—Technical Note—

Forensic Discrimination of Yellow in Similarly Colored Single Wool Fibers by Ultraviolet–Visible–Microspectrophotometry and Thin–Layer Chromatography

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(Received 8 October 2009; accepted 12 January 2010)

During the identification of single fiber evidence, color evaluation is one of the most important examinations. Colors are usually first evaluated by ultraviolet–visible–microspectrophotometry (UV–vis–MSP). If possible, thin–layer chromatography (TLC) or high performance liquid chromatography (HPLC) are conducted after the extraction of dyestuffs from the single fiber. In this report, three woolen single fibers were dyed by similarly colored dyes with structurally different yellow acid dyes and were used to evaluate the discriminatory abilities of UV–vis–MSP and TLC.

Key words: Acid dye, Wool, Vis–microspectrophotometry, Uv–vis–microspectrophotometry, Microspectrofluorometry

Introduction

Fibers, especially single fibers, retrieved from crime scenes, are important trace physical evidence. They are frequently encountered in the forensic science laboratory. These single fibers can prove contact between the victim and suspect, behavior of the suspect at the crime scene and the relation of suspect and/or victim to the crime scene¹⁾. Because they are transferred unconsciously and deposited at the crime scene, they have quite high evidential value in forensic examination. Color is one of the most important characteristics for discrimination of single fibers. Depending on the sample amount, several methods can be utilized for single fiber color comparisons, and multiple method analysis is a requirement from prosecutors.

In Japan, there are 47 prefecture forensic science laboratories and one national institute. All the prefecture forensic science laboratories

are equipped to perform visible – microspectrophotometry (vis–MSP) and the national institute has two instruments to conduct ultraviolet–visible microspectrophotometry (uv–vis–MSP) and one for microspectrofluorometry. If there is not enough sample to allow extraction of dyestuffs, then the sample is analyzed using vis or UV – vis – MSP and / or microspectrofluorimetry^{2–7)}. If the single fibers are longer than 5 mm, then the extraction of dyestuffs for thin-layer chromatography (TLC)^{8,9)} or high performance liquid chromatography (HPLC)^{10–14)} can be considered. Recently, mass spectrometer combined with HPLC (LC/MS)^{15,16)} and pyrolyzer and gas chromatograph (Py-GC/MS)^{17,18)} have been playing an important role in this field.

There are many reports regarding color evaluation of single fibers, but none have evaluated the discriminatory ability of UV–vis–

MSP and compared dyestuffs using TLC or HPLC after extraction from the single fiber. These methods have both advantages and disadvantages for discrimination of dyestuffs. The MSP methods are non-destructive and require quite short fiber lengths, but they cannot give information on the dye components present. In comparison, the dye components can be analyzed by TLC or HPLC when a suitable extraction solvent, developing solution (TLC), column and mobile phase (HPLC) are selected. As previously reported^{10–14}, it is difficult to use dyestuffs extracted from a single fiber to compare cellulose fibers dyed by reaction dyes, especially for black fibers where the color is typically composed of several other dyestuffs colors. In this experiment, we compared the discriminatory ability of vis-MSP, UV-vis-MSP and TLC for wools dyed by yellow acid dyes with known structures and that could not be discriminated using a binocular microscope.

Materials and methods

Sample fibers dyed by yellow acid dyes

The yellow fibers were sourced from Bundesriminalamt (Wiesbaden, Germany). These fibers were kindly supplied as specially colored woolen fibers dyed by similar yellow acid dyes, from dye manufacturer. They were dyed by yellow acid dyes with color index numbers (CI) and Chemical Abstracts compound numbers (CAS) as follows: sample 1, acid dye CI Y-11 (CI 18820, CAS No. 6359-82-6); sample 2, acid dye CI Y-23 (CI 19140, CAS No. 1934-21-0); and sample 3, acid dye CI Y-25 (CI 18835, CAS No. 6359-85-9). Their structures are shown in Fig. 1.

Uv-vis Microspectrophotometry

A MPM-800 type microspectrophotometer (Carl Zeiss, GmbH, Jena, Germany) with a quartz light path was used for vis- and UV-vis-MSP. Samples of single fibers were embedded in glycerin and transmittance strengths were measured in 2.5 nm wavelength steps. Measured wavelength range was 350–740 nm for vis-MSP and 240–740 nm for UV-vis-MSP.

Fig. 1. Structures of yellow acid dyes used in this experiment:

a: CI Y-11 (CI 18820); b: CI Y-23 (CI 19140); c: CI Y-25 (CI 18835).

Extraction and TLC analysis of dyestuffs

Dyestuffs used in coloring the single fibers were extracted according to previously reported protocol⁶. Yellow dyed single woolen fiber was placed in a sealed glass tube (1 mm diameter) with extracting solvent (about 100 μ l of 5 % aq. ammonia) and this was put in a water bath at 100°C for 30 min with sonication. A TLC developing solvent that showed high discrimination for acid dyes was selected: H₂O: 28% aq. ammonia: CHCl₃: methanol (1:1:11: 7, by vol.) according to the previously reported⁸. The TLC plate, Kiesel Gel HPTLC (Merck, Co. Ltd., Germany), without fluorescent indicators was used. The separated dyes were observed by the back lighting method⁸. All chemicals used in this experiment were of analytical grade and purchased from

Wako Pure Chemicals, Co. Ltd. (Osaka, Japan).

Results and Discussions

Vis- and UV-vis-microspectrophotometry

Comparison of the visible transmittance spectra obtained from three yellow fibers allowed discrimination of sample 1 (spectrum a) from samples 2 (spectrum b) and 3 (spectrum c) shown in Fig. 2.

The visible region transmittance spectra between 350–450 nm were different between sample 1 (spectrum a in Fig. 2) and the other two samples. However, between samples 2 (spectrum b in Fig. 2) and 3 (spectrum c in Fig. 2), no significant difference was observed in this region. In the previous report²⁾, intra-sample variation of was observed in the transmittance intensity of single fiber, however showed identical wavelengths for all peaks, above reason the transmittance intensities were not used for differentiation. By only the results obtained from vis-region of microspectrophotometry, color of samples 2 and 3 was identical, however, UV-vis MSP enabled discrimination of this two samples using the ultraviolet region between 240–340 nm, slight differences were observed in the ultraviolet region at 310–330 nm of the transmittance spectra as shown in Fig. 3.

From 2000 years, the importance of ultraviolet region in microspectrophotometric analysis of color of single fiber was reported⁴⁾.

As mentioned, samples 2 and 3 could not be discriminated using only the visible-region between 350–750 nm by vis-MSP. However, this is the type of microspectrophotometric result available in the prefecture forensic science laboratories in Japan. The results obtained here suggest that while using only vis-MSP to identify fiber samples could produce misleading results, adding UV-vis-MSP to the identification protocol eliminates the misinterpretations of similar colored of single fibers.

TLC of extracted dyes and dyestuffs identification

For comparison, the dyestuffs used to the about 5 mm of each yellow colored woolen single

Fig. 2. Spectra from visible region microspectrophotometry of woolen yellow fibers dyed by acid dyes.

a, b and c correspond to the dyes shown in figure 1.

Fig. 3. Spectra from ultraviolet-visible region microspectrophotometry of woolen yellow fibers dyed by acid dyes.

a, b and c correspond to the dyes shown in figure 1.

fiber was extracted by about 30 μ l of 28% aq. ammonia in a sealed capillary tube, 100°C, 30 min in water bath, according to previously reported protocol⁸⁾. Following this extraction, utilized woolen fibers dyed by yellow acid dyes were changed to colorless. This phenomenon

Table 1 R_f values of extracted dyes from wools dyed by Y-11, Y-23 and Y-25.

	Y-11 (a)	Y-23 (b)	Y-25 (c)
<i>Rf</i> values	0.6	0.1	0.33
Developing solvent: H ₂ O : 28% ammonia : CHCl ₃ : methanol (1 : 1 : 11 : 7, by vol.) Plate: Kiesel Gel HPLC plate

Fig. 4. Result of thin layer chromatography of three yellow dyes

- 1: CI Y-11 (CI 18820), 2: CI Y-23 (CI 19140),
3: CI Y-25 (CI 18835)

was considered that the dyestuffs used were completely extracted. The extracted solvent was evaporated under a nitrogen stream at 30°C using a heating block and the residue was re-dissolved in 20 μ l of $CHCl_3$ and spotted onto a HPTLC plate. After development, each spot was observed using backlighting. The R_f values from the TLC plates are detailed in Table 1 and Fig. 4.

Conclusion

When identifying single fiber physical trace evidence, color is one of the most important characteristics to prove their identity. The first step in evaluating color usually involves MSP and comparison of transmittance spectra. TLC can also be used but it involves destruction of the sample with the extraction of dyestuffs from the single fibers. Suitable extraction solvents and developing solvents also need to be selected. However, the results obtained from TLC are

quite useful, because the individual components of the dyes can be clarified. A disadvantage of TLC is that it requires c.a. 5 mm lengths of single fibers. HPLC can supply more detailed information in regard to the dye components, but sometimes dyestuffs are retained on the reversed-phase octadecylsilane (ODS) column used. This is despite pre-examination results of high R_f using ODS-HPTLC plate and developing solvents identical to the mobile phase of HPLC. Furthermore, the dyestuffs could not be eluted during clean up of the column using isopropanol. Results from these experiments indicate that if the fiber is long enough for extraction, the used dyes used can be speculated, then TLC shows high discriminatory ability for similarly colored yellow single fibers. However, in cases when the fibers are quite short, shorter than 3 mm, the most suitable first choice is analysis by UV-vis MSP. Analysis using only depending on the result of vis-MSP could not be eliminated the possibility of misleading the investigation and careful attention is required if only the results of vis-MSP is used for identification.

These color evaluation methods are applied to about 3 mm to 5 mm single fiber samples which diameters were up to 30 μ m, by this reason, quantitative evaluation is impossible. However, the dyestuffs mixture sample c.a. green or black single fibers, etc. shows many spots on TLC plates. But as one of the disadvantages of TLC, this method was totally destructive analysis. For the single fiber analysis, several kinds of another examinations, such as infra-red spectrometry, scanning electron microscopic observation, and elemental analysis by energy dispersive X-ray analysis should be conducted to prove their identity, and for the court trial, all sample consumption should be prohibited.

References

- 1) Suzuki, S., Higashikawa, Y., Sugita, R. and Suzuki, Y.: Guilty by his fiber: suspect confession versus textile fibers reconstructed simulation. *Forens. Sci. Int.* **189**, e27–e32

- (2009).
- 2) Adolf, F-P. and Dunlop, J.: Forensic examination of Fibres (Eds. Robertson, J. and Grieve, M.), pp. 65–86, Taylor and Francis (1999).
 - 3) Suzuki, S., Higashikawa, Y., Suzuki, Y. and Marumo, Y.: Identification of single fiber 4. Microspectrophotometric discrimination of single fibers by ultraviolet visible transmittance profiles. *Ann. Rep. Nat. Res. Inst. Police Sci.*, **46**, 21–32 (1993) (in Japanese).
 - 4) Houck, H. M.: Visual examination of color. *Forens. Sci. Rev.*, **17**, 39–41 (2005).
 - 5) Suzuki, S., Marumo, Y. and Adolf, F. P.: Identification of single fiber 5. Microspectrophotometric discrimination of black single fibers using absorbance profiles of ultraviolet and visible region. *Ann. Rep. Nat. Res. Inst. Police Sci.* **47**, 39–45 (1994) (in Japanese).
 - 6) Suzuki, S., Suzuki, Y., Ohta, H., Sugita, R. and Marumo Y.: Microspectrophotometric discrimination of single fibers dyed by indigo and its derivatives using ultraviolet-visible transmittance spectra. *Sci. & Justice*, **41**, 107–111 (2001).
 - 7) Grieve, M. C. Bierman, T. W. and Schaub, K.: The use of indigo derivatives to dye denim material. *Sci. & Justice*, **45**, 15–24 (2006).
 - 8) Wiggins, K., Palmer, R., Hutchinson, W. and Drummond, P.: An investigation into the use of calculating the first derivative of absorbance spectra as a tool of forensic fibre analysis. *Sci. & Justice*, **47**, 9–18 (2007).
 - 9) Suzuki, S., Higashikawa, Y., Kishi, T. and Marumo, Y.: Identification of a single fiber 1. The extraction and discrimination of dyestuffs by thin-layer chromatography. *Ann. Rep. Nat. Res. Inst. Police Sci.*, **44**, 40–58 (1991) (in Japanese).
 - 10) Wiggins, K. and Drummond, P.: Identifying a suitable mounting medium for the use in forensic fibre examination. *Sci. & Justice*, **47**, 2–8 (2007).
 - 11) Suzuki S., Higashikawa, H., Suzuki, Y. and Marumo, Y.: Identification of a single fiber 2. Analysis of dyestuffs in single fibers by high performance liquid chromatography. *Ann. Rep. Nat. Res. Inst. Police Sci.*, **45**, 36–45 (1992) (in Japanese).
 - 12) Logan, B. K.: Liquid chromatography with photodiode array spectrophotometric detection in forensic sciences. *Anal. Chim. Acta*, **288**, 111–122 (1994).
 - 13) Suzuki, S., Suzuki Y., Higashikawa, Y., Kishi, T. and Marumo Y.: Analysis of dyestuffs in a single fibers by high performance liquid chromatography using multi-wavelength detection method. *Anal. Sci.*, **7** (suppl.) 117–120 (1991).
 - 14) Novotna, P., Pacakova, V., Bosakova, Z. and Stulik, K.: High-performance liquid chromatographic determination of some anthraquinone and naphthoquinone dyes occurring in historical textiles. *J. Chromatogr. A*, **863**, 235–241 (1999).
 - 15) Suzuki, S., Higashikawa, Y., Suzuki, Y. and Y. Marumo, Y.: Identification of single fiber 3. Discrimination of black fibers by high performance liquid chromatography with multi-wavelength detection. *Ann. Rep. Nat. Res. Inst. Police Sci.*, **46**, 46–52 (1993) (in Japanese).
 - 16) Tuinman, A. A., Lewis, L. A. and Sr. Lewis, S. A.: Trace fiber color discrimination by electrospray ionization mass spectrometry: A tool for the analysis of dyes extracted from submillimeter nylon fibers. *Anal. Chem.*, **75**, 2753–2780 (2003).
 - 17) Zhang, X., Boytner, R., Cabrera, J. L. and Lausen, R.: Identification of Yellow dye Types in pre-columbian Andean textiles. *Anal. Chem.*, **79**, 1575–1582 (2007).
 - 18) Causin, V., Marega, C., Schiavone, S., Guardia, V. D. and Marigo, A.: Forensic analysis of acrylic fibers by pyrolysis-gas chromatography / mass spectrometry. *J. Anal. Appl. Pyrolysis*, **75**, 43–48 (2006).
 - 19) Badri, B.: The influence of reactive dyes on the pyrolysis of cotton. *J. Anal. Appl. Pyrolysis*, **81**, 162–166 (2008).