditions of geometry and voltages for our method. Results from these simulations have been tested experimentally, as presented in the second part.

3.1. Simulation

The principle for creating the confining electric field is to implement in the outlet reservoir a third electrode up towards the capillary ends. The performed electric field simulations allowed us to get a precise idea of the necessary conditions (shape of the electrode and voltages). Fig. 4 shows the configurations of the electric field and potential lines surrounding the extremity of one capillary for three different values of the voltage in the detection chamber: $V_{detection}=U_{int}-U_{anode}$ between the additional intermediate electrode potential, U_{int}, and the anode potential, U_{anode}. The total voltage $V_{tot}=U_{cathode}-U_{anode}$ between the cathode potential, $U_{cathode}$, at the capillary inlet and the anode in the cuvette is the same in each case. Already, for a low value of $V_{detection}$, the electric field lines are much better confined than in the case without the additional field (see Fig. 1). However, they are still diverging. On the other hand, for a high potential U_{int}, these lines are compressed or focussed by the surrounding field. Finally, there is a value U^* of U_{int} so that the field ahead of the capillaries is only minimally perturbed. For this value, analyte bands leaving the capillary should continue in a rectilinear movement in the detection chamber. When several capillaries are aligned, the field configuration is obtained from the mono-capillary situation by translation symmetry, as shown in Fig. 5.

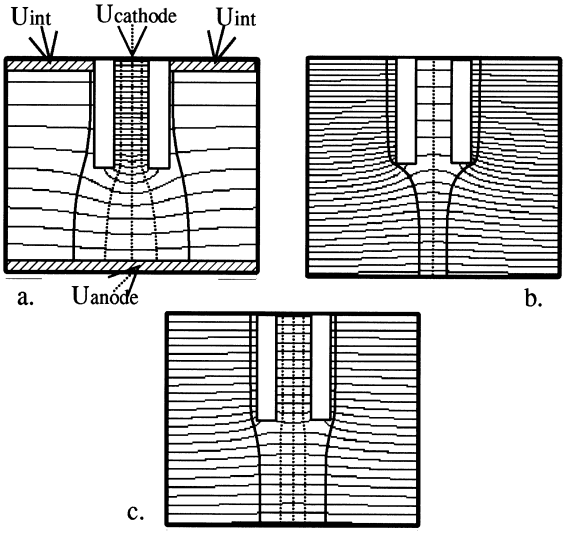

Fig. 4. Equipotential lines and electric field lines at the capillary extremity in between the intermediate electrode and the anode, for three different values of the intermediate electrode potential, U_{int}. The potentials U_{anode} and $U_{cathode}$ are the same in all three cases. Electrodes are indicated only in (a). Dotted lines represent the field coming out of the capillaries and correspond to the electrophoretic trajectories of the analytes. Bold lines are for the field from the intermediate electrode. There is a value U^* of U_{int} so that the field lines from the capillary remain linear. (a) $U_{int}<U^*$, field lines from the capillary are diverging, causing spreading of the analytes bands. (b) $U_{int}>U^*$, the intermediate electrode focuses the analyte bands. (c) $U_{int}\sim U^*$, field lines from the capillary are minimally perturbed.

3.2. Experiment

In order to visualize the different cases of Fig. 4, we used our experimental set-up, applying different voltages on the intermediate electrode. Confinement of the individual electrophoretic lanes in the detection chamber is effectively observed, as shown for two different voltages on the pictures in Fig. 6. The analyte lanes from each capillary remain separated from neighbor lanes even several millimeters down-

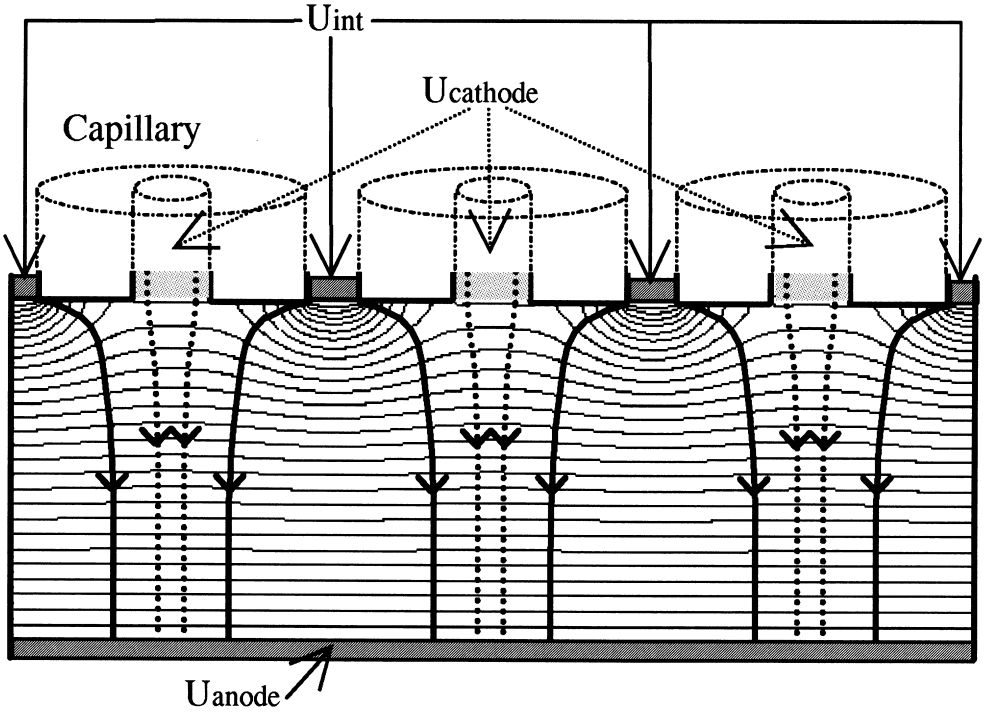

Fig. 5. Array of capillaries with an intermediate electrode. Equipotential and field lines configuration for $U_{int} > U^*$. Same conventions of symbols as in the Fig. 4.

stream from the capillary's extremities. By comparing the two pictures, one can see that the confinement is stronger for a higher potential at the intermediate electrode (Fig. 4b), which is in agreement with our simulations. The best working conditions can be determined only with real separations. This will be done by means of a real detector prototype, which is under construction.

4. Conclusion

We presented an alternative principle to the sheath-flow technique for post-capillary detection. Our approach relies on configuring the electric field in the detection chamber in a way to keep the charged analytes on a rectilinear track. Electrostatic calculations showed that an adequate field configuration can be obtained by using a supplementary intermediate electrode in the detection cuvette. With a first set-up, we could show that, using our principle, the individual analyte lanes are effectively separated in the detection chamber over a sufficient area to allow multicapillary LIF detection.

We expect that, compared to the sheath-flow technique, the absence of laminar hydrodynamic flow in our method will make it more robust and easier to operate. Since band confinement is realized by an electric field, the detection chamber can be filled with buffer as well as with polymer solution and capillaries can be operated with low viscosity separation matrices. Based on these results, we are now assembling a CAE instrument to analyze quantitatively band-to-band separations.

Acknowledgements

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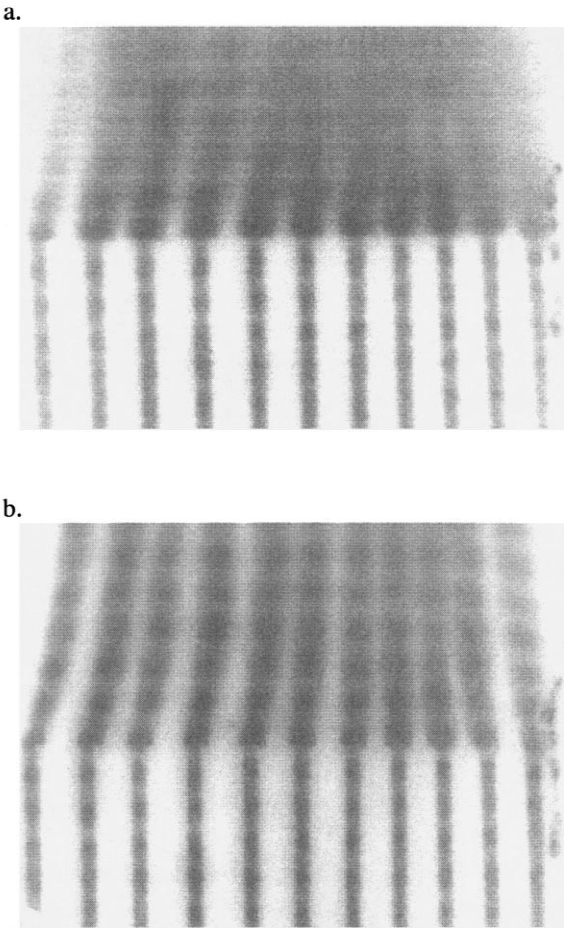

Fig. 6. Fluorescence at the capillary exit with a confining electric field created with an intermediate electrode. The picture is printed as a negative, with black representing illuminated regions. Capillaries are vertically arranged with analyte lanes migrating to the top. (a) Picture for $U_{int} < U^*$, analytes coming out of the capillaries are weakly confined. (b) Picture for $U_{int} > \sim U^*$, the individual lanes are well separated from each other.

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