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A microstructured-capillary electrophoresis method for nitrocellulose detection in dynamite

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Abstract

In this article we present a microstructured-capillary electrophoresis method for the determination of minimal amounts of nitrocellulose in dynamite samples. A 6-hole microstructured capillary (MSC) was selected for the method development. First, the hydrodynamic behaviour of the MSC was studied at varying lengths (31, 41 and 51 cm), which had never been studied before. Next, one of the MSC segments (31 cm) was employed to develop a microstructured-capillary electrophoresis method to determine nitrocellulose in dynamite. In a first evaluation, the dynamite derivatization process was investigated to obtain an efficient derivatization yield. Afterwards, the instrumental parameters, more specifically voltage and capillary cartridge temperature, were optimized. Afterwards, the analytical performance of the developed methodology was assessed. Dynamite analyses during a period of 15 days were performed to establish intra-day (10 analyses) and inter-day (40 analyses) precision, obtaining RSDs lower than 6 and 10%, respectively. Besides, RSDs for migration time (<0.5%), peak asymmetry (<3%), resolution (<3.9%) and S/N (<9%) were obtained. Finally, a diluted sample of dynamite was analysed, detecting amounts of nitrocellulose in the order of 220-400 pg.

Keywords: Capillary electrophoresis; dynamite; forensic analysis; hydrodynamic study; microstructured capillary; optical fiber

Introduction

Capillary electrophoresis (CE) is a widely employed analytical technique which presents high versatility. This property leads to a wide variety of instrumental developments **[1]**. On the one hand, the design of portable CE equipment is a current trend, as it would allow the *in situ* analysis, useful in some analytical fields such as environmental or forensic sciences **[2, 3]**. On the other hand, research effort has focused on the enhancement of CE performance. Apart from general improvements in the automation of the equipment, the capillary where the separation takes place can be thoroughly enhanced to improve the CE performance. In conventional CE methods, this component is usually a fused silica tube of ~300 µm outer diameter (od) with a hole between 50-100 µm inner diameter (id) and coated with polyimide (or other polymers like PTFE) which provides the flexibility needed to handle it **[4, 5]**. Focusing on the geometry, the id and od are two important parameters which can be selected depending on the application. Besides, the detection window can be geometrically modified to enhance the CE sensitivity. For example, bubble cell windows, z-shaped or squared-shaped windows have been proposed to enhance CE sensitivity **[4, 5]**. In addition, capillaries containing a bundle of holes (microstructured capillaries, MSCs) might be employed. These structures resemble a type of optical fibre widely employed in telecommunications, named photonic crystal fibre (PCF) **[6]**. Such is the importance of these structures in chemical applications that they have been used in different chemical and biochemical sensing systems. For example, fluorescent dyes such as fluorescein have been detected through solid core-PCF (SC-PCF). By employing 30 cm of SC-PCF, limits of detection LODs of $3 \cdot 10^{-10}$ M were obtained **[7]**. In other recent work, a hollow core-PCF was employed to measure the Raman spectra of proteins and bacteria in aqueous solution, obtaining sensitivity improvements compared to previous works, and detecting amounts of 106 cells·mL^{-1} **[8]**. Other customized MSCs have been developed for sensing purposes. For example, a capillary with 2700 nanoholes supported with gold nanoparticles was used to detect rhodamine 6G, obtaining LODs of 100 fM **[9]**.

However, the implementation of such structures is limited in commercial equipment, and scarce literature is related to this issue. Small segments (<10 cm) of MSCs and PCFs have been used as separation channels in microchips, taking advantage of the higher heat dissipation of these structures compared to single-hole channels, allowing a voltage increase avoiding Joule effect **[10, 11]**. With regard to conventional CE, different structures have been previously used. For instance, a PCF was recently used by our research group as capillary in CE to detect fluorescein and carry out comparative studies with conventional capillaries, showing migration times and peak efficiencies of the MSC similar to those conventional capillaries, whereas peak asymmetries and linearity were improved with the customized MSCs, when injecting low sample volumes **[12]**. More recently, a new MSC consisting of 6 holes was mainly focused to perform a fundamental study on the hydrodynamic injection. Experimental correction factors were proposed to assure known and controlled sample volumes. Advantages over conventional capillaries such as an increase in sensitivity due to noise reduction, and larger sample capacity in terms of volume injected were demonstrated in this study **[13]**.

Up to date, the use of MSCs has only been studied by using short lengths (<30 cm) in CE microchips and home-made equipment, whereas longer lengths have only been tested in two works **[12, 13]**. However, this parameter is a fundamental variable for CE and more research on MSCs in commercial equipment is needed to explore its real applicability. In fact, a real application of a microstructured-CE developed method for complex samples of relevance has not been demonstrated yet. Dynamite is a commercial explosive containing nitrocellulose, a complex macromolecule that has been previously detected by CE-LIF in other explosives such as gunpowders **[15].** Surprisingly, the detection of nitrocellulose in dynamite samples by separation techniques has not been reported, despite being a widely used explosive for civil purposes, and unfortunately also for terrorist attacks **[14]**. Thus, its analysis and detection presents a real analytical and forensic interest.

Considering the above-described facts, this manuscript aims to i) study the hydrodynamic behaviour of MSCs segments, longer than the previous studied in the literature, in order to study the possible deviations from the theoretical apporach; and ii) show the real applicability of MSCs through the development of a method for the determination of minimal nitrocellulose concentrations contained in dynamite samples.

Experimental

Instrumentation, software and microstructrured capillary

A P/ACE MDQ-Beckman CE system equipped with a LIF detector (4-mW Argon-ion laser with excitation at 488 nm and emission filter of 520 nm) from Beckman Coulter Inc. (Fullerton, CA, US) was used for the experiments. CE runs were acquired in the commercial software Karat 32 from Beckman Coulter (Fullerton, CA, US). CE expert, also from Beckman Coulter (Fullerton, CA, US), was used to calculate the sample volumes injected. The cross section image of the MSC was made with a

Professional Compound Microscope equipped with a 9.1 MP digital camera, from AM Scope (Irvine, CA, US).

The MSC was manufactured following the stack-and-draw technique. Details of this technique for PCF fabrication and the materials employed can be found in previous literature **[13]**. **Fig. 1** depicts the images of the in-house designed and fabricated MSC (longitudinal and cross sections) as well as the MSC inserted in the cartridge. A transparent optical fibre coating product no. 3471-2-136 from DeSolite (Elgin, IL, USA) was the polymer used to coat the MSCs. The geometrical characteristics were: ~360 µm od with polymer, ~210 µm od without polymer, and 23-25 µm id of each one of the 6 internal holes. The MSC was directly inserted in the CE cartridge and the polymer in the detection window zone was not removed as LIF wavelength was able to penetrate through this transparent material. The polymer in both ends of the MSC was removed to cut the extremes with a fibre cutter and obtain clean and perpendicular sections.

Reagents and sample preparation

REAGENTS. Solutions were prepared in ultrapure water from Millipore (Bedford, MA, USA). Acetic and formic acids were supplied from Sigma (St. Louis, MO, USA). Hydrochloric acid was obtained from Scharlab (Barcelona, Spain). Sodium hydroxide pellets were from Scharlau Chemie (Barcelona, Spain). All reagents were analytical grade. For the labelling reaction 8-aminopyrene-1, 3, 6-trisulfonic acid (APTS) was purchased from Fluka (St. Louis, MO, USA) while sodium cyanoborohydride (CBH) 1.0 M in tetrahydrofuran was from Aldrich (Milwaukee, WI, USA).

SAMPLE PREPARATION. A sample of dynamite GOMA 2-ECO, kindly provided by the Criminalistic Service of Guardia Civil (Madrid, Spain) was used to select CE conditions and show the applicability of a microstructured-CE method. As previously reported, nitrocellulose-based explosives can be detected by CE-LIF, with no need of extracting the nitrocellulose contained in them. However, a derivatization with a fluorescent reagent (APTS) is needed to successfully detect the nitrocellulose in these samples **[15]**. Considering that nitrocellulose is a minor component of dynamite, this derivatization procedure was selected and studied to derivatize dynamite. Thus, in order to select the procedure that provided the highest derivatization yield, four samples were prepared by modifying the dynamite quantity and water before derivatization, as can be seen in **Table 1**. Fixed volumes of 2 µL of $1 \cdot 10^{-4}$ $mg \cdot mL^{-1}$ APTS solution in acetic acid (15% v/v, pH =2.18) and 2 µL of 1.0 M CBH in THF was added to samples 1, 2, 3 and 4 to carry out the derivatization process. Note that all samples (1-4) were prepared in duplicate. Then, these solutions were protected from light to avoid the APTS decomposition and heated in a thermostatic bath during 4 h at 65 °C to complete the labelling reaction. Finally, different volumes of ultrapure water were added to obtain the same final volume (50 µL) in all the samples, as can be seen in **Table 1**.

Note that the sample under study (dynamite) is an explosive. Despite its considerable stability, its handling required the following safety considerations. Dynamite samples were always stored at 3 °C. Gloves and protection googles were used during the entire sample preparation. Also, samples were prepared and derivatized in an extractor hood.

Parameters for method development

INSTRUMENTAL PARAMETERS. The total length of the MSC (31, 41 and 51 cm) was studied to determine the correction factor needed to perform a controlled injection.

Besides, different capillary temperatures (20, 25 and 30 °C) were set to test the temperature effect. To perform these experiments, the volume of ultrapure water flushed throughout the capillaries was measured after applying different pressures (4, 8, 12, 20 and 40 psi) during periods of 5 minutes. Each pressure was applied in triplicate and SD of the measurements was calculated. Experimental times to flow 400 µL were recalculated and compared to the theoretical values predicted by Poiseuille's equation ($V = \Delta P \cdot d^4 \cdot \pi \cdot t / 128 \cdot \eta \cdot Lt$), where V represents the volume injected in the MSC (nL); ΔP corresponds to the pressure difference between inlet and outlet vials (Pa); d is the fiber channel id (µm); t is the injection time (s); η is the fluid viscosity (Pa·s); and Lt the capillary or MSC total length (µm). Note that for the MSCs, the resulting V was multiplied for the number of channels (6 in the case of the MSC used).

ELECTROPHORETIC PARAMETERS. The shortest segment (31 cm) and sample 2 were selected for the microstructured CE method development to determine nitrocellulose in dynamite samples. Prior to its first use, conditioning the MSC was necessary. NaOH 1M at 40 psi, 35 °C, 20 min (□100 capillary volumes), ultrapure water at 40 psi, 20 °C, 5 min(□16 capillary volumes) and HCl 1M at 40 psi, 20 °C, 10 min (□33 capillary volumes) were flushed before filling the capillary with the electrophoretic buffer. Between runs, the MSC was washed with ultrapure water, HCl 1.0 M, ultrapure water and the electrophoretic buffer at 40 psi during 5 min, each (□16 capillary volumes). Different voltages (-5, -10 and -15 kV) and temperature cartridge (20, 25 and 30 °C) were tested. The following electrophoretic conditions were finally selected. Sample tray and cartridge were thermostated at 15 °C and 20 °C, respectively. A reverse polarity at -10 kV (providing a current of ~51 µA) was set for the separation. pH of the solution was adjusted to 2 to eliminate the electroosmotic flow. Both non-diluted and diluted dynamite samples were injected at 4 psi during 5 s. To evaluate the method precision, 10 analyses of the dynamite sample plus one blank sample were performed each day, during a period of 4 days, comprising 15 days between the first and the last day. Peak intensities of the eight first peaks were recorded and their RSDs were calculated. Migration time and peak asymmetry (for the second peak of the nitrocellulose profile), resolution (between the first and second peak) and S/N ratio (for the eighth peak of the nitrocellulose profile) were also calculated. Finally, sample 2 was diluted by a factor of 100, and the quantity of nitrocellulose in the aliquot injected was calculated, considering the experimental ratio previously obtained.

Results and discussion

Hydrodynamic injection study

As stated in our previous study **[13]**, experimental times to flow a fixed volume did not fit the theoretical values predicted by Poiseuille's equation when using low pressures. These phenomenon was attributed to either an increase in wet perimeter which could lead to a higher resistance of the liquid passing through the 6 holes, or non-homogeneities along the fibre roll. Hydrodynamic injection has never been studied for capillaries longer than 31 cm, thus the first experiment focused on studying the possible deviations from Poiseuille's equation previously reported in the literature **[13]** as a function of capillary length. For this study, different segments of MSC were taken (31, 41 and 51 cm) and experimental times to flow a fixed volume were obtained by applying different pressures. **Fig. 2** shows the three graphics corresponding to the different MSC lengths studied. As can be seen, theoretical and experimental times were similar for the three structures when applying high pressure values. However, experimental results were higher than the predicted values when using low pressures, confirming the deviation previously observed **[13]**. Besides, the experimental ratio, R ($t_{experimental}/t_{theoretical}$) was calculated for each pressure and graphics are in the inset of **Fig. 2**. As can be seen, R ~1 were obtained for high pressures. Nevertheless, for low pressures (e.g. 4 psi) R resulted in 1.44±0.12, 1.24±0.04, and 1.56±0.19, for the 31 cm (**Fig 2.A**), 41 cm (**Fig 2.B**) and 51 cm (**Fig 2.C**) length MSCs, respectively. Higher ratios were expected when increasing MSC lengths, but this tendency was not clearly evidenced. On the contrary, influence of the temperature was also studied (20, 25 and 30 °C), taking the 31 cm long MSC segment, and results (see **Fig. 3**) evidenced that temperature did not influence on the ratios, even at low pressures (R values of 1.20±0.11, 1.14±0.04 and 1. 15±0.20 for 20, 25 and 30 °C . Given these results, the calculation of this ratio is recommended to estimate the real volume injected in the capillary, overall when attempting sample quantification using hydrodynamic injection.

After studying the capillary length, it was decided to select the shortest segment for subsequent analysis, as shorter capillary lengths provide faster separations and lower backpressure to flush the liquid inside the microstructure.

Method development and application

Once the segment had been chosen and the hydrodynamic injection controlled, a microstructured-CE method able to detect minimal nitrocellulose concentrations in dynamite samples was developed. As previously said, the derivatization was the first procedure before analysing the samples. Derivatization temperature was set at 65 °C and two different times (4 and 6 hours) were tested. Derivatization yield did not vary between these times, thus the shortest time was selected. Then, 8 different samples (see **Table 1**) were prepared and derivatized. In this case, different water volumes and dynamite amounts were employed in order to obtain the most appropriate relative amounts which increased the derivatization yield. Derivatization of samples which were subjected to water addition before the process (1 and 3) did not show the typical nitrocellulose profiles previously reported in the literature **[15]**, indicating a poor derivatization yield, thus samples 2 and 4 were selected for subsequent analysis. As there were not appreciable improvements when increasing the amount of dynamite (2 mg or 4 mg), sample 2 (2 mg of dynamite) was taken to carry out the selection of the most appropriate separation voltage and cartridge temperature. Separation voltage (-5, -10 and -15 kV) and capillary cartridge temperature (20, 25 and 30 °C) were studied as CE instrumental variables, and values were selected by comparing visually the resulting electropherograms. A separation voltage of -10 kV provided well-resolved signals without compromising the analysis time, thus it was selected for subsequent analysis. Regarding the capillary cartridge temperature, 20 °C was selected as no peak overlapping was evidenced, whereas for 25 °C and 30 °C peaks comigrated, impoverishing the CE performance. Note that this temperature was set for the cartridge, and real capillary temperature was not calculated. Considering that the electrolyte had low conductivity and the internal diameter of the MSC holes was 25µm, large deviations from the set temperature were not expected, as previously reported in the literature **[16]**. Besides, previous literature demonstrated that separation current for microstructures was similar to conventional capillaries with similar cross section, thus deviations from the microstructure geometry (and therefore also expected currents) were not contemplated **[12]**.

After selecting the most convenient separation voltage and temperature, the method performance was assessed through the calculation of some CE parameters. For this purpose, 10 analyses were made in one day, during 4 non-consecutive days. **Fig. 4** depicts the average electropherograms from one day (**Fig. 4A**) and from the 40 analyses made during the analysis period of 15 days (**Fig. 4B**), with their respective standard deviations (relative to peak intensity) along the electropherogram. Note that APTS signal was removed (from min. 0 to min. 5) to focus on the visualization of the nitrocellulose peaks.

Precision was calculated as RSD values of peak intensities for the height first peaks. Acceptable intra-day precision (10 analyses per day, RSDs lower than 6.4%) and inter-day precision (taking the 40 analysis made during a period of 15 days, RSDs always lower than 10%) were obtained.

In addition to the precision study, other analytical parameters were calculated to show the performance of the method. **Table 2** shows the resulting average values of the electropherogram parameters studied, with their respective RSDs. These values were calculated for the peak showing the highest intensity (second peak of the electrophoretic profile, see **Fig. 4A**). Migration times were similar among days, and RSDs under 0.5 % were obtained in all cases, values even lower than those obtained with conventional capillaries and other MSC prototypes employed in our previous studies **[13]**. Regarding peak asymmetries, values obtained were near the unity. Resolution values were above 6.8 in all the cases, indicating that the two first peaks were well resolved. Finally, S/N ratio values were calculated for the eighth peak (see **Fig. 4A**), obtaining S/N values ranging from 150 to 300, with RSDs from 3 to 9 %, thus assuring at least the visualization of the first eight peaks of the nitrocellulose profile.

Once assessed the method performance, the last experiment focused on the detection of nitrocellulose at minimal levels, which may be of high interest for forensic purposes. Previous fundamental studies on this MSC indicated some important advantages, such as the noise reduction and the larger capacity of volume injected in the MSC **[13]**, which are in fact useful improvements to enhance sensitivity in the pursued application. Therefore, sample 2, which contained 2mg of dynamite, was diluted by a factor of 100 and analysed. **Fig. 5** depicts the resulting electropherogram from this new diluted sample (containing 0.02 mg of dynamite). Bearing in mind the injection conditions (4 psi during 5s), a volume of 4 nL was introduced in the MSC. As these analyses were made with the segment 1 of MSC (31 cm long), the ratio R = 1.44 was considered (See **Fig. 3**). After recalculation, an estimated volume of 3.5 nL was injected into the system. According to the manufacturer, the percentage of nitrocellulose contained in dynamite samples ranged from 3 to 5 %. Taking this into account, the amount of nitrocellulose detected ranged approximately between 250-400 pg.

Therefore, the microstructured CE method developed was able to detect minimal nitrocellulose concentrations contained in dynamite GOMA-2-ECO, being the first time that this compound is detected in dynamite samples without requiring a nitrocellulose extraction, and achieving LODs low enough for real casework.

Conclusions

Regarding the hydrodynamic studies and despite possessing more complex internal structures than conventional capillaries, MSCs can also be employed using longer capillary lengths of at least, 41 and 51 cm. Again, deviation from Poiseuille's equation was observed, but a clear tendency of this deviation as a function of the total length was not conclusive. Due to this reason, this study is recommended prior using MSCs if analyte quantification is desired.

Focusing on its real applicability, the shortest segment (31 cm) was used to successfully determine nitrocellulose in dynamite samples. Through the method development and performance assessment, we have proved that MSCs can be used as capillaries in commercial CE equipment, obtaining reproducible results when analysing samples of high complexity, such as dynamites. The sensitivity of the microstructured CE method has been proved by detecting minimal nitrocellulose concentrations, at pg levels, in dynamite GOMA 2 ECO.

Having demonstrated the use of MSCs for the analysis of complex samples, this study opens up new research on MSCs to develop other microstructured-CE methods, by optimizing the internal geometry and enhance analytical performance of CE.

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Figure Captions

Fig. 1. MSC used in this study. A) longitudinal section; B) cross section without polymer; C) cross section with polymer(in colour on the web only)

Fig. 2. Experimental vs theoretical times to flow 400 µL of pure water at different pressures. Vertical bars depict the SD of each value. Capillary temperature 20°C. A) Lt= 31 cm, i.d. 24.5±0.9 (x6) µm; B) Lt = 41 cm, i.d. 23.7±0.9 (x6) µm; C) Lt= 51 cm, i.d. 23.9±0.5 (x6) µm. (in colour on the web only)

Fig. 3. Ratios ($t_{experimental}/t_{theoretical}$) obtained with different temperatures (20, 25 and 30 °C) using the same 31 cm long MSC fragment. Each line corresponds to a different temperature applied. (in colour on the web only)

Fig. 4. Average electropherograms of dynamite. A) intra-day precision (average from 10 replicates made in the same day); and B) inter-day precision (average from the 40 electropherograms made during a period of 15 days). SD for both average electropherograms also depicted (considering the SD of peak intensities). Derivatization conditions described in the experimental section. Electrophoretic conditions: buffer, 1.0 M Formiate buffer (pH 2.0); Capillary temperature, 20 °C; Sample temperature, 20 °C; Separation voltage, -10 kV; LIF detection at 488 nm (λex) and 520 (λem). MSC dimensions, Lt, 31 cm (effective length, 21 cm); id, 24.5±0.9 µm, 6 holes. (in colour on the web only)

Fig. 5 Complete electropherogram of dynamite 2 diluted by a factor of 100. Derivatization conditions described in the experimental section. Electrophoretic conditions, as indicated in Fig. 4. (in colour on the web only)

Table 1. Optimization of the sample preparation. Dynamite (mg) and volume of reagents (in µL) added to study the efficiency of the derivatization process.

Sample[1]	Nitrocellulose (mg)	H_2O[2] (µL)	APTS (µL)	CBH (µL)	H_2O[3] (µL)	Final Volume (µL)
1	2	10	2	2	36	50
2	2	-			46	
3	4	10			46	
4	4	-			36	

[1] Each dynamite sample was prepared in duplicate.

[2] Water added before the derivatization process.

[3] Volume of water added after the derivatization.

Table 2. Average values of the analytical parameters obtained for each day in the analysis of dynamite (sample 2).

Day[a]	Mig. Time (min) [b]	Asymmetry [b]	Resolution [b]	S/N [c]
1	5.6 (0.1)	0.97 (2.3)	6.8 (3.9)	288 (9)
2	5.4 (0.3)	1.01 (3.0)	7.7 (0.1)	160 (3)
3	5.4 (0.5)	1.06 (2.6)	7.8 (3.2)	157 (3)
4	5.6 (0.2)	1.02 (2.2)	7.02 (1.0)	199 (3)

[a] Each value corresponds to the average value from 10 replicates under the same conditions (RSDs indicated in parenthesis). [b] Calculated for the second peak from the electrophoretic profile of nitrocellulose. [c] Calculated for the eighth peak of the electrophoretic profile of nitrocellulose.

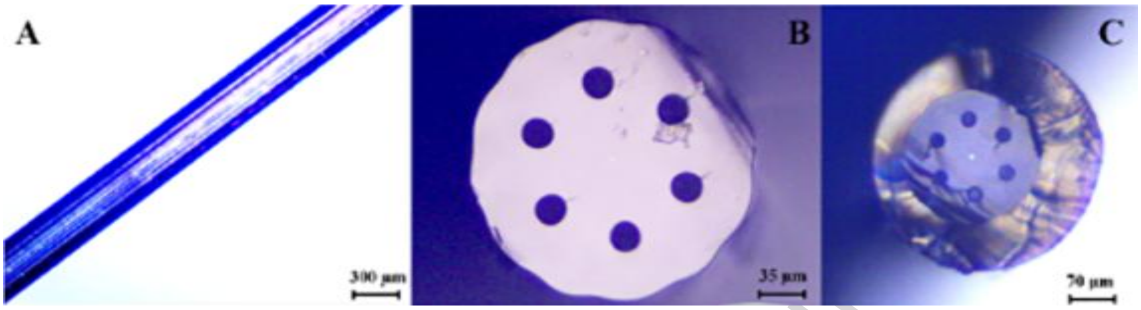
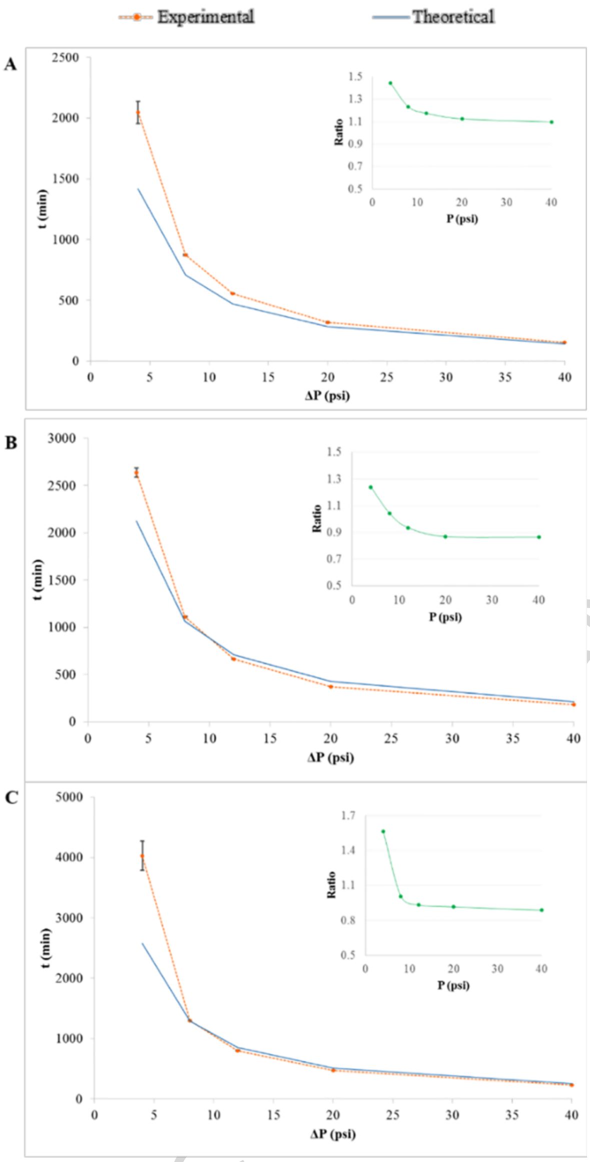


Figure 1


Experimental
Theoretical
A
t (min)
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2000
1500
1000
500
0
0
5
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40
ΔP (psi)
Ratio
1.5
1.3
1.1
0.9
0.7
0.5
P (psi)
B
3000
2500
2000
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C
5000
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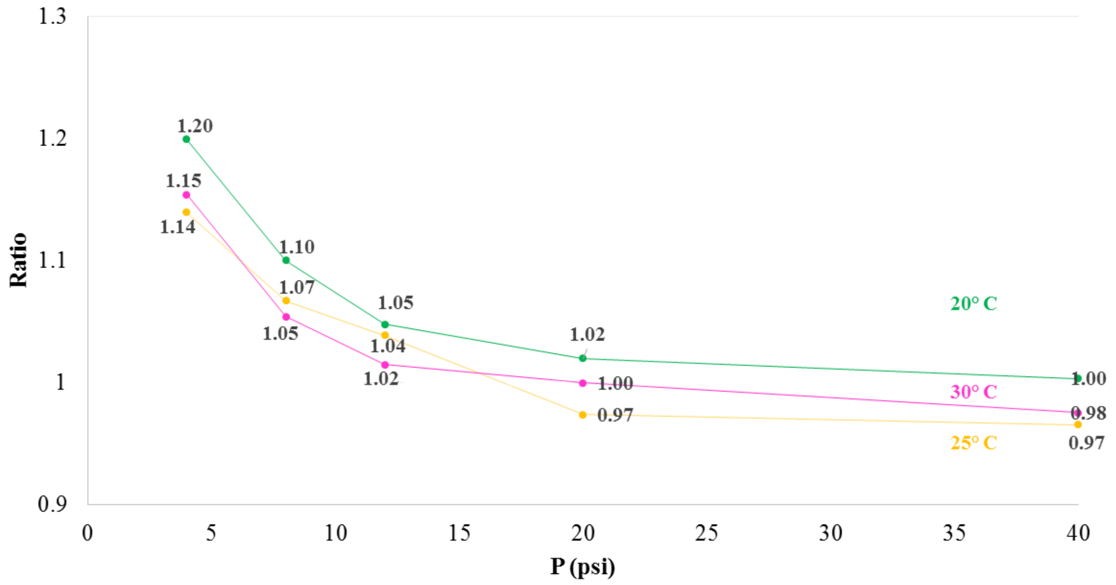
2000
1000
1.7
1.4
1.1
0.8
0.5

Figure 2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1.2
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1
0.9
0
5
10
15
20
25
30
35
40
P (psi)
1.20
1.15
1.14
1.10
1.07
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1.05
1.04
1.02
1.02
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0.97
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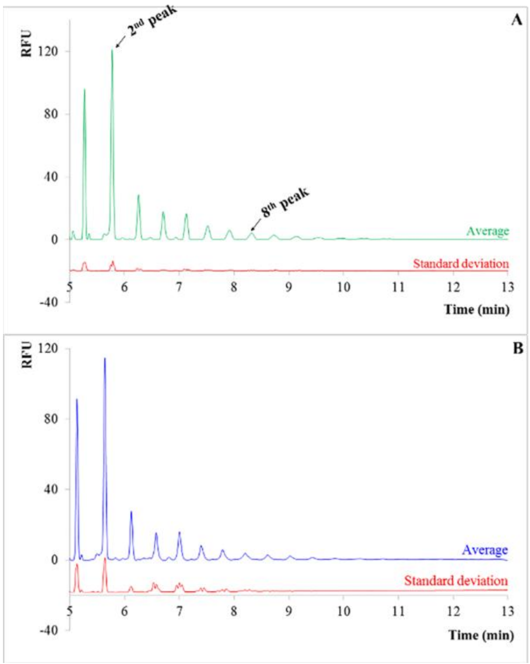
30° C
25° C

Figure 3


A
RFU
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0
-40
2nd peak
8th peak
Average
Standard deviation
5
6
7
8
9
10
11
12
13
Time (min)
B
RFU
120
80
40
0
-40
Average
Standard deviation
5
6
7
8
9
10
11
12
13
Time (min)

Figure 4

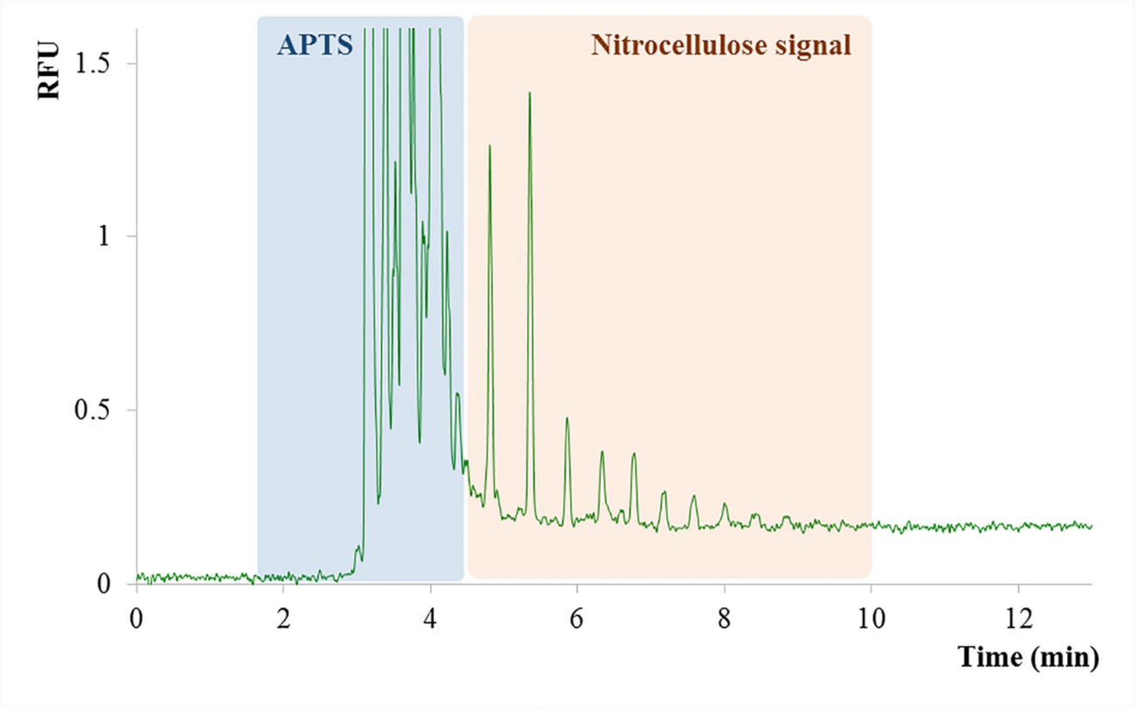

Figure 5

Highlights

- A microstructured capillary is employed to develop a method for dynamite analysis
- Hydrodynamic studies are recommended to calculate real volumes injected
- Method was developed in terms of derivatization and CE instrumental parameters
- Nitrocellulose at pg levels was detected in dynamite samples
- A real forensic application is demonstrated for future casework