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Optimized Photonic Crystal Fibers supporting efficient Capillary Electrophoresis

M. Calcerrada *[a], C. García-Ruiz[a], P. Roy[b], M. Gonzalez-Herraez[c]
[a]Dept. de Química I, Universidad de Alcalá, C. Universitario, 28871, Madrid (Spain);
[b] Xlim Fibres Photoniques, Dept. UMR 7252-CNRS, Université de Limoges, 87000, Limoges (France);
[c] Dept. de Electrónica, Universidad de Alcalá, C. Universitario, 28871, Madrid (Spain)

ABSTRACT

In this paper we present preliminary results on the use of Photonic Crystal Fibers (PCFs) in a conventional capillary electrophoresis system to separate and detect fluorescent species. PCFs show interesting advantages over conventional capillaries for this application, including larger surface-to-volume ratio and potential for higher resolution with comparable sensitivity. Our results illustrate some of these advantages, and we point out the need for stringent tolerances in the fabrication of specific PCFs for this application.

Keywords: Chemical sensing and sensors; Laser induced chemistry, Micro-structured Fibers (MSF), Photonic Crystal Fibers (PCF)

1. INTRODUCTION

Photonic crystal fibers (PCFs) have experimented a huge evolution since their discovering and nowadays their properties make them attractive for new applications in diverse fields [1]. PCFs are being used as new components in analytical techniques, such as chips and biochips for chemical detection [2,3,4]. Among the remaining analytical techniques, capillary electrophoresis (CE) is an efficient and economical one capable of separating tens of components simultaneously using small amounts of samples and reagents. In addition, it possesses a high versatility and usually better resolutions than other separation techniques [6]. Fig. 1 shows a basic scheme of a CE system. Conventionally, this technique is based on the separation of ions of diverse interest through the application of a high voltage across a polymide-coated glass capillary (usually 20-100cm length and 50-75µm of inner diameter (i.d.)). The separation of the ions is accomplished on the basis of their charge mass ratio. While separating, the species travel along the capillary and are detected through a wide variety of techniques such as Differential Absorption or Laser Induced Fluorescence (LIF). Detection is usually done by transversal illumination on the capillary by making a window on the polyimide coating. Results are presented in an electropherogram and substances appear as peaks of different widths and heights.

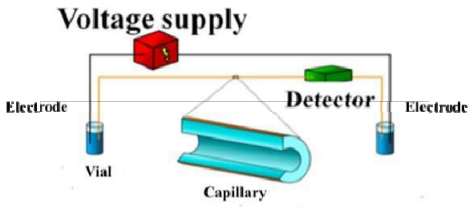

Fig. 1. Basic scheme of a CE system

High voltages and narrow capillaries offer higher speed and less band-broadening, which produces a better performance and results. However these extreme conditions may give rise to peak overlapping if the time is not enough to achieve entire separations or low sensitivity if the sample introduced is minimal due to the capillary narrowness. In addition, the Joule effect in the capillary (heat generation due to the current) is also an important limitation when attempting higher resolutions as the temperature gradient affects the mobility of the ions and gives rise to wider peaks in the electropherogram [6]. Although PCFs have been used in this field [7], these structures have not been used directly in commercial equipment. However, some premises make PCFs interesting devices to be applied in conventional CE: on one hand, PCFs can be seen as a bundle of capillary tubes that would allow a better resolution (because of the narrow capillaries) but injecting total volumes similar to conventional capillaries, hence achieving not only good sensitivity but

also better resolution between peaks; on the other hand, the favorable surface-to-volume (S/V) ratio in PCFs, which relates the total inner surface ($2\cdot\pi\cdot r\cdot h$) according to a specific capillary volume ($\pi\cdot r^2\cdot h$), which implies that there is more surface available for heat dissipation. In this paper we demonstrate the advantageous use of PCFs in conventional CE equipment for the detection of chemical species.

2. EXPERIMENTAL SETUP

A conventional CE System equipped with a LIF detector (4-mW Ar laser emitting at 488 nm and detection filter at 520 nm) was used for CE detection. A conventional PCF, a custom-made PCF (that we will refer to as Smart Micro-Structured Capillary, SMSC), a conventional capillary and a transparent-coated capillary were compared for the electrophoretic detection. For both the PCF and the SMSC an equivalent conventional capillary was theoretically calculated, in such a way that this structure would have the same total cross area than those of the PCF and the SMSC. Table 1 summarizes geometrical parameters of all the devices employed and Fig. 2 depicts some images of these structures. Fluorescein disodium salt was the analyte of study, as this compound emits fluorescence at 520 nm and absorbs at 490 nm. An aqueous borate buffer (sodium borate/boric acid 0.3/0.4% (m/m)) was used for the electrophoretic separation. NaOH 1M was used for the capillary conditioning. Ultrapure H_2O was used to prepare the solutions.

Table 1. Geometrical parameters of PCFs and SMSCs used

	No. Holes	Channel i.d. (µm)	Total cross area (µm2)	S/V	Equivalent capillary	
					id (µm)	S/V
(a) SC-PCF	84	4.3±0.2	1220	0.930	39	0.101
(b) SMSC	85	5.6±0.3	2094	0.714	52	0.077
(c) Capillary	1	50±3	1964	0.08	-	-
(d) T-Capillary	1	50±3	1964	0.08	-	-

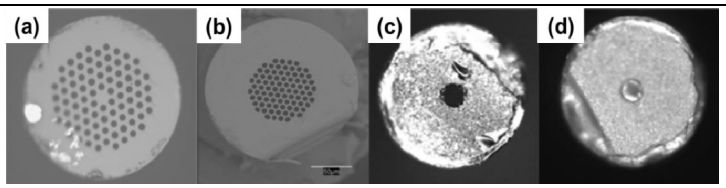

Fig. 2. Devices used as capillaries in the experimental setup. (a) and (b) correspond to SEM images of PCF and SMSC. (d) and (e) correspond to optical microscopy images from the polyimide-coated and transparent coated conventional capillaries respectively.

Fluorescein aliquots were introduced hydrodynamically and experimental conditions are described individually for each experiment as they depended on different parameters according to Poiseuille's equation:

$$V = \frac{\Delta P\cdot d^4\cdot\pi\cdot t}{128\cdot\eta\cdot Lt} \tag{1}$$

Where V represents the volume injected in the capillary or fiber (nL); ΔP corresponds to the pressure difference between inlet and outlet vials (Pa); d is the capillary or fiber channel i.d.(µm); t is the injection duration (s); η is the fluid viscosity (Pa·s) assumed 0.001 (water viscosity at normal conditions); and L_t the capillary/fiber total length (µm) [12].

To interpret the results, typical parameters used in CE were recorded and treated, such as: current (between the inlet and outlet extremes of the capillary or fiber, measured in µA); migration time (minutes required for an analyte moving from the sample introduction point to the detection point); peak area (which is directly proportional to the analyte mass injected); linearity (R^2) of the analytical method (ability, within a given range, to obtain peak areas directly proportional to the amount of analyte in the sample, necessary for an accurate quantification of the analyte); asymmetry (dimensionless measurement of peak tailing usually made at 5 or 10% of the maximum peak height) and signal to noise (S/N) ratio (measurement that compares the level of a signal to the level of the background noise);

3. EXPERIMENTAL RESULTS

A conventional PCF was initially used as a proof of concept. The PCF geometrical parameters and appearance can be seen in Table 1 and Fig. 2(a). As this device was not designed for an electrophoretic separation, geometrical parameters were quite different from conventional capillaries, fiber-equivalent capillaries (39 µm i.d.) were not at our disposal and therefore direct comparisons with them were not possible. However, these first results did corroborate the possibility of using micro-structured devices in conventional equipment. Fig. 3 shows one resulting electropherogram of a fluorescein analysis as a representative example. Signals presented tails and baseline noise (enlarged in Fig. 3) comparable to those obtained with conventional capillaries. Current between extremes and migration time were repeatable with deviations of 0.4% and 0.3% respectively. Area variations were higher, resulting in deviations of 16%. Fig. 3 also shows a graph relating volume injected against signal area (methodological calibration). As can be seen, results followed a linear tendency, with a R^2 value of 0.9981. This fact proved the linearity of the method in the range studied using a conventional PCF.

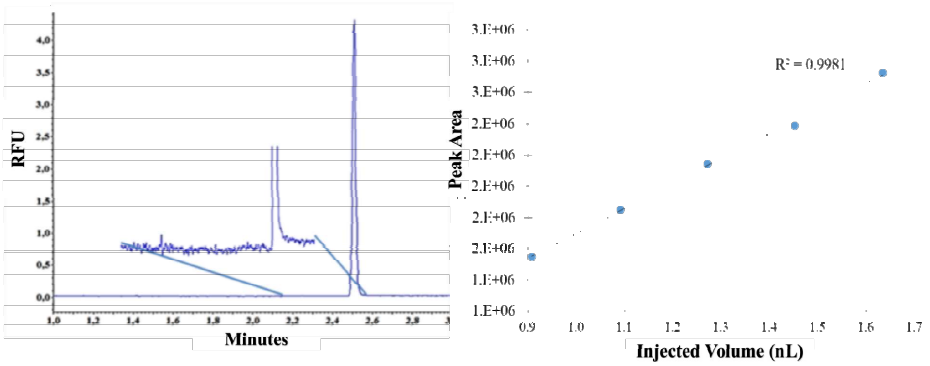

Fig 3. Fluorescein signal using a conventional SC-PCF. Separation Buffer: sodium borate/boric acid (0.3, 0.4%, m/m), pH 8.6. Conditions: separation length 20cm, total length 30cm, separation voltage 12 kV, temperature 25°C and LIF detection at 488 nm excitation and 520 nm emission. Injection: 1psi, 22s (0.4 nL)

After demonstrating the use of conventional PCFs in CE, a new device named Smart Micro-structured Capillary (SMSC), hybrid between optic fiber and capillary, was designed by adapting PCF fabrication technology to the external physical properties found in capillaries, which are desirable in CE. Fig. 2 (b) shows a SEM image from the design and Table 1 summarizes geometrical parameters. The SMSC was manufactured through the well-established stack-and-draw technique by using typical PCF materials (SiO_2 and polymer as jacket). In this way, transparent jacket avoided the need for a detection window, necessary in polyimide-coated capillaries when using optical detectors. Contrary to conventional PCFs, this SMSC was made without core in order to achieve more homogeneous pressure force and heat dissipation along the entire SMSC. In addition, this device presents 360 µm outer diameter, similar to conventional capillaries and being then more appropriate to introduce them in the CE cartridge, and 85 holes of 5.6 µm, corresponding to a total cross section of a ~50 µm i.d. equivalent-conventional capillary, as can be seen in Table 1. Taking advantage of this equivalence, comparisons between this new device and two conventional capillaries (50 µm i.d. polyimide-coated and transparent-coated) were performed. Comparisons were made through methodological calibrations on each one of the structures. For the conventional capillaries, pressure injections of 0.1 psi at different times (1, 2, 3, 4, and 5 s, three replicates for each time) were made. Those conditions resulted in injection volumes of 0.4, 0.79, 1.19, 1.58 and 1.98 nL respectively, according to Eq. 1. However, for the SMSC, both injection pressure (0.5 psi) and injection times (10, 20, 30, 40, 52, 60, 70 and 80 s, also three replicates for each time) were higher as more powerful conditions were needed to inject reasonable volumes of: 0.59, 0.79, 0.98, 1.18, 1.38 and 1.57 nL, respectively.

Averages of the three replicates for each injection time were used to graph the results obtained. In Fig. 4 peak areas, peak asymmetries and S/N ratios from each structure are shown as a function of volume injected. Area was expected to increase linearly when increasing sample volume. However, when injecting small volumes (0-2 nL), conventional capillaries did not follow a tendency as linear as the one obtained when using the SMSC. In terms of the peak asymmetries (which measure the asymmetry in the peak recorded: the closer to unity, the more symmetric will be the signal and less peak-tailing will be obtained), similar values were obtained for all structures when injected volumes were near 2 nL, but closer-to-ideality asymmetries were evidenced for SMSC when small volumes were injected (0-1.8 nL).

Finally, S/N ratio was calculated for the sequences and is also graphed in Fig. 4, and surprisingly these were an order of magnitude higher when using the SMSC. An electropherogram and its enlargement are depicted in Fig. 4 for a clear comparison of the results obtained.

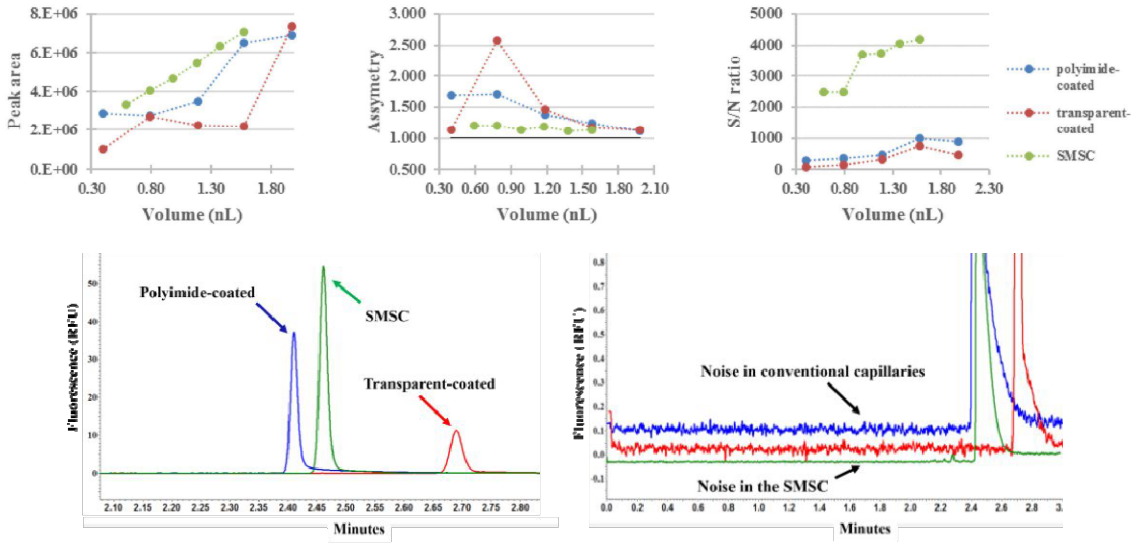

Fig. 4. Peak area, asymmetry and S/N ratio as a function of the injected volume for the three structures employed. Below an electropherogram and its enlargement at baseline for three analyses carried out with the three structures. Injection conditions: for the polyimide-coated and transparent-coated 0.1 psi during 3 s (1.19 nL); for the SMSC 0.5 psi during 60 s (1.18 nL).

4. CONCLUSIONS

In this paper a new application of PCFs in the field of analytical chemistry has been proposed. Conventional PCFs were directly used as capillaries in commercial CE equipment, obtaining acceptable results without the need of a detection window. After, a new optimized design (named SMSC) was also successfully used in the equipment. When the SMSC was compared to the current component in CE (usually a polyimide-coated capillary), improvements such as: a more linear tendency, closer-to-ideal asymmetries and higher S/N ratios were experimented in the range under study, and all without the need of a detection window, needed in the polyimide-coated capillary. When comparing the SMSC to the transparent-coated capillary, improvements were even clearer. These improvements could be of great importance when one comes across complex separations of minimal samples with non-resolved peaks and trace-compounds. It is expected that more precise designs could improve the present results. New designs will be made in order to carry out more comparisons and using more complex samples. We point out that the fabrication tolerances should be very tight as high homogeneity in the inner holes should be indispensable to avoid band broadening.

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