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A microdestructive capillary electrophoresis method for the analysis of blue-pen-ink strokes on office paper

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ABSTRACT

This manuscript describes the development of a capillary electrophoresis (CE) method for the detection of acid and basic dyes and its application to real samples, blue-pen-ink strokes on office paper. First, a capillary zone electrophoresis (CZE) method was developed for the separation of basic and acid dyes, by studying the separation medium (buffer nature, pH and relative amount of additive) and instrumental parameters (temperature, voltage and capillary dimensions). The method performance was evaluated in terms of selectivity, resolution (above 5 and 2 for acid dyes and basic dyes, respectively, except for two basic dye standards), LOD (lower than 0.4 mg/L) and precision as intraday and interday RSD values of peak migration times (lower than 0.6%). The developed method was then applied to 34 blue pens from different technologies (rollerball, ballpoint, markers) and with different ink composition (gel, water-based, oil-based). A microdestructive sample treatment using a scalpel to scratch 0.3 mg of ink stroke was performed. The entire electropherogram profile allowed the visual discrimination between different types of ink and brands, being not necessary a statistical treatment. A 100% of discrimination was achieved between pen technologies, brands, and models, although non-reproducible zones in the electropherograms were found for blue gel pen samples. The two different batches of blue oil-based pens were also differentiated. Thus, this method provides a simple, microdestructive, and rapid analysis of different blue pen technologies which may complement the current analysis of questioned documents performed by forensic laboratories.

1. Introduction

Questioned documents and their analysis are of relevant importance for forensic laboratories, as different crimes such as forgery, frauds, suicide letters, or even terrorist attacks involve them as evidence. Proof of the wide range of analyses required in these samples is demonstrated in a recent review [1]. The determination of ink composition from blue pens is of special importance, probably due to the number of caseworks leaving them as evidence. Despite the existence of novel analytical instrumentation, compared to the past years, the analysis of questioned documents is often a difficult task. Basically, this adversity is not originated in the analysis itself, but in the results interpretation considering the sample background. One of the most important compounds present in a questioned document are often the compounds contained in the inks. As long as ink formulations are under patent, the identification of compounds can be tedious. In addition, modern manufacturing processes are able to produce large batches of pens with identical ink composition, which makes the differentiation or individualization challenging. These facts, together with other conditions affecting the sample, such as the environment (temperature, light), interferences from the support (office paper for example) or simply the pressure applied during the writing process, make complex the interpretation of the results. Besides, it should be noted that inks degrade over time, and this uncontrollable process must be considered [2]. Finally, these samples play an important role in forensic caseworks related to forgery, and therefore destruction must be minimal in order to preserve the evidence for the court or future analysis.

Given the challenges in ink analysis, different methodologies have been developed [1]. Each technique can provide different results and has its limitations, advantages and disadvantages. Mass spectrometry (MS) techniques have the possibility of unequivocally identifying the species contained in inks, and sometimes are non-destructive methods, but these are expensive and require more resources than other techniques [3]. Recently, spectroscopic

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techniques have shown their potential to characterize inks, although they might not have sufficient discrimination power, and their sensitivity is lower. For these techniques, the use of the entire spectra without the identification of compounds, also called spectral fingerprint, is a common practice for discrimination purposes [4]. Separation techniques have a destructive character, but they can separate compounds in complex mixtures, and even traditional modes, such as thin layer chromatography, still constitute the official method for ink analysis [5].

Focusing on separation techniques, capillary electrophoresis (CE) is attractive for the analysis of questioned documents: it possesses high versatility, is less expensive than other separation techniques, needs minimal amount of sample, and also provides higher sensitivity than other techniques like vibrational spectroscopy, which can be of interest for differentiation processes; yet CE has been less explored for the discrimination of pen inks than other techniques as HPLC or MS [1]. Various CE modes have been proposed for the analysis of dyes, pens and ink strokes on paper. Reader can consult **Table SM1** in the supplementary material file for details on the number of samples, quantity needed, preparation, and separation media employed for these studies. Regarding non-aqueous CE (NACE), few studies have been carried out to analyze dyes and pens. A. Fakhari et al. optimized a NACE method which allowed the separation of different basic dyes (standards). Then, 8 different ballpoint and fiber tip pens (7 blue and 1 black) were analyzed. With the developed method, RSD values lower than 2% in migration times were obtained. These standards were also identified in real samples when using MS as a complementary technique. [6]. H. Zou et al. also employed a NACE method with a similar background electrolyte (BGE). 120 black ballpoint pens were clustered in 6 groups, and good RSDs in migration times (0.63%) and peak areas (3.38%) were obtained [7]. Another possibility is the use of micellar electrokinetic capillary chromatography (MECK). J. A. Zlotnick et al. showed preliminary data on the analysis of black rollerball pens using a borate buffer with SDS. Despite being a limited set, the method proved to be another alternative for the analysis of these samples [8]. Later, J. Mania et al. developed a procedure for the analysis of blue and black ballpoint pens and fountain pens. The method was firstly applied to different blue and black standard dyes. The optimized extractant (water:pyridine 1:1 (v/v)) was useful for the extraction of ballpoint pens (22 samples) and fountain pens (9 samples) but invalid for gel pens (10 samples). Different electropherogram profiles were obtained depending on the type of pen [9]. More recently, J. D. Brewer et al. developed two methods to analyze black and blue inks (10 samples) from ballpoints [10,11]. However, each method consisted in two different CE analysis of the same sample with different conditions in order to analyze separately anions and cations. Besides, the BGE employed in both cases was quite complex. The cationic method provided more discrimination. However, the UV–vis and MS spectra were needed to unequivocally differentiate between the samples. On the other hand, with the anionic method, no significant differences were found for the set of blue ballpoint pen samples. Note that apart from dyes, other compounds such as guanidine or copper phtalocyanine were detected and identified [10]. Finally, MECK has also been used for the analysis of inks from inkjet printers. During the last years, P. Kościelniak et al. have developed comprehensive MECK methodologies for the analysis of color and black inkjet printing inks [12–15], as well as CE-methods for the examination of black inkjet printing inks [16]. As can be seen in **Table SM1**, the BGE, containing SDS, has also been used to successfully extract the inks from the paper. On the basis of the UV–vis spectrum of each peak, dyes were clustered in cyan, magenta and yellow, and other peaks (from additives) were considered for discrimination. Colored samples were clustered in 25 different groups, whilst black printing inks were grouped in 3 different groups, demonstrating also a reproducible method.

Regarding capillary zone electrophoresis (CZE), K. Tsutsumi et al. applied a method based on a borate buffer to analyze water-soluble black pens (including rollerball and marking pens). This method allowed the differentiation among most of the samples despite obtaining poor reproducibility in the peak areas, identifying also methyl violet and direct black 154 dyes for the set under study [17]. Later, C. Vogt et al. analyzed blue and black fountain pens by CZE and UV–vis and fluorescence detection. Separation was performed in a basic medium to assure ionization of the species. 12 different pens (6 blue and 6 black) were analyzed. A poor reproducibility was obtained for migration times, especially for the last peaks. In addition, interferences from the paper were evidenced when using UV–vis detection. Thus, stacking procedures were recommended for sample preconcentration [18]. Subsequently, authors applied the method to a set containing 5 more samples, obtaining similar results [19]. On the other hand, C-M Shin et al. compared two CE methods (one MECK and other CZE) to analyze one blue ink taken directly from a printing cartridge. Authors found the CZE method useful for ageing purposes, while the MECK allowed more discrimination power. However, this methodology was only tested in one blue ink taken from the pen cartridge [20]. Finally, A. M. López-Montes et al. developed a CZE method to analyze synthetic dyes, by employing a BGE composed of 50 mM ammonium acetate and 15% (v/v) acetonitrile. Despite not being applied to pens for forensic purposes, the method showed a wide range of dyes successfully detected [21].

The methods above described have proved the successful performance of CE for the analysis of dyes and pens, showing the versatility of the technique in terms of modes and separation media employed. However, some of the reported methods have focused solely in the determination/study of the dyes; others have analyzed cationic or anionic species alone; or have focused only in one pen technology and/or type of ink. Also, during the last years, attention has been fundamentally paid to complex modes of CE [6–16], being the most basic mode (CZE) less used. Therefore, this study aims to develop and evaluate a simple CZE-DAD method for the separation of blue inks, and its application to the microdestructive analysis of ink strokes written on office paper. To achieve this, three specific objectives were pursued:

(i) The development of a new CZE-DAD method for the separation of acid and basic dyes by studying the separation medium and instrumental parameters;
(ii) The evaluation of the analytical performance of the developed method in terms of selectivity, LODs and precision; and
(iii) The application of the method to ink strokes written on office paper from blue pens of diverse nature (gel, water-based and oil-based inks).

2. Material and methods

2.1. Instrumentation and software

The optimization of the method, as well as the application to real samples, was performed in a CE commercial equipment PA 800 plus from Beckman Coulter Inc. (Brea, CA, US). Detection of the species was carried out through a DAD detector equipped with a deuterium lamp ranging from 190 to 600 nm, also from Beckman Coulter inc. (Brea, CA, US).

For the separation of the species, two different capillaries were employed. A conventional fused-silica polyimide-coated capillary of 50 μm internal diameter (id) and another of 25 μm id (both capillaries with 360 μm outer diameter) from Polymicro