



Determination of the nitrogen content of nitrocellulose by capillary electrophoresis after alkaline denitration

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ABSTRACT

Nitrocellulose (NC) is a nitrate cellulose ester polymer whose nitrogen content determines its physical and chemical properties and its industrial applications. For the first time, capillary electrophoresis (CE) was used to quantify nitrite and nitrate ions released after the alkaline hydrolysis of NC to determine its nitrogen content. This article focuses on the development and validation of the CE method adapted to the determination of these anions in basic matrices in 3 min total runtime. Molybdate anion was used as internal standard. The matrix effect in sodium hydroxide solution was statistically studied, demonstrating that no significant effect occurred in 0.5 M sodium hydroxide solution. RSDs on normalized migration times ($n=20$) were 0.05% for both anions, while RSDs on normalized corrected areas ($n=20$) were 0.8% and 0.7% for nitrite and nitrate ions, respectively. The selectivity of the CE method was checked in the presence of 10 other anionic species potentially present in post-blast residues. Excellent linearities for normalized corrected areas ($R^2 > 0.999$, residual standard deviations of ca 0.05) were obtained for both anions in the range 5–100 mg L⁻¹. Finally, the optimized CE method was successfully applied to calculate the denitration yields of two NC standards belonging to the non-explosive and explosive classes, and to determine the nitrogen content of a NC contained in a single-base gunpowder. CE, with its speed, low running costs, and simplicity of use, appears as a valuable alternative to ion chromatography for the nitrogen content determination of pure NCs and NC-based explosives, and more generally, for the determination of nitrite and nitrate ions in other highly alkaline matrices.

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1. Introduction

Cellulose nitrate, commonly known as nitrocellulose (NC) is a cellulose ester polymer prepared by the nitration of cellulose, a natural polymer composed of β -1,4 anhydroglucose units. Each dehydrated glucose monomer of cellulose has three reactive hydroxyl groups which are available for esterification [1,2]. Following this esterification reaction, hydroxyl groups of cellulose are replaced by nitro groups. This reaction is realized using various ratios of nitric and sulfuric acids, and cellulose. The acidic medium and the source of cellulose used for synthesis influence the degree of substitution (DS)

and therefore the nitrogen content of the final NC. Theoretically, the maximum nitrogen content which can be achieved is 14.14%. However, in practice, the nitrogen content cannot exceed 13.8% [1,2]. Fully nitrated cellulose having a DS of 3 cannot be synthesized because, before completion of this reaction, unstable compounds such as sulfuric esters of cellulose are formed [2].

Physical and chemical properties and industrial application of NC depend on its nitrogen content. Indeed, NCs with a nitrogen content less than 12.5% are widely used as raw material in daily use products (printing inks, paints, lacquers, varnishes and filter membranes), whereas highly-nitrated NCs (nitrogen content > 12.5%) are employed in the manufacturing of explosive materials such as smokeless gunpowders and dynamites. Therefore, and also for regulation reasons, methods for the determination of nitrogen content in NCs are needed. To date, the reference method for this determination is still the long and tedious Devarda's method, based on alkaline denitration of NC in the presence of hydrogen peroxide, reduction of the released nitrate ions into ammonia by Devarda alloy, and back-titration of sulfuric acid

Abbreviations: BGE, background electrolyte; CE, capillary electrophoresis; DBP, dibutylphthalate; DNT, dinitrotoluene; DPA, diphenylamine; DS, degree of substitution; EOF, electroosmotic flow; HDMB, hexadimethrine bromide; IC, ion chromatography; LOD, limit of detection; LOQ, limit of quantitation; NC, nitrocellulose

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added in excess to ammonia by sodium hydroxide [3]. However, for direct NC analysis, Vibrational spectroscopy [4,5], mass spectrometry [6–8], gas chromatography [9–13], reversed phase [14,15], ion [16–19] and size exclusion [20,21] chromatography, and capillary electrophoresis [22,23] have all been used. NCs are always present in gunpowders, which are commonly classified according to the number of energetic materials in their formulation: (i) single-base propellants, which mainly contain NC, (ii) double base propellants, consisting of NC and nitroglycerin, and (iii) triple base propellants, containing NC, nitroglycerin, and nitroguanidine [24,25]. Various additives such as stabilizers, flash suppressants, deterrents, plasticizers, and inert material are added to enhance performance. Although NC is the main constituent of gunpowders, few studies focusing on the analysis of NC in NC formulations are available in literature, probably because of difficulties encountered in sample treatment.

As NCs are widely used in industry, large quantities of unusable or waste NCs are generated and have to be destroyed by inexpensive, efficient, and environmentally suitable processes. Various chemical and biological pathways have been used to remove NCs [26,27]. Better treatment efficiencies are obtained when both pathways are combined [28–30]. Unlike cellulose, NC is very resistant to microbial degradation due to the conformational rigidity of the molecule [18]. The chemical processes are based on alkaline or acid hydrolysis. However, acid denitration of NC is much slower than alkaline hydrolysis [29]. The reaction between NC and an alkaline agent leads to denitration and breakdown of the structural backbone of cellulose. Following this reaction, various organic and inorganic products are released [28]. Among the post-hydrolysis products, nitrite and nitrate ions are the main compounds containing nitrogen. Previous works considered the effect of temperature, reaction time, type and concentration of bases on the denitration yield [17,18,28,29,31,32]. Results showed that NaOH and KOH at comparable concentration levels are more effective than $\text{Ca}(\text{OH})_2$ and NH_4OH [17,18,32]. Alkaline denitration without hydrogen peroxide has also been used as an analytical route to determine NC nitrogen contents [18], as it is likely safer to control than in the presence of that. So far, in all of these studies, nitrite and nitrate concentrations were monitored by ion chromatography (IC).

Capillary electrophoresis (CE) is commonly employed for inorganic ion analysis, as a simple alternative to IC, particularly in environmental field. Especially, CE has already been reported for the determination of nitrite and nitrate ions [33–45]. As the absolute mobilities of both anions are rather close, acidic background electrolytes are preferably used, to take benefit from the weakly acidic properties of nitrite anion. As compared to IC, the use of CE for the analysis of nitrite and nitrate ions presents advantages such as simpler sample preparation, shorter analysis times, miniaturized operational conditions, and much lower running cost.

This paper focuses on the development of a new CE method adapted to the simultaneous analysis of nitrite and nitrate ions contained in basic matrices such as those originating from the alkaline hydrolyses of NCs. This method was successfully optimized with hydrolyzed NC standards. For the first time, it was then applied to NC extracted from a gunpowder to determine its nitrogen content. Applications of this to the determination of nitrite and nitrate anions in other basic matrices can also be contemplated.

2. Materials and methods

2.1. Standards and electrolytes

NC standards with nitrogen contents of 11.14% (NC1, $\text{DS}=2.0$, $M_w=20,000 \text{ g mol}^{-1}$) and 13.42% (NC2, $\text{DS}=2.8$, $M_w=69,000 \text{ g mol}^{-1}$) were given by the Central Laboratory of Police Prefecture

(Paris, France). NC standards were received with a damping agent (water for NC2 or ethanol for NC1) content of approximately 30%. Sodium nitrite (>97%), sodium nitrate (>99%), sodium perchlorate (99%), sodium chlorate (99%), sodium chloride (>99.5%), sodium iodide (99.5%), sodium bromide (99%), oxalic acid (99%), hexadimethrine bromide (HDMB) used as electroosmotic flow (EOF) reversal agent, and sodium molybdate dihydrate ($\geq 99.5\%$) used as internal standard were supplied by Sigma-Aldrich (Saint-Quentin-Fallavier, France). Sodium sulfate (>99.5%) and sodium thiocyanate ($\geq 98.5\%$) were supplied by Merck (Fontenay-sous-Bois, France). Concentrated volumetric solutions (ConvL Normadose[®]) of 1 M sodium hydroxide used as base for NC hydrolysis were purchased from VWR (Fontenay-sous-Bois, France).

Stock solutions at 1000 mg L^{-1} of nitrite, nitrate, and molybdate ions were prepared weekly by dissolving the appropriate amount of sodium salt in ultra-pure water delivered by a Direct-Q3-UV system (Millipore, Molsheim, France) and stored at 4°C . HDMB solution at 1 g L^{-1} was prepared weekly by dissolving 0.01 g in 10 mL of ultra-pure water and stored at 4°C . Sodium phosphate monobasic (>99%) and sodium phosphate dibasic (>99%) from Sigma Aldrich were used for electrolyte preparations. Stock solution of 77.4 mM (200 mM ionic strength) phosphate buffer was prepared by mixing the appropriate amount of sodium phosphate monobasic and sodium phosphate dibasic in ultra-pure water. Background electrolytes (BGE) were prepared daily by dilution from the stock solution at 200 mM ionic strength and filtered through a $0.20 \mu\text{m}$ cellulose acetate membrane (VWR).

2.2. Instrumentation

An oven (model UNB100) from Memmert (Schwabach, Germany) was used to remove damping agent from NC samples. Alkaline hydrolysis experiments were achieved with a block heater (model SBH 130D) from Stuart (Staffordshire, UK) capable of receiving twelve 10-mL glass tubes.

Two capillary electrophoresis instruments were employed in this study. An Agilent HP³DCE system (Massy, France) equipped with a photodiode array (PDA) detector was used for the development of the CE method and a Beckman Coulter[®] P/ACE MDQ system (Beckman Coulter, Villepinte, France) equipped with a PDA absorbance detector was used for the validation of CE method and real samples analysis. Instrument control and data acquisition were performed using Chemstation B.02.01 (Agilent) and 32 Karat[®] 8.0 (Beckman Coulter) softwares.

2.3. Electrophoretic procedures

Polymicro Technologies bare fused-silica capillaries purchased from Photonlines (Marly-Le-Roi, France) with an inner diameter of $50 \mu\text{m}$ and an outer diameter of $365 \mu\text{m}$ were used throughout this study. Conditions retained as optimum on Beckman instrument for subsequent validation were as follows. New capillaries were conditioned by successive flushes with 1 M NaOH, 0.1 M NaOH, ultra-pure water, HDMB solution at 1 g L^{-1} and finally the BGE, each under 14.5 psi ($\sim 1 \text{ bar}$) for 10 min (15 capillary volumes for a 60 cm long capillary). The optimized BGE consisted of 38.7 mM (100 mM ionic strength) sodium phosphate buffer at pH 7.7. Between each run, the capillary was flushed with HDMB solution under 14.5 psi for 1 min (ca 1.5 capillary volumes) followed by the BGE under 14.5 psi for 2 min (ca 3 capillary volumes). Samples were introduced into the capillary by hydrodynamic injection for 3 s at 0.5 psi ($\sim 35 \text{ mbar}$) ($< 0.3\%$ of capillary volume). The selected voltage was -30 kV (reversed polarity) and the capillary cartridge was thermostated at 25°C . The detection wavelength

was 210 nm with the reference set at 310 nm and bandwidths set at ± 10 nm and ± 40 nm. The rate of data collection was 8 Hz. After the last analysis of the day, capillary was rinsed with water under 14.5 psi for 10 min and stored overnight in the apparatus with both ends dipped in water.

2.4. Alkaline hydrolysis of NC standards

Before hydrolysis, to remove wetting agent, about 40 mg of wetted NC were placed in weighted 10-mL glass tubes and dried at constant weight in an oven at 100 °C for 2 h. Next, tubes containing dry NC were stored for 25 min in a glass desiccator to reach room temperature. The glass tubes containing dry NC were weighed again to obtain the exact amount of NC used for the assays of alkaline hydrolysis. Then, five milliliters of a fixed concentration of sodium hydroxide solution were carefully added into each tube containing NCs and the tubes were screw-capped. The stopped tubes were placed in the block heater to allow hydrolysis to proceed for a fixed time at a fixed temperature within the studied ranges (1–4 h, 60–90 °C). When the experiment was over, the tubes were transferred to an ice bath for 15 min to quickly cool down the reaction mixture and thus stop denitration. After 25 min at room temperature, 250 μ L of the hydrolysates were transferred into volumetric flasks of 10 mL containing 200 μ L of the internal standard solution at 1000 mg L⁻¹ and supplemented to the mark with ultra-pure water. Finally, the hydrolysates were analyzed by CE to determine nitrite and nitrate ion concentrations. A filtration step was not necessary because the fiber dissolution was total.

2.5. Single-base gunpowder extraction and alkaline hydrolysis

A single-base gunpowder (containing NC, diphenylamine (DPA), dibutylphtalate (DBP), dinitrotoluene (DNT), potassium sulfate, calcium carbonate, and graphite) was provided by the Central Laboratory of Police Prefecture (Paris, France). Prior to hydrolysis, the single-base gunpowder was subjected to solvent extractions to isolate NC from the sample matrix, applying a protocol that was adapted from [24]. Fig. 1 summarizes the useful steps to isolate NC from single-base gunpowder. About 0.2 g of gunpowder was introduced into a glass beaker. Then, 5 consecutive solvent extractions were needed to remove organic and ionic components: three extractions with methanol (18 mL, sonication for 10 min at 35 °C) and two extractions with water (18 mL, sonication for 10 min at 35 °C). Most of the solid NC was recovered after decantation, divided approximatively equally into three previously weighted 10-mL glass tubes, and dried at constant weight in an oven at 100 °C for 2 h. After the drying step, the glass tubes containing dry NC were weighed again to get the exact amount of NC used to implement alkaline hydrolysis. Next, 5 mL of 3 M sodium hydroxide solution were added into each tube and the tubes were screw-capped. The glass tubes were placed in the block heater to allow hydrolysis to proceed for 2.5 h at 75 °C. At the end of the hydrolysis, the tubes were transferred in an ice bath for 15 min to quickly cool down the reaction mixture and thus stop denitration. After 25 min at room temperature, 2500 μ L of the hydrolysates were transferred into volumetric flasks of 10 mL containing 200 μ L of

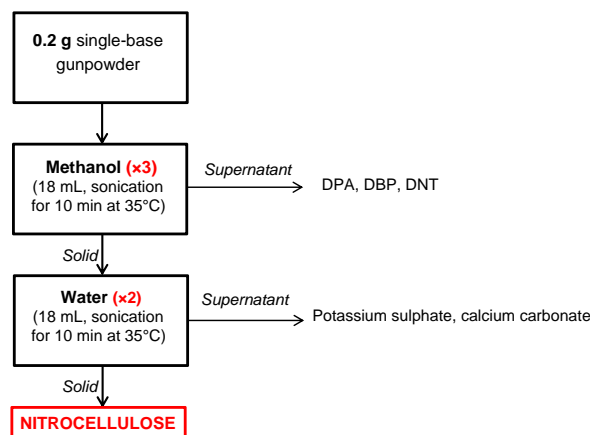


Fig. 1. Diagram summarizing the different steps to extract NC from single-base gunpowders composed of NC, dinitrotoluene, diphenylamine, dibutylphtalate, salts (potassium sulfate and calcium carbonate), and graphite.

the internal standard solution at 1000 mg L⁻¹, and supplemented to the mark with ultra-pure water.

2.6. Safety considerations

NC in dry state is a highly flammable solid that ignites easily and burns explosively when is concealed. This is why it should always be stored wet. NCs having nitrogen content higher than 12.5% are classified as explosives. NC can be ignited by flame, heat, shock, friction, sparks or static electricity. Its explosive propensity depends on the nitrogen content. The higher the nitrogen content, the more easily it can explode. Special attention was paid to keep NC samples wet when stored in a refrigerator and to weigh them after drying in the desiccator to avoid any shock. Security glasses and protection clothes were worn by the operator. In addition, weighing of dry matter was always performed under the assistance of a person equipped with a water vaporizer to prevent from any fire start. Working under less than 30 mg dry matter considerably reduces hazard risks. Indeed, it is worthy to note that no incident has occurred over more than 100 assays that have been realized to date under these experimental conditions.

2.7. Calculation of the denitration yield of alkaline hydrolysis of NC standards

Quantitation of nitrite and nitrate ions in the post-hydrolysis solution was performed by the external standardization calibration method with aqueous standard solutions of nitrite and nitrate ions having concentration from 5 to 100 mg L⁻¹ (5 concentration levels). Molybdate anion was used as internal standard. Analyses were carried out in triplicate and the calibration curves were obtained by plotting normalized corrected areas (analyte peak area divided by its migration time and by the internal standard peak area divided by its migration time) against concentration and using the least-squares regression method. The denitration yield was calculated from nitrite and nitrate concentrations, the nitrogen content given by the manufacturer, and the exact amount of dry NC used for the assay of alkaline hydrolysis, according to Eq. (1):

$$\text{Yield} = \frac{\% N_{\text{experimental}}}{\% N_{\text{manufacturer}}} = \frac{([\text{NO}_2^-]fM_N V_{\text{NaOH}}/M_{\text{NO}_2} \times 10^6 + [\text{NO}_3^-]fM_N V_{\text{NaOH}}/M_{\text{NO}_3} \times 10^6)}{\% N_{\text{manufacturer}} \times m_{\text{NC}}} \times 100 \quad (1)$$

where %N experimental is the nitrogen content obtained experimentally, %N manufacturer the nitrogen content given by the manufacturer, $[\text{NO}_2^-]$ and $[\text{NO}_3^-]$ the concentrations of nitrite and nitrate ions in mg L^{-1} , f the dilution factor, M the molecular mass, V_{NaOH} the volume of sodium hydroxide in mL and m_{NC} the amount of dry NC in gram.

2.8. Calculation of the nitrogen content of NC contained in gunpowder

The nitrogen content of NC contained in the single-base gunpowder was calculated according to Eq. (2):

$$\% N_{\text{experimental}} = \frac{\left(([\text{NO}_2^-] f M_{\text{N}} V_{\text{NaOH}}) / M_{\text{NO}_2} \times 10^6 \right) + \left(([\text{NO}_3^-] f M_{\text{N}} V_{\text{NaOH}}) / M_{\text{NO}_3} \times 10^6 \right)}{m_{\text{NC}} \times \eta} \times 10^4 \quad (2)$$

where $[\text{NO}_2^-]$ and $[\text{NO}_3^-]$ were the concentrations of nitrite and nitrate ions in mg L^{-1} , f the dilution factor, M the molecular mass, V_{NaOH} the volume of sodium hydroxide in mL, m_{NC} the amount of dry NC in gram and η the denitration yield.

2.9. Statistical approach of matrix effects

To study matrix effects, five repeated injections for five concentration levels (5, 10, 20, 50, and 100 mg L^{-1}) of nitrite and nitrate ions in ultra-pure water for the standard calibration and three repeated injections at five concentration levels (5, 10, 20, 50, and 100 mg L^{-1}) in sodium hydroxide at 0.5 mol L^{-1} were carried out. The statistical comparison of the regression lines (residual variances, slopes, intercepts) was performed applying appropriate tests using Excel[®] software (Microsoft Corporation, Courtaboeuf, France).

3. Results and discussion

3.1. CE method development

Various methods for the determination of nitrite and nitrate ions by CE have been developed [33–45]. Among these, some of them involved the use of low pH electrolytes. Indeed, both anions have very close absolute mobilities (74.6 (nitrite) and 74.1 (nitrate) $\times 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ at 25 °C [46]) and the use of low pH electrolytes can selectively decrease the mobility of nitrite ions due to its weakly acidic property ($\text{pK}_a=3.22$ at 25 °C [46]), thus improving the resolution of this pair of ions, in the migration order of nitrate first, nitrite next. For the purpose of this work and in spite of the fact that nitric and nitrous acids are completely dissociated at pH higher than 5.3, acidic electrolytes were not advocated owing to the highly basic sample matrices of NC hydrolysates. Sodium phosphate buffer was selected as BGE for its high UV transparency and its buffering capacity at pH close to its pK_a (7.2 at 25 °C) at a constant pH value of 7.7. Separations were performed at a pH value of 7.7 to promote the presence of monohydrogen phosphate ion (absolute mobility, $61.5 \times 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ at 25 °C [46]) with respect to dihydrogen phosphate ion (absolute mobility, $35.1 \times 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ at 25 °C [46]), and thus better adapted co-ion mobility to analyte mobilities, resulting in improved peak symmetries. The effect of BGE pH was not investigated around this pH value, as it was expected not to influence selectivity, because of the full dissociation of nitric and nitrous acids. To significantly reduce the analysis time, EOF

was reversed using a dynamic coating of the capillary by hexadimethrine bromide, so that nitrite and nitrate ions will migrate in the same direction as the EOF. Method development was performed with the Agilent instrument. Capillary length was varied within the range of from 35 to 50 cm and BGE ionic strength within the range of from 10 to 100 mM, in order to find out baseline separation while keeping short analysis time. Eventually, a resolution of 2.8 between nitrite and nitrate ions (in that order) was achieved in less than 2.5 min using a 60 mM ionic strength phosphate buffer, pH 7.7, with a 50 cm long capillary (41.5 cm to the detector) and the temperature set at 25 °C. The maximum voltage that can be applied under these conditions without noticing detrimental effect of Joule heating was –16 kV. Samples

were introduced into the capillary by hydrodynamic injection for 3 s under 35 mbar, which represents a sample volume of the order of 0.3% of capillary volume, preventing band spreading. Before each analysis, the capillary coating layer was regenerated by applying a rinse step with a HDMB solution at 1 g L^{-1} under 960 mbar for 2 min. This markedly improved migration time repeatability (see Section 3.2.5). Maximum absorbances for nitrite and nitrate ions were obtained at 210 and 202 nm, respectively. As hydroxide ion, which was present at high concentration in the targeted sample matrices, strongly absorbs below 205 nm, a single detection wavelength, 210 nm, was preferred for the determination of both ions of interest. Among contemplated anions meeting mobility and absorbance requirements for use as internal standard (molybdate, thiocyanate and oxalate), molybdate was selected, as it was the only one not being potentially encountered in post-blast residues.

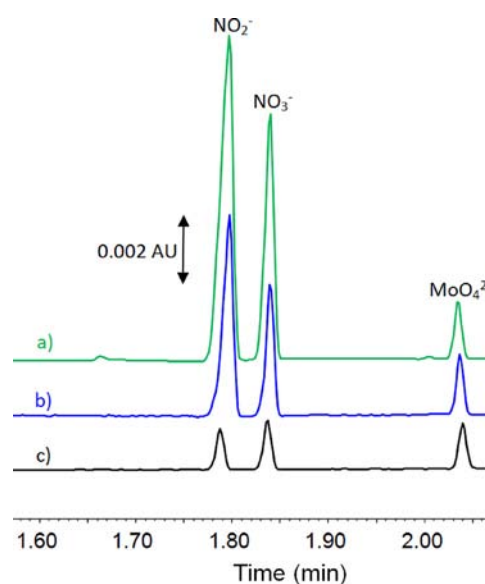


Fig. 2. Electropherograms of (a) a standard mixture of nitrite, nitrate, and molybdate ions at 5 mg mL^{-1} in water, (b) an alkaline hydrolysate of NC2 standard diluted 40 times with water and spiked with MoO_4^{2-} at 20 mg mL^{-1} , and (c) an alkaline hydrolysate of gunpowder extract spiked with MoO_4^{2-} at 20 mg mL^{-1} and diluted 4 times with water. CE conditions: bare-fused silica capillary, 50 μm ID \times 60 cm (detection at 50 cm); electroosmotic flow reversal with 1 g L^{-1} HDMB; BGE: 100 mM ionic strength sodium phosphate buffer, pH 7.7; temperature: 25 °C; applied voltage: –30 kV; hydrodynamic injection, 0.5 psi, 3 s; detection: UV at 210 nm. Hydrolysis conditions: 3 M NaOH for 2.5 h at 75 °C.

Table 1

Linear regression calibration parameters for nitrite and nitrate ions for five concentration levels between 5 and 100 mg mL⁻¹. Experimental conditions: see Fig. 2.

Parameter	Ions	
	Nitrite	Nitrate
Slope ($b \pm s_b$)	0.0989 ± 0.0007	0.1118 ± 0.0006
Intercept ($a \pm s_a$)	0.0742 ± 0.0345	0.1005 ± 0.0320
R ²	0.9998	0.99991
Residual standard deviation	0.0532	0.0492

For routine analysis, the method was transferred from the Agilent to the Beckman apparatus, in view of method transfer to the laboratory of police (LCPP). Capillary length and BGE ionic strength were adjusted to keep comparable resolution and analysis time. The best final separation conditions were found to be 100 mM ionic strength phosphate buffer at pH 7.7 with a 60 cm long (50 cm to the detector) capillary, thus affording still higher buffering capacity with respect to highly alkaline matrices. The applied voltage could be raised to -30 kV, thanks to more efficient liquid cooling of this apparatus. The length of the detection window was 800 μm. Other parameters were kept unchanged. A typical electropherogram of a standard mixture of nitrite and nitrate ions, along with the internal standard (molybdate anion), is depicted in Fig. 2a. As can be seen, the whole separation, including molybdate ion, is completed within 2 min, which is much faster than IC (ca 14 min [18]).

3.2. Method validation

3.2.1. Linearity

Calibration curves were obtained using 5 standard solutions prepared in ultra-pure water in concentrations ranging from 5 to 100 mg L⁻¹. This concentration range allows the determination of both ions in the NC hydrolysates obtained from NC standards (diluted 40 fold before analysis) or real formulated samples. The linearity of the method was evaluated by plotting normalized corrected areas against analyte concentration and using the least-squares method. Concentrations and normalized corrected areas correlated linearly in the studied range, as testified by correlation coefficients, and residual standard deviations given in Table 1.

3.2.2. Limits of detection (LOD) and quantitation (LOQ)

The LODs in standard mixtures were evaluated as a concentration of nitrite and nitrate ions corresponding to a signal to noise ratio of 3. LODs of 1.1 and 0.8 mg L⁻¹ were obtained for nitrite and nitrate ions, respectively. The limits of quantitation (LOQs) in ultra-pure water, based on a signal-to-noise ratio of 10 were 3.5 and 2.5 mg L⁻¹ for nitrite and nitrate ions, respectively. These values were sufficiently low for the determination of nitrite and nitrate ions in the NC hydrolysates. This is why no attempt was undertaken to lower them further.

3.2.3. Matrix effects

To evaluate a possible influence of basic matrices on quantitation, the calibration curves obtained from least-squares method with standard solutions prepared both in ultra-pure water and in 0.5 M sodium hydroxide were statistically compared. This concentration was deemed high enough taking into account the dilution factor of 40 that can be applied to NC hydrolysate samples, which would make this diluted basic matrix consistent with an initial sodium hydroxide concentration of 20 M. It is very likely that this concentration largely outpaces those to be used to hydrolyze NCs.

The setup of this comparison includes three successive steps: (i) the residual variances of the regressions must be homogeneous (comparison using a bilateral *F*-test), (ii) if the residual variances of the regression are homogeneous, the slopes of the regression lines are compared (bilateral *t*-test using the pooled standard-deviation of the slopes as denominator) and (iii) if the slopes are equals, the intercepts are compared (bilateral *t*-test using the pooled standard-deviation of the intercepts as denominator). Then, the two calibration curves are considered as comparable only if residual variances, slopes, and intercepts of the regression lines are not significantly different. All statistical tests were performed with a first-kind risk set at 5%. No significant matrix effect was observed for both nitrite and nitrate ions at this level of hydroxide ion concentration. So, it can be concluded that calibration curves obtained in pure water can be used to quantify nitrite and nitrate ions in the alkaline hydrolysates of NCs. These conditions may also be of interest for other applications in basic matrices.

3.2.4. Selectivity

The method was developed with a mixture containing only nitrite and nitrate ions plus molybdate ion, used as internal standard. Common post-blast residues potentially contain other anions that may interfere with the separation and detection of nitrite and nitrate ions [47]. Therefore, bromide, iodide, thiocyanate, cyanate, oxalate, azide, chloride, sulfate, perchlorate, and chlorate ions were tested at the 10 mg L⁻¹ level. From these, only bromide, iodide, azide, oxalate, and thiocyanate absorb at 210 nm and can therefore be detected. In addition to this, it was checked the presence of the UV-transparent anions (cyanate, chloride,

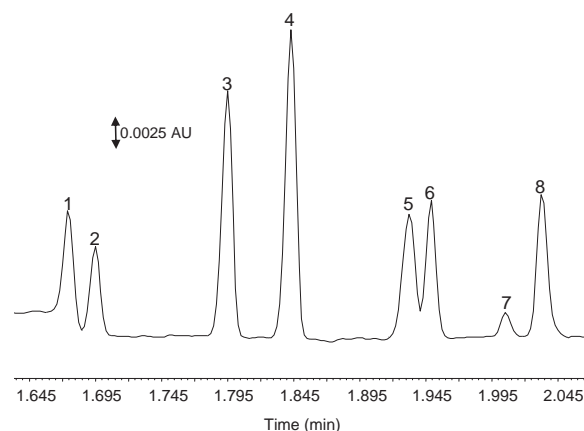


Fig. 3. Electropherogram of a standard mixture of nitrite, nitrate, and molybdate ions + 10 supplementary anions, potentially found in post-blast residues, all at 10 mg mL⁻¹ in water. CE conditions: see Fig. 2. Identification: bromide (1), iodide (2), nitrite (3), nitrate (4), azide (5), oxalate (6), thiocyanate (7), and molybdate (8). Cyanate, chloride, sulfate, perchlorate, and chlorate are not detected.

Table 2

Repeatabilities on migration times, normalized migration times, corrected peak areas, and normalized corrected areas for a standard mixture of nitrite, nitrate, and molybdate ions at 20 mg L⁻¹ each in 0.5 M sodium hydroxide. Molybdate ion was used as internal standard to normalize peak areas and migration times. Experimental conditions, see Fig. 2.

RSD (%) (n=20)	Ions	
	Nitrite	Nitrate
Corrected areas	3.1	3.1
Normalized corrected areas	0.8	0.7
Migration times	0.3	0.3
Normalized migration times	0.1	0.1

sulfate, perchlorate, and chlorate) neither perturb the baseline at the migration time of the three anions of interest, nor alter their peak areas. Fig. 3 presents the resulting electropherogram of a standard mixture of the 13 anions. Eventually, none of these added anions interfered with the separation of nitrite, nitrate, and molybdate ions, showing the good selectivity of the CE method. The advantage of using direct UV detection, which leaves many potentially interfering anions undetected, over indirect UV detection of classical CE methods is clearly emphasized here.

3.2.5. Repeatability

Repeatabilities on migration times, normalized migration times, corrected peak areas and normalized corrected areas are given in Table 2 for a standard mixture of nitrite, nitrate, and molybdate ions at 20 mg L⁻¹ each in 0.5 M sodium hydroxide. Satisfactory results were obtained, with RSDs on normalized corrected areas less than 1%. These data also show the interest of using the internal standard method to compensate for migration speed and injected volume variations. It is also worthy of note that RSDs on corrected areas higher than 5% were obtained in the absence of a refreshment of the capillary coating between each injection (see Sections 2.3 and 3.1), although HDBM is usually considered in the literature [48] as a semi-permanent coating agent.

3.3. Application to real samples

3.3.1. Hydrolysis of NC standards

The method previously validated was then applied to the determination of nitrogen contents of NC standards. Based on the results published in literature, sodium hydroxide concentration, reaction time and temperature have significant effects on the NC denitration yield [17,18,26–29]. To get the highest and most reproducible denitration yields, the effects of these three parameters on denitration yields were studied. The extreme values for each of the three parameters were selected according to the data obtained in literature and preliminary hydrolysis studies. For sodium hydroxide, the low and high concentration levels were chosen to obtain complete solubilization of NC at 8 g L⁻¹ (40 mg/5 mL) at the end of the hydrolysis, to minimize perturbations of the denitration reaction by too viscous alkaline media, and be sure

Table 3
Denitration yields (% ± one standard deviation, *n*=3) obtained for NC1 and NC2 after alkaline hydrolysis at 65 °C with 3 M NaOH and for different reaction times.

NC	Reaction time (h)	Denitration yield (%)
NC1	1	79 ± 2
	2.5	84 ± 2
	4	80 ± 2
NC2	1	82 ± 1
	2.5	83 ± 1
	4	83 ± 1

Table 4
Denitration yields (% ± one standard deviation, *n*=3) obtained for NC1 and NC2 after alkaline hydrolysis with 3 M NaOH for 2.5 h and at different temperatures.

NC	Temperature (°C)	Denitration yield (%)
NC1	60	76 ± 2
	75	84 ± 2
	90	81 ± 2
NC2	60	82 ± 1
	75	83 ± 1
	90	84 ± 1

Table 5

Denitration yields (% ± one standard deviation, *n*=3) obtained for NC1 and NC2 after alkaline hydrolysis at 75 °C for 2.5 h and with different concentrations of sodium hydroxide.

NC	[NaOH] (M)	Denitration yield (%)
NC1	1	81 ± 2
	3	84 ± 2
	5	81 ± 2
NC2	1	82 ± 1
	3	83 ± 1
	5	84 ± 1

Table 6

Nitrogen content of the NC used in a gunpowder as obtained from three alkaline extractions followed by CE (this work), and as given by the manufacturer (Devarda's method).

	Nitrogen content (%) This work	Nitrogen content (%) Manufacturer data
Hydrolysis 1	13.56 ± 0.06	13.20 ± 0.05
Hydrolysis 2	13.73 ± 0.06	
Hydrolysis 3	13.25 ± 0.04	

not to encounter matrix effect during CE analysis. Accordingly, experiments were carried out with sodium hydroxide concentrations ranging from 1 to 5 M. Reaction time was varied between 1 and 4 h. Indeed, a minimum of 1 h was necessary to ensure good solubility of cellulosic backbone at the end of the denitration and reaction times greater than 4 h did not appear reasonable. Finally, NC hydrolysis were carried out by varying temperature in the range of 60–90 °C. The maximum value was set at 90 °C to prevent from NC decomposition and minimize explosion hazards. Temperatures lower than 60 °C led to too long hydrolysis times to reach high denitration yields. The effects of temperature, sodium hydroxide concentration, and reaction time on the denitration yields were studied separately with the other two parameters held constant and the results are summarized in Tables 3–5. A typical resulting electropherogram obtained from NC2 hydrolysate is shown in Fig. 2b. For all conditions studied, the obtained denitration yields (calculated according to Eq. (1)) ranged from 76% to 84%, and the RSDs on the denitration yields calculated from the three repetitions of the hydrolysis protocol were 1.8% and 1.4% for NC1 and NC2, respectively, which appears to be very satisfactory.

Based on the denitration yields obtained under the nine investigated hydrolysis conditions, the effects of sodium hydroxide concentration, reaction time, and temperature on denitration yields cannot be clarified further by this monivariate study.

3.3.2. Hydrolysis of NC contained in gunpowder

A single-base gunpowder containing a highly-nitrated NC was first extracted three times independently, next each extract was hydrolyzed according to the protocol described in Section 2.5, and finally each hydrolysate was analyzed three times using the optimized CE method. Alkaline hydrolysis was performed with 3 M sodium hydroxide, at 75 °C and for 2.5 h, as these conditions led to the same denitration yield, whatever the nitrogen content of the NC (see Tables 3–5). A typical electropherogram of the hydrolyzed gunpowder extract is presented in Fig. 2c. As can be seen, no additional peak from the matrix interferes with these of nitrite and nitrate ions in the hydrolysate, thus making it possible quantitation of these anions. The nitrogen content of the NC used in this gunpowder was calculated from the determination of

nitrite and nitrate ions and a value of 0.83 for the denitration yield under the conditions employed (see Tables 3–5), according to Eq. (2) and the results are reported in Table 6. As expected, the obtained nitrogen contents are greater than 12.5%, demonstrating the presence of an explosive NC. The mean value of nitrogen content obtained from the three independent extractions is 13.5%, with a standard-deviation of 0.3% (RSD, 1.8%). The RSDs on normalized corrected peak areas for nitrite and nitrate ions were 0.5% and 0.4%, respectively ($n=3$), while the RSDs on corrected migration times for nitrite and nitrate ions were 0.07% and 0.05%, respectively ($n=3$). The experimental nitrogen content obtained from this work is in close agreement with that claimed by the manufacturer (determined by Dewarda's method), thus demonstrating the effectiveness of this new whole protocol (alkaline hydrolysis followed by CE) for the determination of the nitrogen content of NCs contained in formulated samples.

4. Conclusions

A new and simple CE method was developed for the quantitation of nitrite and nitrate ions released by the alkaline hydrolysis of NCs. This method is based on the use of a 100 mM ionic strength, pH 7.7 phosphate buffer, able to accommodate highly alkaline sample matrices (0.5 M sodium hydroxide), and allowing direct absorbance detection of both anions of interest, which provides a high degree of selectivity. The method was validated with respect to linearity, limits of detection and quantitation, repeatability, and selectivity. In addition to this, no significant matrix effect was observed with 0.5 M sodium hydroxide. This CE method, which compares favorably with IC, was successfully used for the study of the alkaline hydrolysis of pure NCs and the determination of the denitration yields, obtained under various conditions of sodium hydroxide concentration, temperature, and time. It was next applied to the determination of the nitrogen content of a NC used in a single-base gunpowder and results in close agreement with the value given by the manufacturer were obtained. Nevertheless, the monivariate approach implemented so far to study the denitration reaction of NC in alkaline medium has not allowed to clarify the influence of the above-mentioned three parameters, and to identify the conditions providing the highest denitration yield. Also, a design-of-experiment approach is underway in our lab to unravel the actual effects of these factors, and the present CE method, due to its speed, cost-effectiveness, miniaturization, and simplicity, appears to be the method of choice to monitor this reaction.

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