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Differentiation and dating of gel pen ink entries on paper by laser desorption ionization- and quadruple-time of flight mass spectrometry

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ABSTRACT

The approaches for differentiation and dating of gel pen ink entries have been investigated by laser desorption ionization-time of flight mass spectrometry (LDI-TOF-MS) and high performance liquid chromatography-quadruple-time of flight mass spectrometry (HPLC-Q-TOF-MS). 45 kinds of black and blue gel pen ink entries were differentiated individually by the profiles of their LDI-MS spectra. The dye components in the black and blue ink entries have been identified by thin layer chromatography and HPLC-Q-TOF-MS methods. The degradation processes of the dye components in the ink entries under various aging conditions have been probed by LDI-MS approach. The results showed that the variations of relative intensities for the main dye components have a close relationship with aging time, and the degradation of the main dye components were significant under natural storage conditions, which can provide important evidences for dating of the ink entries on paper.

1. Introduction

Gel pens are common writing instrument all over the world since they were first manufactured in 1984 by Sakura Color Products Corp. of Japan [1,2]. Differentiation and dating of gel pen ink entries can provide rich information for tracking their origins and offer scientific evidences and clues for determining the questioned documents [3,4]. Therefore, it has a great significance to develop and establish the related analytical methods for forensic examination of the inks.

Gel pens utilize the water-based inks, which contained dyes or pigments as colorants, water as vehicles, resins, surfactants and other additives [2]. The differences of the compositions from product to product can be used to differentiate the inks [2–4] and the variations of components for ink entries on paper can track the dating of the documents [3–5]. Ion-paring high performance liquid chromatography (IP-HPLC), gas chromatography (GC) and related techniques were very useful in the examination of gel pen ink entries on paper [3,4,6,7]. They can separate the components of the inks, such as dye components, and classify the inks depending on their compositional differences [3,6], and determine the relative ages of the ink entries according to the variations of the ink components, such as evaporation of volatile solvents [7], degradation of the dyes [3,6] or other additives. Generally, the inks must be extracted from the documents before chromatographic analysis, and in this case, the samples would be partially or completely destroyed. Additionally, the efficiency of the extraction procedure would influence the final results.

Quick and nondestructive analytical technologies are more necessary in forensic examination of the ink entries on documents. Spectroscopic methods, such as Fourier transform infrared spectrometry [8], Raman spectroscopy [9,10], visible and near infrared reflectance [11], and microscope [12], can provide much information for differentiation of the gel pen inks, and sometimes determine the dye components [9]. However, the low sensitivity and less discriminating power of the spectroscopic approaches limited their application for observing exactly the changes in composition of pen inks. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to discriminate the pen inks by elemental analysis [13], while it cannot provide the evidences for dating of the ink entries on documents.

Mass spectrometric techniques are more promising for forensic examination in terms of its high discrimination power, especially coupled with HPLC [6], and it can qualitatively identify the

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components of the ink entries on documents [14–16]. Mass spectrometry with an ion source called Direct Analysis in Real Time can detect the ink components in nondestructive way [17]. Laser desorption ionization mass spectrometry (LDI-MS) is also a nondestructive approach for forensic examination of inks on documents [18–21] and utilized to discriminate the gel pen ink entries on paper and identify their dye components [21,22] in recent years.

In this report, the approach of differentiation for 45 kinds of gel pen ink entries on paper (40 black and 5 blue) were investigated by LDI-MS method according to their compositional differences. Several dye components of the gel pen ink entries were identified by HPLC-Quadrupole-Time of Flight mass spectrometry (HPLC-Q-TOF-MS) and thin layer chromatography (TLC) methods. The degradations for the dye components of the gel pen ink entries on paper under artificial and natural aging conditions were probed by LDI-MS. The methods developed were rapid and nondestructive and the results obtained can provide scientific evidences for differentiation and dating of gel pen ink entries on documents.

2. Experimental

2.1. Reagents and instruments

Basic blue 7 was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan), and Rhodamine 6GDN, Rhodamine B and Basic blue 26 were from Shanghai Jingchun chemical Co. (Shanghai, China). HPLC grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). The other reagents were of analytical grade and from Beijing Beihua Fine Chemical Limited Liability Company (Beijing, China). Water for buffer preparation was prepared by Milli-Q filtration system from EASYpure LF compact ultrapure water system (Barnstead Corp., Boston, U.S.A).

Ultraviolet apparatus was from Haimen QL-Lab (Jiangsu, China). Basis pH Meter PB-21 was from Sartorius (Goettingen, Germany). Liquid handling pipettes (20–200 μL and 100–1000 μL) were from Eppendorf Research (Hamburg, Germany). 0.2 μm Millipore filters were from Microdyn-Nadir Ltd. (Frankfurt, Germany).

2.2. Sample collection and pretreatments

Forty five kinds of black (40) and blue (5) gel pens were collected from different manufacturers at home and abroad. The 25 black gel pens from abroad were numbered as I1 to I25, and the 15 homemade black and 5 blue gel pens were labeled as J1 to J15 and G1 to G5, respectively, according to the time sequence obtained (see Table S1 in Supplementary information).

The straight ink lines were drawn on ordinary A4 copying paper for preparation of the samples. For natural aging samples, the ink line strokes were stored in darkness at room temperature. The black gel pen I24 and blue gel pen G2 were randomly selected, and their freshly prepared ink entries were exposed to UV light at 254 nm or fluorescent lamp (40 W) at a vertical distance (about 10 cm), respectively. 5 cm ink entries were cut to small pieces and extracted by 1.0 mL dimethyl formamide (DMF) for 12 h and then filtered through a 0.22 μm Millipore film prior to MS and TLC analysis for determination of their dye components.

2.3. LDI-TOF-MS method

The mass spectra of the gel pen ink entries were collected on a microflex matrix-assisted laser desorption/ionization (MALDI) time of flight (TOF) mass spectrometer (Bruker Daltonic, Germany) equipped with a 337 nm nitrogen laser in positive or negative modes. The parameters were as follow: ion source, IS1, 19 kV; IS2, 15.7 kV; lens, 9.7 kV; reflector, 20.0 kV; frequency, 60 Hz; detector gain, 3.8×; and sample rate, 2.0 GS/s. All spectra were collected in reflecting mode with a delayed extraction time of 160 ns. The spectral range were from $m/z = 0$ to 1700. To make every sample desorbed and ionized adequately, a variable attenuator was placed between the laser and the sample, with which the operative laser irradiance was regulated conveniently. Mass spectra of 50 laser shots at a single location in positive mode or 100 in negative mode were collected and averaged. For every gel pen, nine averaged spectra were obtained from 9 stroke lines at different points individually to guarantee the homogeneity of samples.

The laser energy was optimized based on the principles to balance the intensity of main molecular ion, peak resolution and the signal to noise ratio for the gel pen ink entries, and the results showed that the energy range from 37% to 45% was suitable to collect the spectra for the ink entries. In the optimized conditions, the relative intensities of the main peaks in the mass spectra of gel pen ink entries have high reproducibility, and relative standard deviations (RSD) for 5 determinations were below 3%.

2.4. HPLC-ESI MS method

The microTOF-Q II Mass Spectrometer (Bruker Daltonic, Germany) with the Agilent Technologies 1200 HPLC system (California, USA) was used for HPLC–MS/MS analysis of the dye components in the gel pen ink entries. The column for separation was Agilent ZORBAX SB-C18 (2.1 × 150 mm, 5 μm). The mobile phases were 20 mmol/L ammonium bicarbonate (eluent A, pH 7.0, adjusted by ammonia) and acetonitrile (eluent B), and the linear gradient was from 95% A to 5% A. The column temperature was kept at 25 °C and the flow rate of the mobile phase was 0.25 mL/min. The high-resolution Mass Spectrometer was calibrated by sodium formate (500 μL 0.1 mol/L NaOH solution were mixed with 500 μL 10% formic acid in volumetric flask, and then isopropyl alcohol aqueous solution (90:10,v/v) were added to 10 mL). The dry temperature was set to 180 °C. The other instrument parameters, such as the flow rates of the dry gas and the Nebulizer gas, the capillary voltage and the collision energy, were optimized depending on the samples.

2.5. TLC method

The solvent system for separation of the DMF extracts for ink entries I24 and G2 were mixtures of *n*-butanol, ethanol, water and glacial acetic acid (6:2:2:1). Silica GF_{254} plates (Yinlong brand, China) were used as solid phase.

3. Results and discussion

3.1. Differentiation of the gel pen ink entries on paper by LDI-MS

Gel pen inks contained dyes and additives, such as surfactants which enabled the inks to have certain ability of penetration and to promote air seasoning on paper [2]. The compositional differences of the inks reflected on their LDI-MS spectra and the information obtained can be used to distinguish the ink entries on paper [14,16,18–20,23–25].

In order to observe the interferences of the blank paper, the MS spectra for five common types of copying paper from China, including Xinle, Jiayin, Sanyi, Gaopinle, and Jinlinwang, were collected both in positive and negative mode. The results showed that these blank papers have the similar spectra in positive mode (see Fig. S1 in Supplementary information), while they have no obvious peaks in negative mode. In the m/z range of 100–1200, there were only two moderate intensity peaks, $M^+ = 372$ and