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Short Communication

Separation of crystal violet dyes and its application to pen ink analysis using CZE and MEKC methods

A crystal violet (CV) standard was irradiated under a Hg–Cd lamp for different exposure times to obtain various N-demethylation products. CZE effectively separated the photodegradation products based on molecular weight differences. In contrast, micellar EKC (MEKC), using SDS as the surfactant, was ineffective because the binding constants of the demethylation products and SDS were too close for separation. Nevertheless, MEKC analysis of ink has applications in forensic science because MEKC separated neutral components in the inks. Thus, MEKC can be used to obtain an ink “fingerprint” since each ink is unique depending on the location and time it was made. In contrast, CZE is useful for dating inks because CV is the primary ink dye and it photodegrades slowly.

Keywords: Crystal violet / CZE / Ink / MEKC / Methyl violet

Received: October 16, 2007; revised: December 6, 2007; accepted: December 7, 2007
DOI 10.1002/jssc.200700509

1 Introduction

Ballpoint pen inks are viscous, prohibiting the use of HPLC and GC in their analysis, although these methods have played important roles in related fields [1–5]. Ballpoint pen inks are manufactured from a wide variety of materials that exhibit different chemical properties. Currently, methyl violet (MV) is the primary dye used in the synthesis of blue and violet inks. In general, MV is a mixture of tetramethyl-, pentamethyl- and hexamethyl-para-rosaniline (PR). Hexamethyl-PR is also known as MV 10B, or as crystal violet (CV); pentamethyl- and tetramethyl-PR are known as MV 6B and MV 2B, respectively (Fig. 1). By blending the different varieties of MV, the dye maker can create various shades of ink. In fact, under visible-light irradiation, CV photodegrades to form a series of N-demethylation products, including PR (a magenta dye); use of TiO₂ catalysts accelerates photodegradation of CV after irradiation [6–8]. Compositional information about inks, such as the degree of photodegradation of the dyes, may be used in the investigation of counterfeiting, fraud, forgery, and other crimes. Thus far, infrared spectroscopy [9–11], Raman spectroscopy [11–13], infrared luminescence [14, 15], and MS [16–18] are valuable tools for the examination of documents. In this study, the CV standard was irradiated to obtain various N-demethylation products that were then separated and characterized using CZE and micellar EKC (MEKC). These methods were also investigated for their abilities to determine MV in ballpoint pen ink samples.

2 Materials and methods

2.1 Chemicals

Phosphoric acid, sodium phosphate (dibasic), MV, methanol, CV, cetrimonium bromide (CTAB), and ACN were obtained from Acros (New Jersey, USA). SDS, sodium phosphate (monobasic), and α-cyano-4-hydroxycinnamic acid (CHCA) were obtained from Sigma (St. Louis, MO, USA). Hydroxylamine hydrochloride and citric acid were purchased from Fluka (Bauch, Switzerland) and Yakuri Pure Chemical (Osaka, Japan), respectively. Sodium hydroxide and sodium chloride were purchased from J. T. Baker (Mallinckrodt Baker, USA). All other chemicals were of analytical grade and were commercially available.

2.2 Apparatus

The CE/UV setup, using a JASCO 870-CE detector (λ⁺ = 209 nm) and data acquisition system, was similar to a previously described setup [19]. The detailed methods of the linear TOFMS, including the spectrophotometer design (modified Wiley-McLaren, R. M. Jordan, Grass
Valley, CA), the laser source (Nd: YAG, 355 nm), and the data acquisition system previously have been described in detail [20]. A Shimadzu Hg–Cd lamp was the light source (λ_{max}, 546 nm) in the artificial photodegradation experiments; concentration level of the test CV standard solution was 100 ppm, located at 15 cm in distance in the front of the light.

3 Results and discussion

3.1 Photodegradation and separation of MV dyes

CV (MV 10B) is an important dye with applications in many fields. CV is a well-known pH-indicator; acid aqueous solutions appear violet to blue or yellow, depending on pH values, whereas alkaline aqueous solutions are colorless due to the formation of the carbinol base. To optimize the conditions for separation, the MV (10 ppm; a mixture of MV10B, 6B, and 2B) and PR (10 ppm) standards were tested under different CE conditions. The findings show that the separation efficiency depends on the CE solution, including the type of buffer and the pH of the solution. This is because a mixture of primary, secondary, and tertiary amines, in general, is difficult to be separated. We found that, in this case, the use of phosphate solution (at lower pH value) provides better separation efficiency than that of citrate or borate solution. Figure 2 shows typical electropherograms of the MV and PR standards obtained by CZE under various pH conditions with an applied voltage of +15 kV (electropherograms a–d: pH 8.6, 6.9, 4.5, and 2.2, respectively, prepared using

Figure 1. Chemical structures of CV (MV 10B), MV 6B, MV 2B, and PR.

Figure 2. CZE electropherograms of MV and PR standards obtained by CZE under various pH conditions; electropherograms a–d: pH 8.6, 6.9, 4.5, and 2.2, respectively. CE conditions: capillary, 50 μm id; total/effective length, 80/60 cm; applied voltage, +15 kV; sample concentrations: MV (10B, 6B, 2B) and PR, each 10 ppm; solution: 60 mM phosphate.
a phosphate solution). At higher pH values, the EOF was increased, leading to a poor separation since the analytes moved too quickly to be separated (electropherograms a and b). The optimal pH was 4.5, as shown in electropherogram c; the migration order followed the mass per charge ratio \( m/z \). However, at lower pH values, the \( m/z \) ratios of MV dyes and PR are changed. For CV (MV 10B), excess acid blocked the amino groups, and as a result, dication (blue) and trication (yellow) compounds formed. The formation of multivalent cations altered the mass-to-charge ratio \( m/z \), reversing the migration order, as shown in electropherogram d (pH 2.2). However, under conditions of acid excess, PR reacted with phosphoric acid, causing disintegration of the PR peak, as shown in electropherogram d. The inset shows that this broad zone was increased after spiking with higher concentration (30 ppm) of PR, confirming that a low pH environment is not suitable for separating PR from dye mixtures. In fact, MV may be a natural photodegradation product of CV. Low purity CV may be contaminated with MV. In order to fully optimize the separation conditions for the photodegradation products of CV, various CE buffers were examined. The test samples were obtained by irradiating the CV standard (100 ppm) under a Hg–Cd lamp for several days. Figure 3A shows the CZE separation obtained for the artificial degradation products (electropherograms a–d: before exposure, 3, 6, and 12 days, CE conditions as described in Fig. 2-c). Additional peaks gradually appeared in the separation of the artificial degradation products. The inset shows the dating ratio of these dyes. To complement the CZE analysis, the artificial degradation products were also examined using MALDI-TOFMS (matrix, CHCA; laser source Nd: YAG, 355 nm), as shown in Fig. 3B (major fragments of CHCA are indicated as *). During the degradation process, methyl groups were slowly removed and replaced with protons, leading to a loss of 14 amu. When the exposure time was longer than 14 days, six methyl groups were lost to give a mass peak at 288 \( (m/z) \), as shown in the inset of Fig. 3B. This mass peak may represent PR; meanwhile, the mass peak of CV \( (m/z, 372) \) almost disappeared. Thus, CZE and MALDI-TOFMS are useful for examining the degradation
of CV and especially for the dating of inks that contain CV. This is also helpful for backdating by demonstrating that the ink on a document was not available until after the document was purportedly produced.

3.2 Comparison of a blue ink analysis by CZE and MEKC

An ink sample was obtained from a commercial blue ink ballpoint pen, diluted in methanol and immediately analyzed. Figure 4-a shows a typical CZE electropherogram of the ink; CE conditions were the same as those described in Fig. 2-c. The ratio of CV: MV 6B:MV 2B was very similar to that observed for the photodegradation products of CV, as shown in Fig. 3B-g. Thus, it was concluded that the major dyes in the blue ink of a commercial ballpoint pen are MV dyes, which could be prepared from MV or from natural photodegradation of CV. However, CZE does not allow for observation of numerous neutral ingredients, such as oils, desiccants, plasticizers, waxes, and other additives. Thus, CZE is not suitable for separating neutral ingredients in the ink. In fact, a broad band was evident at ~35 min migration time. In other words, CZE is useful for examining the degree of degradation, i.e., ink dating, but is not helpful for the recognition of different types of inks. In contrast to this, MEKC allows for distinction between inks. Zlotnick and Smith [21] have been reported on the preliminary analysis of some black rollerball pen inks using the MEKC method, in which an SDS containing borate buffer was used. Based on this, however, we found that the use of a phosphate solution also provided satisfying separation efficiency. Figure 4B b–d shows typical MEKC electropherograms of the ink, using SDS as the surfactant in an acidic CE solution. Applied voltage was ~15 kV in each case. In electropherogram 4b, the buffer solution consisted of 60 mM SDS and 30 mM H3PO4 in a water/ACN/methanol (80:10:10, v/v) solution. In electropherogram 4c and d, the solutions were changed to a water/ACN (90:10, v/v) and aqueous solution, respectively. It is found that only few peaks can be observed when an aqueous solution was used. This suggested that numerous neutral ingredients in ink are hydrophobic materials. This is the reason why organic modifiers are important and needed for their separation. It can also be imagined that a nonaqueous MEKC mode could be useful for ink analysis since it can provide different types of ink “fingerprints” in forensic science. The inset shows MEKC electropherogram of standard solution for comparison; CE condition was described as electropherogram 4b. It is found that numerous neutral ingredients were separated in electropherogram 4b. Unfortunately, the peaks of MV violet dyes (including MV-10B, -6B, and -2B) could not be separated. This is because the binding constants of MV dyes and SDS were too similar [22] in these testings. The conditions for separation of the MV dyes varied, changing the pH, increasing the migration time, adding organic solvents, and using different types of surfactants (CTAB). Changing the separation conditions did not allow for separation of the MV dyes. However, taking an optimistic view of this, CZE and MEKC each has a unique advantage in ink analysis. The data obtained from MEKC can be used as a fingerprint to identify an ink source, whereas CZE is useful for dating inks by measuring the degree of photodegradation.

4 Concluding remarks

This study successfully evaluated different methods of pen ink analysis, CE (under CZE and MEKC modes) and MALDI-TOFMS. Various N-demethylation products of CV were separated using CZE and identified by means of MALDI-TOFMS. These methods are useful in dating inks. Unique CE-patterns, including migration times, related intensities of neutral materials detected by MEKC can be
used to create ink fingerprints in forensic science. The methods discussed in this paper may solve problems that are frequently encountered in pen ink analysis.

This work was supported by grants from the National Science Council of Taiwan under contracts no. 95-2745-M-003-001.

5 References