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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

$M^+ = 575$, which can be referred to the whitening agents for paper, Basic Violet 3 and Pigment Phthalocyanine Blue [14,20] respectively. These signals can be distinguished with those of the gel pen inks and have little influence on the determination of the gel pen ink entries.

The mass spectra for 45 gel pen (40 black and 5 blue) ink entries on paper were collected by LDI-MS in both positive and negative modes. The 40 black gel pen ink entries can be classified into two groups according to the presence or absence of the information for the surfactants in their spectra. From the spectra of these ink entries, it has been found that 30 black gel pen ink entries contained surfactants, while the spectra for the 10 black and 5 blue ink entries only have the information of dyes.

3.1.1. Differentiation of the gel pen ink entries containing surfactants

Fig. 1 shows the representative LDI-MS spectra of the ink entries on paper collected in positive (Fig. 1a) and negative (Fig. 1b) modes, respectively. It can be seen from the Figure that a series of peaks appeared as normal Gaussian distribution and the mass differences of the neighboring peaks were 44 units (m/z), which was the characteristics of polyoxyethylene or poly(ethylene glycol)(PEG) [15,18]. Some ink entries have two or more series of peaks (such as I10 in Fig. 1a) and the mass differences between the series were 16 units (m/z), an oxygen atom. It can also noted from the profiles of the MS spectra that the mass numbers and the maximum intensities of the peaks were different for various gel pen ink entries and the information can be used to differentiate them. The mass numbers of the maximum intensity for 30 black gel pen ink entries on paper are summarized in Table 1.

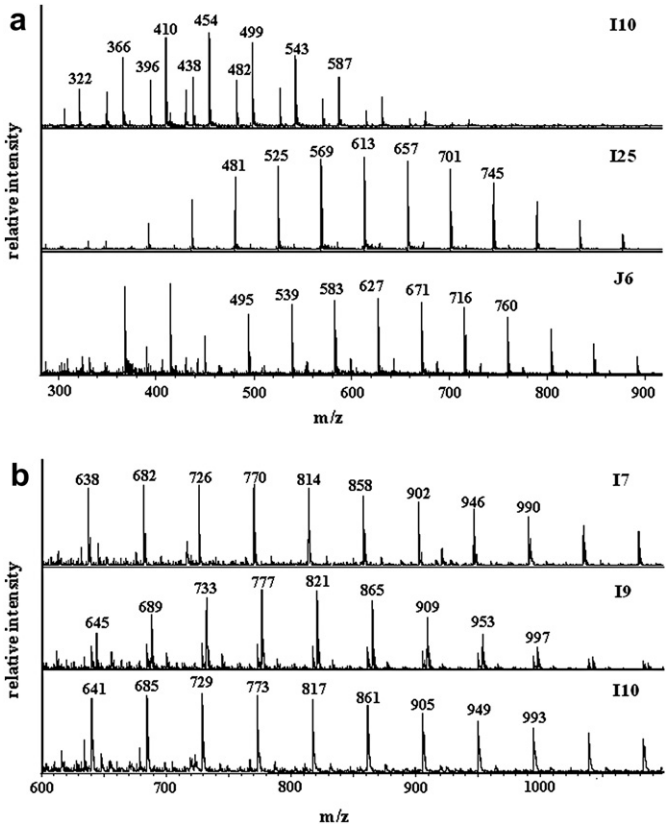

Fig. 1. The representative LDI-MS spectra of the black gel pen ink entries collected in positive (a) and negative (b) modes.

Table 1
The mass numbers of maximum intensities for the 30 black gel pen ink entries.

Serial no.	Positive mode	Negative mode	Serial no.	Positive mode	Negative mode
I3	479 ± 44n, 495 ± 44n, 503 ± 44n	–	I25	613 ± 44n	–
I4	859 ± 44n	–	J1	350 ± 44n, 366 ± 44n	508 ± 44n, 522 ± 44n, 536 ± 44n
I5	796 ± 44n	–	J2	260 ± 44n, 276 ± 44n	–
I7	453 ± 44n	814 ± 44n	J4	685 ± 44n	–
I9	763 ± 44n, 779 ± 44n	821 ± 44n	J5	533 ± 44n, 549 ± 44n	773 ± 44n
I10	438 ± 44n, 454 ± 44n	773 ± 44n	J6	1080 ± 44n	–
I13	507 ± 44n, 523 ± 44n	–	J7	774 ± 44n, 790 ± 44n	769 ± 44n
I14	805 ± 44n, 821 ± 44n	–	J8	627 ± 44n, 643 ± 44n	479 ± 44n
I15	669 ± 44n	–	J9	394 ± 44n, 410 ± 44n	769 ± 44n
I17	–	726 ± 44n	J10	627 ± 44n	–
I18	438 ± 44n, 454 ± 44n	–	J11	641 ± 44n	–
I19	948 ± 44n	–	J12	262 ± 44n	–
I20	1328 ± 44n, 1344 ± 44n, 1358 ± 44n	–	J13	818 ± 44n	–
I21	645 ± 44n	–	J14	489 ± 44n	817 ± 44n
I23	619 ± 44n	–	J15	–	449 ± 44n, 463 ± 44n

The data in Table 1 shows that there is only one series of peaks in the LDI-MS spectra collected in positive mode for the ink entries I4, I5, I15, I19, I21, I23, I25, J4, J6, J10, J11, J12 and J13, and it is interesting to note that there is no MS signal for these ink entries when collected in negative mode. There are more series of peaks in the MS spectra in positive mode for 13 kinds of ink entries, such as I3, I9, I10, I13, I14, I18, I20, J1, J2, J5, J7, J8 and J9. For some ink entries (I7, I9, I10, J1, J5, J7, J8, J9 and J14), the surfactants can be detected both in positive and negative modes, while those of ink entries (I17 and J15) can only be detected in negative mode. It can also be noted from Table 1 that the mass numbers of the maximum intensities for various ink entries are different and the 30 black gel pen ink entries can be differentiated individually from the profiles of their LDI-MS spectra.

3.1.2. Differentiation of the gel pen ink entries absence of surfactants

The LDI-MS spectra for 10 black and 5 blue gel pen ink entries, which were absence of surfactants, were collected in positive mode and their main precursor ions and relative intensities in the m/z range from 300 to 900 are summarized in Table 2. From the data as

Table 2
The main peaks and their relative intensities in the LDI-MS spectra of 10 black and 5 blue gel pen ink entries.

Serial no.	Main peaks and intensities	Serial no.	Main peaks and intensities
I1	468(100); 404(66); 580(37)	J3	331(100); 347(80); 393(69)
I2	393(100); 347(45); 509(21)	I22	414(100); 357(74); 468(26)
I6	347(100); 509(78); 671(35)	G1	603(100); 356(78); 559(56);
I8	392(100); 376(84); 409(49)	G2	456(27); 470(100)
I11	414(100); 430(25); 332(23)	G3	328(100); 492(42); 603(33)
I12	328(100); 393(95); 564(65)	G4	575(100)
I24	478(100); 443(2)	G5	548(100); 718(76); 740 (26)
I16	414(100); 430(13); 442(13)		

Table 3
The accurate mass numbers and potential dyes for the ink entries I24 and G2.

Serial no.	Mass (detected)	Formula	Mass (theoretical)	Deviation (mDa)	Potential dyes
I24	478.3219	$C_{33}H_{40}N_3$	478.3217	0.2	Basic blue 7
	443.2322	$C_{28}H_{31}N_2O_3$	443.2329	0.7	Rhodamine 6GDN
					Rhodamine B
G2	470.2592	$C_{33}H_{32}N_3$	470.2591	0.6	Basic blue 26

shown in Table 2, there are three main peaks (relative intensities are above 10%) in the MS spectra for 9 kinds of black ink entries and their base peaks were m/z = 468, 414, 393, 347, 392, 328 and 331, respectively. Six out of the nine ink entries can be distinguished for their base peaks. Three kinds of ink entries have the same base peaks m/z = 414 (I11, I16 and I22), while they can be differentiated by differences of the relative intensities (I11 and I16) and mass numbers (I11, I16 and I22) for other two components. The ink entry of I24 has only two peaks, the dye components, which would be identified in the following section. The base peaks for the five blue gel pen ink entries were 603, 470, 328, 575 and 548, individually, and they can be discriminated directly from their MS spectra.

3.2. Identification of the dye components for the gel pen ink entries by HPLC-Q-TOF-MS

The gel pen ink entries (I24 and G2) were extracted with Dimethyl Formamide (DMF), and the extracts were separated with thin layer chromatography (TLC). Three spots with colors of blue, red and yellow have been found in the TLC chromatogram (Figure not shown) of the ink entry for I24 and it illustrated that there are three kinds of dye components in the extract of this ink entry. Similarly, the TLC result showed that there was only one obvious spot for the extracts of ink entry G2.

In order to qualitatively determine the dye components, and the DMF extracts of the ink entries were analyzed by HPLC-Q-TOF-MS. For the black gel pen ink entry of I24, there are two main peaks (Figure not shown) in the chromatogram, and it inferred that the two components were dyes. Meanwhile, there is only one main component in the chromatogram for the blue gel pen ink entry G2. The potential molecular formulas of the three components were obtained from their accurate molecular mass and isotopic distributions, and the results were summarized in Table 3.

From Table 3, it can be seen that the accurate molecular mass for the potential formulas matched well with those detected and the deviations were below 1 mDa, which was within the instrument errors. For ink entry of I24, the molecular formula of $C_{33}H_{40}N_3$ corresponds to a blue dye, Basic blue 7, and that of $C_{28}H_{31}N_2O_3$ to two kinds of red dyes, Rhodamine 6GDN and Rhodamine B, which were identical with those obtained by TLC method. To confirm the structures of these dyes, the MS spectra for the DMF extract of the ink entry I24 (see Fig. 2a) and the three standard dye samples (Figure not shown) were analyzed with direct injection of the samples in positive mode, and the MS/MS spectra for the precursor ions were collected (see Fig. 2b–f).

The MS/MS spectrum for the precursor ion (m/z = 478.3219) detected in ink entry I24 (Fig. 2b) was identical with that of Basic blue 7 (Fig. 2d). As shown in Fig. 2b and d, there are six main ion fragments. The fragment ion m/z 449 was elimination of an ethyl (CH_3CH_2–) group from the molecular ion 478, and fragments m/z 434 and m/z 405 were loss of a CH_3CH_2NH– group and of CH_3CH_2– and CH_3CH_2NH– groups from the parent ion, respectively. It can also deduce that the fragments m/z 329 and m/z 285 originated in eliminating a molecule of diethylaniline from the precursor ion of Basic blue 7, and subsequent loss of CH_3CH_2NH– group, respectively. The results confirmed that the dye component in the ink entry was Basic blue 7.

Fig. 2c, e and f showed the MS/MS spectra for the precursor ion of the dye component (m/z = 443.2322) in the ink entry I24 and the two potential dyes Rhodamine B and Rhodamine 6GDN, respectively. There are two main fragment ions in the MS/MS spectra of the ink entry I24 and Rhodamine B, the parent ion 443 and its fragment ion 399, lose of a carboxyl group (–C=O) from the parent ion. The main fragment ions were 443, 415 for Rhodamine 6GDN, which was obviously different from those of the ink entry for I24. It indicated that the dye component in the ink entry was Rhodamine B.

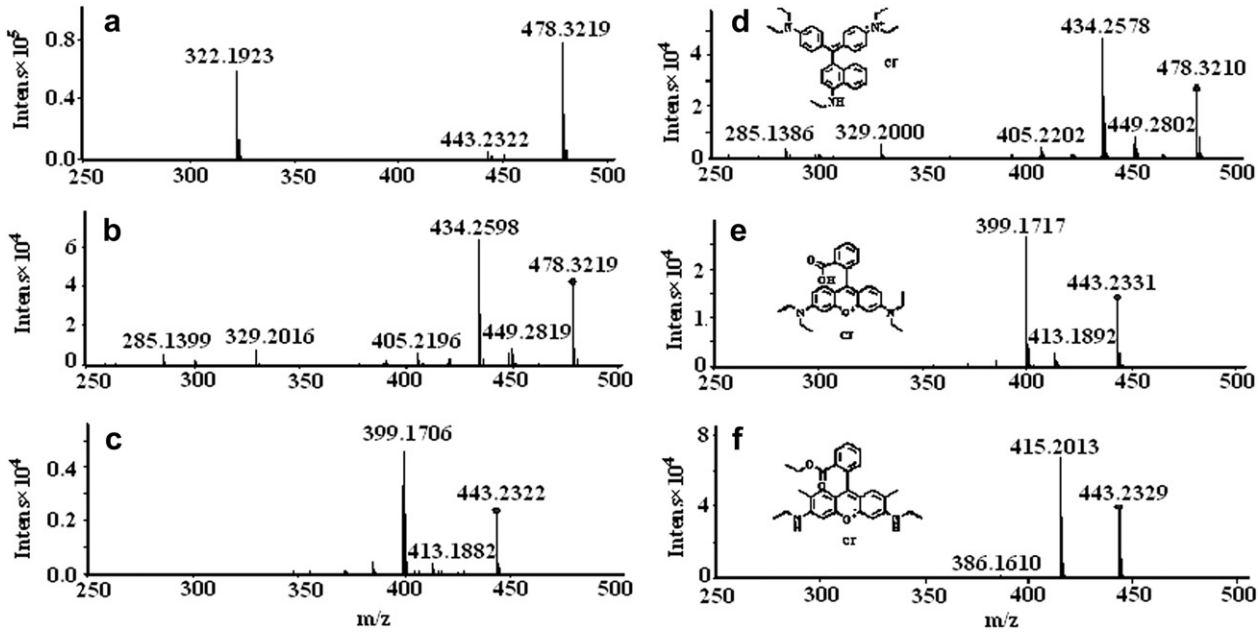

Fig. 2. The MS spectra for the DMF extract of the ink entry I24 (a) and the MS/MS spectra for its precursor ions (b and c); the MS/MS spectra for the precursor ions of the standard dyes, Basic blue 7 (d), Rhodamine B (e) and Rhodamine 6GDN (f).

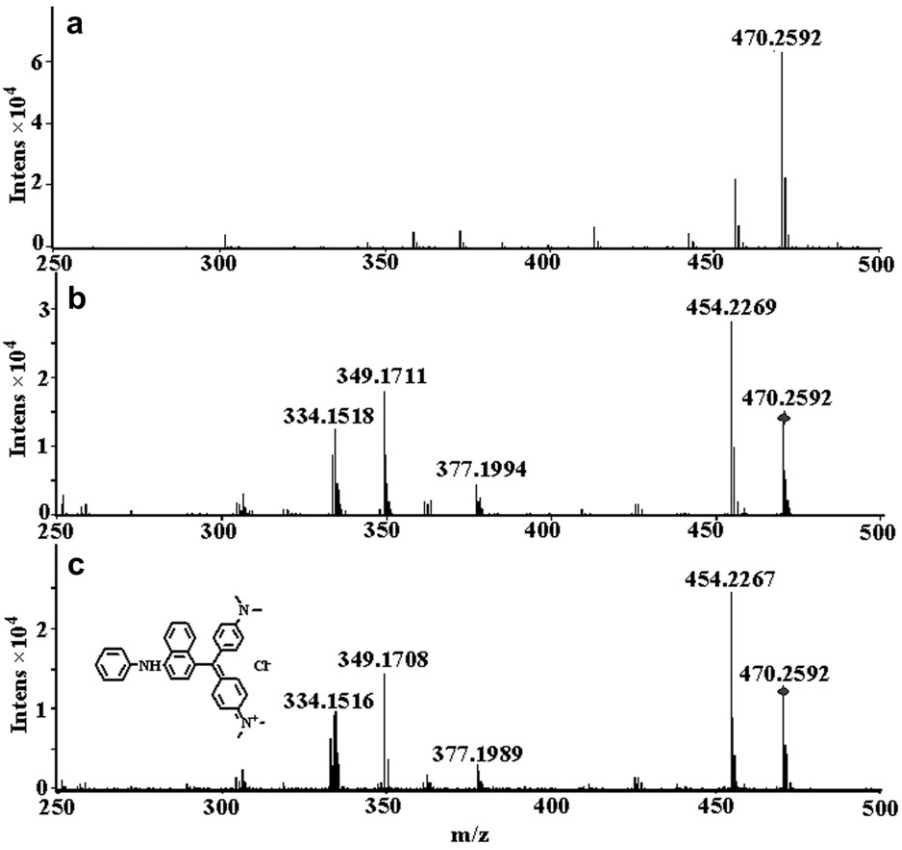

Fig. 3. The MS spectra for the DMF extract of the ink entry G2 (a) and the MS/MS spectra of its precursor ions (b) and the potential dye, Basic blue 26 (c).

The MS spectra for the DMF extract of the ink entry G2 (see Fig. 3a) and the standard dye sample Basic blue 26 (Figure not shown) were analyzed with direct injection of the samples in positive mode, and the MS/MS spectra for their precursor ions were collected (see Fig. 3b and c). It can be seen that the MS/MS spectra for the dye component of G2 ink entry and standard sample of Basic blue 26 have same fragment features. The positive parent ion ($m/z = 470$) of Basic blue 26 lost a methane (CH_4) to give the ion m/z 454, and from which further loss of a dimethylaniline obtained the fragment ion 334. The fragment ions m/z 349 and 377 were originated in eliminating a molecule of dimethylaniline or aniline from the parent ion, respectively.

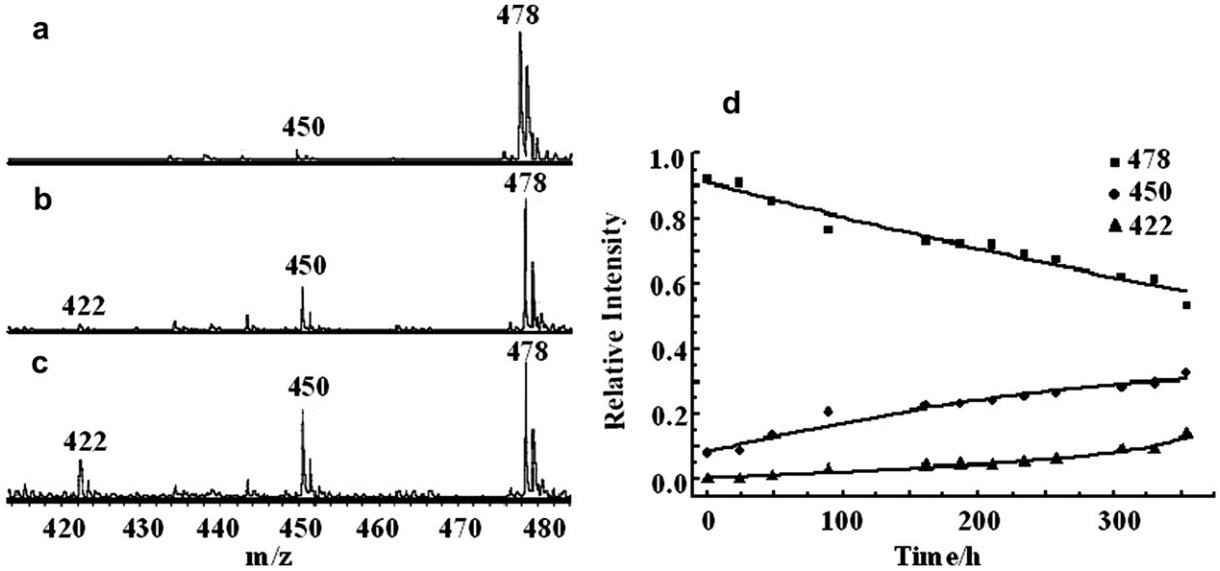

Fig. 4. The representative LDI-MS spectra of the I24 gel pen ink entries before and after exposed to UV light for 0 h (a), 162 h (b) and 354 h (c), and the curves for the relative intensities of the Basic blue 7 and its degradation products versus aging time (d). $n = 5$, RSD < 3% for $m/z = 478$ and RSD < 7% for $m/z = 450$ and 422.

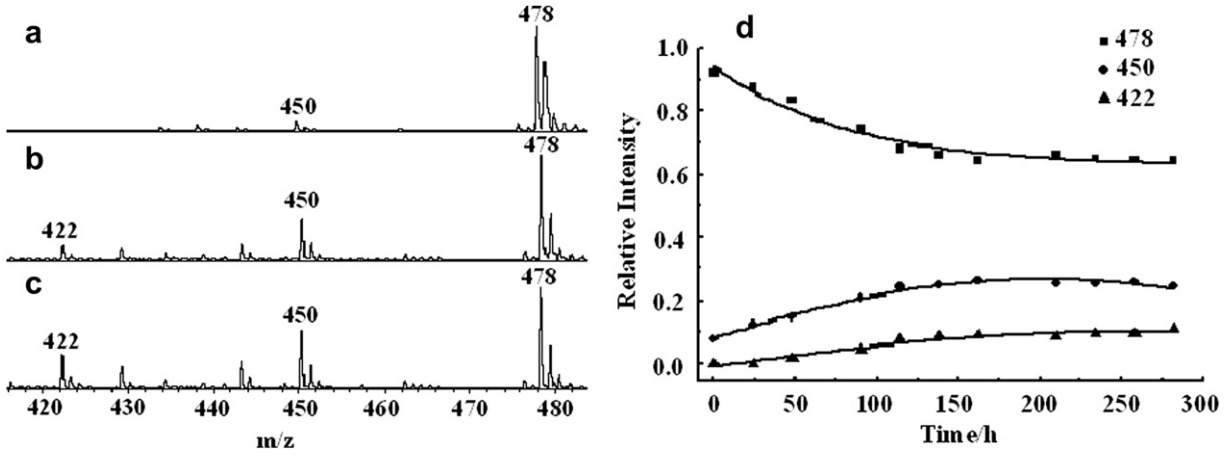

Fig. 5. The representative LDI-MS spectra of the I24 gel pen ink entries before and after exposed to fluorescent lamp for 0 h (a), 162 h (b) and 282 h (c), and the curves for the relative intensities of the Basic blue 7 and its degradation products versus aging time (d). $n = 5$, RSD < 3% for $m/z = 478$ and RSD < 7% for $m/z = 450$ and 422.

3.3. Degradation of the dye components under artificial light aging conditions

The degradation of the dye components for gel pen ink entries of I24 and G2 under artificial light conditions were investigated by LDI-MS in positive ionization mode. Fig. 4 shows the representative MS spectra of the I24 gel pen ink entries before and after exposed to UV light for a period of time and the variations of the relative peak intensities for the dye component and its degradation products. As shown in Fig. 4, there are two main degradation products of Basic blue 7 ($m/z = 478$), $m/z = 450$ and 422. The degradation product ($m/z = 450$) may be originated in the replacement of an ethyl group from the molecule of Basic blue 7 with an H atom [23], and subsequent loss of an ethyl group from the product $m/z = 450$ and addition of an H atom resulted in another degradation product of $m/z = 422$. It can be seen from Fig. 4b and c that the intensities of the degradation product ($m/z = 450$) obviously increased with prolonging the aging time, accompanying the reduction of the intensities for the main dye component, Basic blue 7, and it further demonstrated that the degradation product originated from Basic blue 7. Another degradation product ($m/z = 422$) emerged when the aging time reached to 50 h, and thereafter, its intensities gradually rose with increasing the aging time. In order to illustrate the variations of the dye components in the ink entries, the relative intensities of the Basic blue 7 and its degradation products (ratios of peak height for each component to total peak heights for Basic blue 7 and the degradation products) with various aging time were plotted (see Fig. 4d). The results showed that the relative intensities of the dye component were linearly reduced with prolonging the aging time, while those of the degradation production correspondingly increased. The linear relationship between the relative intensities and the aging time provides a chance for preparation of the reference samples for determining the age of the black ink entries.

Fig. 5 shows the LDI-MS spectra of I24 ink entries before and after exposure to fluorescent lamp and the variations of the relative intensities with aging time for the Basic blue 7 and its degradation products. As shown in Fig. 5, the decomposing characteristics of the dye component in the ink entries exposed to fluorescent lamp were similar with those under UV light condition, while the extents of degradation were relatively inferior due to the weaker energy of fluorescent lamp comparing with that of UV light. It can be seen from Fig. 5d that the relative intensities of Basic blue 7 and its degradation products versus aging time were not linear, and the variations of the intensities were more significant in the beginning, and then became gentle when the aging time was longer than 100 h.

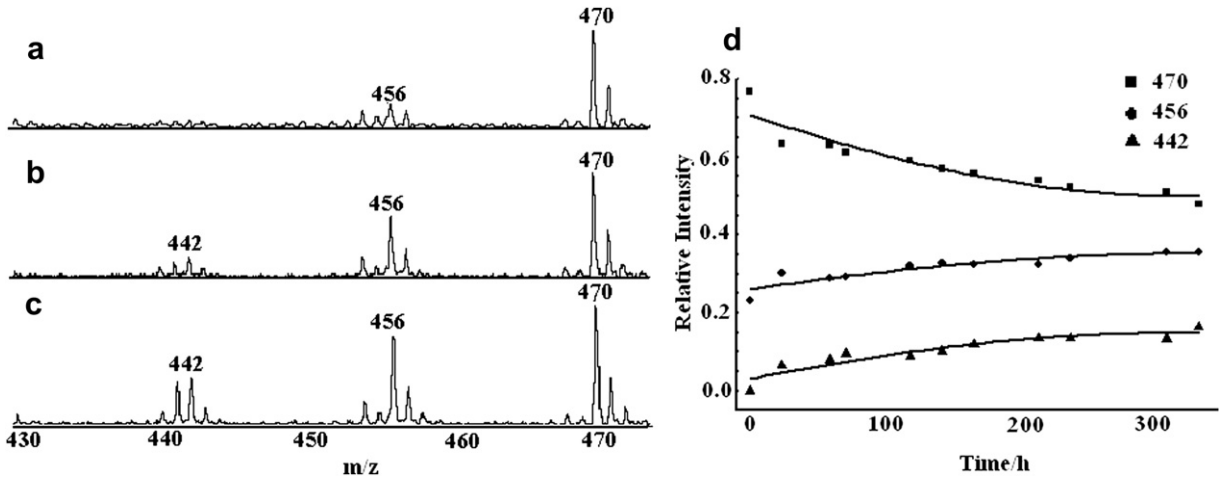

Fig. 6. The representative LDI-MS spectra of the G2 blue gel pen ink entries before and after exposed to UV light for 0 h (a), 168 h (b) and 336 h (c), and plots for the relative intensities of the Basic blue 26 and its degradation products versus aging time. $n = 5$, RSD < 3% for $m/z = 470$ and RSD < 7% for $m/z = 456$ and 442.

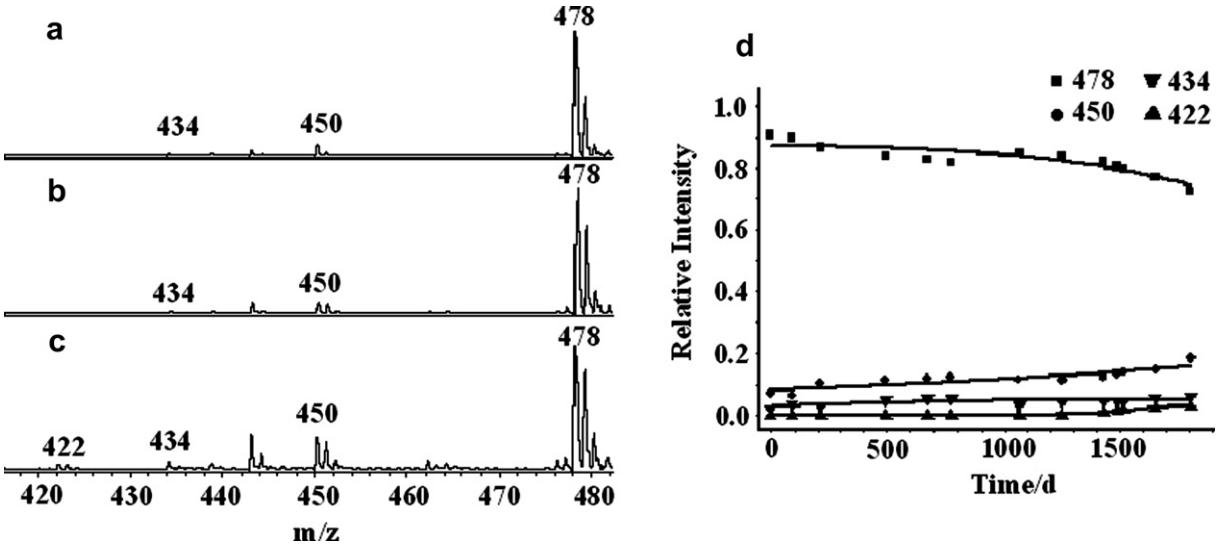

Fig. 7. The representative LDI-MS spectra of the I24 black gel pen ink entries stored in natural condition for 0 d (a), 707 d (b) and 1926 d (c) and the relative intensities of the Basic blue 7 and its degradation products versus natural aging time. $n = 5$, RSD < 3% for $m/z = 478$ and RSD < 10% for $m/z = 450$, 434 and 422.

Fig. 6 shows the representative LDI-MS spectra of the blue ink entries (G2) before and after exposure to UV light for 168 h and 336 h and the curves of the relative intensities for the dye components versus aging time. From the MS spectra of the ink entry G2 (Fig. 6a), it can be noticed that there are two main peaks (m/z 470, 456) in the mass range of 430–480, and the component with $m/z = 470$ was Basic blue 26. A third component ($m/z = 442$) has been appeared when the ink entries exposed to UV light for 50 h. Comparing with control sample, the intensities of the main dye component, Basic blue 26, significantly decreased with the aging time, while those of the components ($m/z = 456$ and 442) obviously increased accordingly (Fig. 6b and c). The results inferred that the components ($m/z = 456$ and 442) originated from the degradation of the Basic blue 26. It can be supposed that the loss of a CH_3– group from Basic blue 26 and addition of an H atom obtained the component $m/z = 456$, which subsequent eliminated a CH_3 and added an H atom gave another component $m/z = 442$. From the aging curves as shown in Fig. 6d, it can be seen that the relative intensities of Basic blue 26 linearly decreased with prolonging the aging time, and those of the degradation products correspondingly increased.

The degradation features of the G2 ink entries under fluorescent lamp were similar with those under UV light (Figure not shown), while the changes of the relative intensities for the components were relatively minor.

3.4. Degradation of the dye components under natural aging conditions

The black and blue gel pen ink entries (I24 and G2), stored under natural aging conditions, were analyzed by LDI-TOF-MS method. Fig. 7 shows the MS spectra of the ink entries stored for different period of time and variations of the relative intensities for the dye components in the ink entries. The precursor ions ($m/z = 478$, 450 and 422) in the MS spectra of the ink entries (see Fig. 8c) were same with those demonstrated under artificial aging conditions, and another precursor ion $m/z = 434$ originated in the loss of a CH_3CH_2NH– group from $m/z = 478$, as illustrated in the MS/MS spectra of Basic blue 7. It can be noted from the Fig. 7 that the degradation of the main dye component ($m/z = 478$) under natural storage conditions were relative slow comparing with those when exposed to UV light or fluorescent lamp. The degradation product

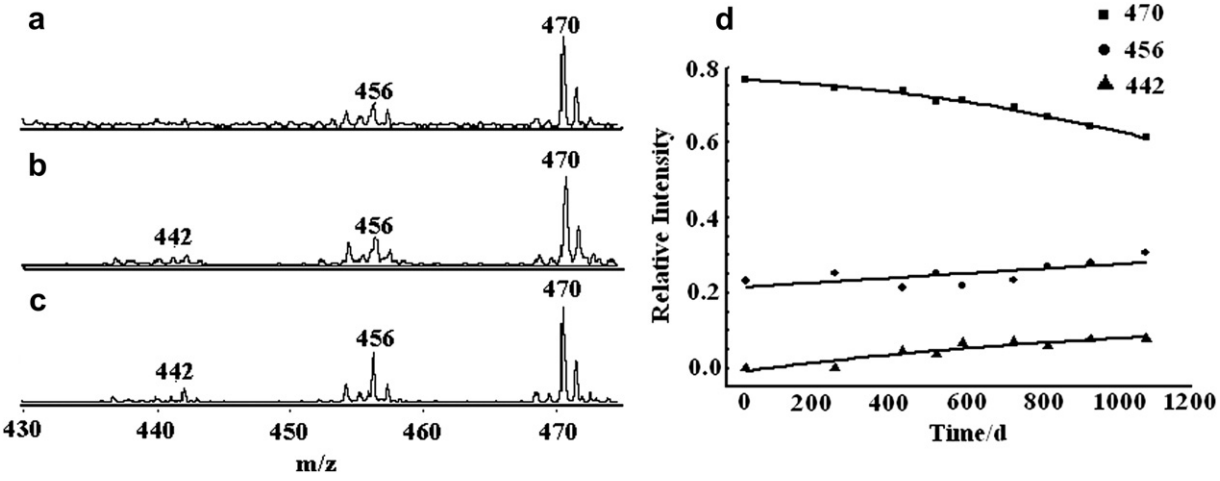

Fig. 8. The representative LDI-MS spectra of the G2 blue gel pen ink entries stored in natural condition for 0 d (a), 585 d (b) and 1080 d (c), and the relative intensities of the Basic blue 26 and its degradation products versus natural aging time. $n = 5$, RSD < 3% for $m/z = 470$ and RSD < 7% for $m/z = 456$ and 442.

($m/z = 422$) was not emerged until the ink entries stored in natural condition for more than 700 days, while the product was formed when the ink entries exposed to artificial light conditions for 25 h. The relative intensities of the main dye component, Basic blue 7, gradually decreased with the aging time, and those of $m/z = 450$ slowly increased (see Fig. 7d). It can also be seen from Fig. 7d that the relative intensities for the other two components ($m/z = 434$ and 422) also slightly increased with the aging time, and it indicated that the two components originated from the degradation of the Basic blue 7.

Although the degradation rate for the Basic blue 7 was slow, the variations of its relative intensities were significant in a long period of time, and about 20% of the dye component has been degraded in natural storage condition for 1803 days (approximately 5 years). So, it is possible to determine the age of the ink entries from the variations of its relative intensities.

Fig. 8 shows the LDI-MS spectra of the blue ink entries (G2) before and after stored in natural conditions and the variations of the relative intensities for the dye components. The three components in the mass spectra were same as those illustrated in Fig. 6. It can be seen from Fig. 8d that the degradation of the main dye component, Basic blue 26, was significant under natural storage conditions, and its relative intensities were linearly decreased with prolonging the aging time, while those of the component ($m/z = 456$) gradually increased. The degradation characteristics of the dye components provided a chance to probe the ages of the blue ink entries. The component of $m/z = 442$ appeared when the ink entries stored in natural conditions for about 400 days, and then its intensities slightly increased with the aging time.

4. Conclusions

The approaches for differentiation and dating of the gel pen ink entries on paper by LDI-TOF-MS method have been developed. 40 Black and 5 blue gel pen ink entries on paper have been individually discriminated based on the differences of their LDI-MS spectra. The developed approaches were rapid and nondestructive, and suitable to differentiate the gel pen ink entries on paper. The degradation mechanisms of the dye components in the gel pen ink entries under artificial light and natural storage conditions have been investigated by LDI-MS method, and the results showed that the dye components underwent a significant degradation. The changes of the dye compositions in the ink entries were almost linear with the aging time, and it indicated that the LDI-TOF-MS method was a feasible mean to be potentially used for dating the gel pen ink entries on questioned documents.

Appendix A. Supplementary information

Supplementary information associated with this article can be found in the online version, at doi:10.1016/j.dyepig.2012.03.005.

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