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TECHNICAL NOTE

QUESTIONED DOCUMENTS

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Characterization and Dating of Blue Ballpoint Pen Inks Using Principal Component Analysis of UV–Vis Absorption Spectra, IR Spectroscopy, and HPTLC

ABSTRACT: The ink of pens and ink extracted from lines on white photocopier paper of 10 blue ballpoint pens were subjected to ultraviolet–visible (UV–Vis) spectroscopy, infrared (IR), and high-performance thin-layer liquid chromatography (HPTLC). The R_f values and color tones of the bands separated by thin-layer chromatography (TLC) analysis used to classify the writing inks into three groups. The principal component analysis (PCA) investigates the pen responsible for a piece of writing, and how time affects spectroscopy of written ink. PCA can differentiate between pen ink and ink line indicates the influence of solvent extraction process on the results. The PCA loadings are useful in individualization of a questioned ink from a database. The PCA of ink lines extracted at different times can be used to estimate the time at which a questioned document was written. The results proved that the UV–Vis spectra are effective tool to separate blue ballpoint pen ink in most cases rather than IR and HPTLC.

KEYWORDS: forensic science, ink, ultraviolet–visible spectroscopy, infrared, high-performance thin-layer chromatography, dating, principal component analysis

Ink analysis is an important forensic procedure that can reveal useful information about questioned document. Modern inks contain many substances can improve ink characteristics (1–3). The most important component is the coloring material which comes in the form of dyes, pigments, or their combination. Dyes are soluble in the liquid body of the ink which is also known as the vehicle. On the other hand, pigments are finely ground multimolecular granules that are insoluble in the vehicle. The vehicle can consist of oils, solvents, and resins whose composition affects the flowing and drying characteristics of the ink. Other substances used for finely tuning the characteristics including driers, plasticizers, waxes, greases, soaps, and detergents (4).

The techniques regarding the analysis of inks can be divided into nondestructive and destructive approaches. The nondestructive method, however, is preferable but it is limited to the forensic examiner. Among these, the use of infrared (IR) absorption and luminescence spectra (2) carried out in a video spectral comparator is highly successful particularly for black inks. Other nondestructive techniques are Raman (5), surface-enhanced resonance Raman spectroscopy (6), and ultraviolet–visible (UV–Vis) microspectrophotometry (2).

For blue ballpoint pen ink, in particular, the UV–Vis absorption spectroscopy or IR spectroscopy is immensely informative and the spectrum from around 200 to 700 nm in UV–Vis spectroscopy or 4000 per cm to 500 per cm in IR spectroscopy is reliable, and quantitatively measures the mix of dyes in that sample. This technique is straightforward to execute and provides fairly rapid and repeatable results.

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The destructive analysis start by removal of a small section of the ink line followed by solvent extraction of the ink opens up many more avenues of analysis. In particular, the chromatographic separation of ballpoint pen ink into its constituent component dyes has proved a highly productive method, not only for the comparison of inks but also for the matching of an ink to a database of chromatograms (7–14). Thin-layer chromatography (TLC) is widely used as it is rapid and requires no sophisticated instrumentation (1,5). The limited resolution and difficulties with quantification of TLC technique have led to the application of high-performance thin-layer liquid chromatography (HPTLC) (4) and capillary electrophoresis (6) to ink analysis.

Recently, chemometrics and, in particular, multivariate analysis, has been used successfully in a limited number of cases to aid the forensic examination of inks. Principal component analysis (PCA) was one of the applied methods (4) in their interpretation of visible spectrophotometry data from a range of blue ballpoint pen inks.

The wide array of materials used in inks coupled with possible contamination from the writing surface confronts forensic ink chemists with a complex analytical challenge to carry out this type of analysis. But the aim of most analyses is to determine whether two pieces of written text originated from the same ink; therefore, comparison of different writing inks on a document is the main goal of the most investigations. The present work aims to evaluate the potential for the use of PCA in the forensic analysis of blue ballpoint pen inks, using UV–Vis spectrophotometry data, IR spectroscopy, and HPTLC. This study evaluates the discrimination between different pen inks and to identify the pen responsible for a piece of writing by matching with database of ink spectra, and examines the effect of the time on the spectroscopy of the written ink.

Methods and Techniques

Principal Component Analysis

PCA is a multivariate statistical method which allows a large number of sample data sets to be described in terms of a much smaller number of principal components. The first principal component describes the gross average features of the data sets while the second and subsequent principal component introduce further specific features of decreasing significance. A consequence is that the data set for each sample may be constructed by forming an appropriate linear combination of the principal components. This technique facilitates classification, and individualization of sample data sets in an objective and reproducible fashion.

In this work the PCA is applied to a set of UV–Vis absorption spectra, X, obtained from the ink solutions under examination. Each column in the matrix X represents a spectrum, as a function of wavelength, for a particular sample. These spectra are each normalized to a mean of zero and unity standard deviation prior to the PCA calculation, as this ensures that intensity variations as a result of concentration or instrumental effects are removed. The core of the PCA calculation is solving the following equation (15).

$$X = S * L$$

where, S is the scores matrix and L is the loadings matrix. The principal components are given by the columns of S and L while the rows represent the coefficients for each principal component contributing to each original, standardized spectrum within X. The eigenvalues produced reflect the contribution of that principal component to the spectrum. The PCA calculations were carried out within Microsoft Excel (Microsoft Corp., Redmond, WA) incorporating the multivariate add-ins (16).

Experimental Methods

A set of 10 blue ballpoint pens were purchased from markets in Egypt, in March 2008. All pens were allocated a reference number during this study, namely, (1) ROTO, (2) PRIMA FORMA, (3) SCHNEIDER, (4) REYNOLDS, (5) FABER-CASTELL, (6) ZA B 1000, (7) ZEBRA, (8) STICK, (9) GHM 501/P, and (10) Bic. Experimental work was carried out on both the ink from each pen and its ink extracted from lines produced on white photocopier paper. Two standard reference dyes, crystal violet (CV) and methyl violet (MV), were used.

The UV–Vis spectroscopy was carried out on a Shimadzu UV–Vis spectrometer (SHIMADZU SCHWEIZ GmbH, Switzerland) using quartz cuvettes with a path-length of 1 cm. Sample concentrations were optimized to provide a sample absorption maximum of around unity. Ink was taken from each pen by dipping a thin capillary tube into the pen barrel and then depositing the ink removed into a sample tube containing ethanol. Ink lines were extracted from paper by cutting very small round on the paper with a sharp blade and then placing it into the solvent. This extracted sample was used for both TLC and UV–Vis spectra. All spectra were scanned from 200 to 700 nm and all range was used in the PC analysis. The spectra of three types of pen inks were shown in Fig. 1. The scan interval of 1 nm was used. Each spectrum used for analysis therefore contained 501 data points.

The IR spectra were recorded using Perkin-Elmer spectrophotometer (PerkinElmer, Rodgau, Germany); the scanned spectra were covering the frequency range 500–4000 per cm and all range was used in the PC analysis. The spectra of three types of pen inks were illustrated in Fig. 2.

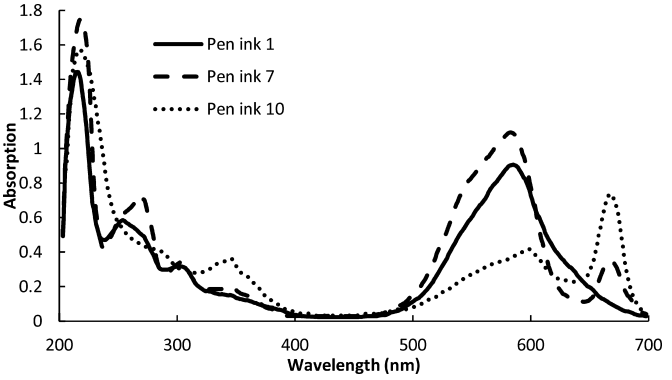

FIG. 1—*The UV–Vis spectra for three types of pen inks 1, 7, and 10.*

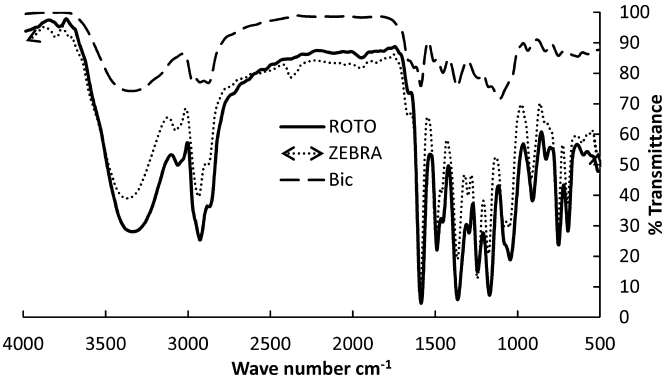

FIG. 2—*The IR spectra for three types of pen inks 1, 7, and 10.*

TLC was carried out using Silica gel/TLC-cards with layer thickness 0.2 mm and 20 × 20 cm aluminum cards. Ten ballpoint pen samples were soluble in ethanol solution. All of spots were 0.6 cm in diameter and the distance between them is 0.5 cm. The origins were at 1 cm from the bases of the plates. The developing solvent was Butanol/Ethanol/Water (5:0.5:1.5). The uniform origin position on the plates, proper loading at the origin, and the solvent saturation in the developing tank were kept during all of the TLC analyses to obtain a high reproducibility.

The HPTLC silica gel plate 60 and CAMAG horizontal developing chambers (Muttenz, Switzerland) were used at the central laboratory (Faculty of Pharmacy, Alexandria University, Egypt) for chromatographic separation of dyes and pigments in ink entries. A CAMAG Linomat IV instrument offered semi-automatic sample application for qualitative and quantitative analyses. Comparison was carried out on a CAMAG TLC Scanner by measurements of reflection in absorbance mode are measured at six wavelengths (215, 305, 347, 547, 585, and 665). Spectra recording, peak detection, and semi-quantitative data were calculated by winCATS software (CAMAG). Ten ballpoint pens and there extracted ink lines were analyzed simultaneously with a standard spot of MV and CV.

Results and Discussion

UV–Vis Spectroscopy Data

PC analysis for the full pen set: UV–Vis spectra were measured for all inks that directly extracted from the 10 pen types and MV and CV as standard reference dyes. The data recorded from 200 to 700 nm were subjected to PC analysis using five components. The first three PCs described 98.69% of the data points. Analysis is

most readily achieved by plotting the loadings for PC3 versus PC1 and PC3 versus PC2 (Fig. 3). Such plots show that the 12 spectra of 10 pens and MV and CV fall into four clusters of varying compression. Both plots include a clearly separated area A (Bic pen no. 10) from the other ones. A second cluster (labeled B) containing two pens (7 and 9) is also well clear of the rest and all spectra are distinct. The third cluster (labeled R) contains the two reference dyes MV and CV. The remaining pens are fairly closely clustered into one group labeled C.

Bearing in mind that PCA works by calculating the weights given to the orthogonal features of each spectrum of the complete set of pens, the differentiation between the pens under investigation based on PC2 and PC3 will be enhanced if these complete sets have similar weights as PC1. This can be achieved by removing some pens (reduced pen set) that are readily discriminated, purely on the PC1 loading. Hence, this PCA calculation was repeated on the set of 1–6, eight pens where clusters A, B, and reference dyes MV and CV are excluded. The results show that the first three PCs described 99.79% of the data. In this case the pen classification can be seen on the basis of PC1, PC2, and PC3 loadings, where these PCs clearly show four groups of pens for the reduced pen set.

These plots show four clusters, labeled D, E, F, and G discriminated on the bases of three PCs. In the previous calculation, these four classes were not so clearly discriminated and were encircled as a single cluster (C) with negative PC3 loadings as shown in Fig. 3. In class D, pen 1 is well discriminated from each other groups. In group E, pens 2 and 8 are closely clustered while they can be discriminated along the PC2 axis. In group F, pens 3 and 6 are also closely clustered while they can be discriminated with the PC3 loadings. Finally, in group G, pens 4 and 5 are also closely clustered but they cannot be discriminated with any PC loadings. As G is a tight cluster, it is reasonable to expect similar variability in the parameters for each of the inks within that group, as they all contain very similar dye components. These conclusions are only indicative but nevertheless this analysis provides a useful indicator of the discriminatory power of that method.

PC analysis for ink lines extracted from the full pen set: The UV–Vis absorption spectra for the ink lines extracted were analyzed also by PCA method. The dyes CV and MV are not been included in this calculation, where only the first three PCs modeled 99.69% of the spectral data. The results of this analysis are best described by a loadings plot of PC3 versus PC1 and PC3 versus PC2. Although the clusters of points are similar in this graph to the corresponding pen inks data (Fig. 3), the scaling and detail are slightly different. This is simply a consequence of the fact that the PCA are specific to the full data set employed for any particular analysis. The discrimination of the inks lines extracted agrees, however, with the previous pen ink analysis: group A, with positive values of PC3, consists of pen 10 while a further group B, with negative PC3, comprises pens 7 and 9, and the remaining pens are fairly closely into group C.

The second application of PCA involves removing of 7, 9 (cluster B), and 10 (cluster A) pens and repeating the calculation. As expected, the first three PCs also describe the spectral features in a more complete fashion (99.89%). The plots of PC3 versus PC2 and PC3 versus PC1 show quite discrimination of pens in group C into possibly four clusters. The first class D contains pen 1 with positive PC2. Also, all the other groups are well differentiated as shown in the group E that contains pens 2 and 8 along PC2 axis. The group F contains pens 3 and 6 are differentiated along PC3 axis. Also group G containing pens 4 and 5 can be differentiated along PC3 axis. Pens 2 and 6 can be clustered in one group where pens 8, 4, and 5 are presumably gathered in one grouped. Consequently, it is suggested the PC loadings may be used to classify and individualize each pen ink or extracted ink line on the basis of PCA.

PCA of analysis for ink pen and ink line: Attempts have been made to apply PCA on the UV–Vis spectroscopy of the blue ballpoint pen inks and their extracted line inks to distinguish between them. The spectral data for pen inks and the corresponding pen lines without MV and CV were subjected to PCA on the basis of potentially five principal components. The results show that the first three principal components described 98.94% of the spectral features. The plot of PC3 versus PC2 (Fig. 4), shows two main points: (i) the data can be grouped into two different groups (pen ink and ink line) and (ii) the loadings of ink lines are more scattered than that of pen inks.

The corresponding scores for the first three principal components (Fig. 5), show that the first PC reflects the dominance of the doublet peak (shoulder overlapped with a peak) coming from the MV dye. In addition, the presence of complementary absorbing dyes at the low wavelength region and a deep red absorbing component around 660 nm are evident. The PC2 moves negatively along the wavelength axis to slightly higher wavelengths while the secondary absorption moves toward the violet/UV range. It also enhances the deep red peak. Also, a positive PC3 moves the complementary absorption toward the violet region, but with little effect than PC2, and it has less effect on the doublet peak or on the red end of the spectrum. The quite link between the first PC and the MV or CV spectra normalized to a mean of zero and unity standard deviation illustrates how the optical absorption of each pen ink, and therefore its color, can be characterized by the relative scores for each of these principal components.

Pen identification using PC analysis: An approach will be discussed to establish the potential of PC analysis of UV–Vis spectroscopy data for individualizing the inks under investigation. It may be used to identify a questioned ink from a database of spectra. Although the loadings and analysis are quantitative of each ink

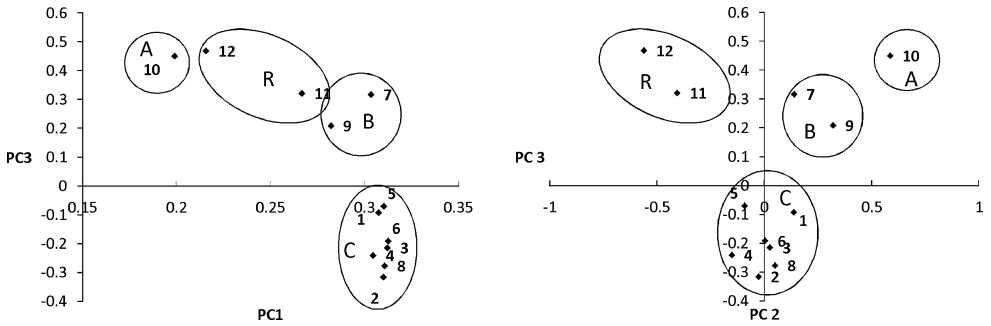

FIG. 3—*Loadings of the PCA for the pen ink of the full pen set.*

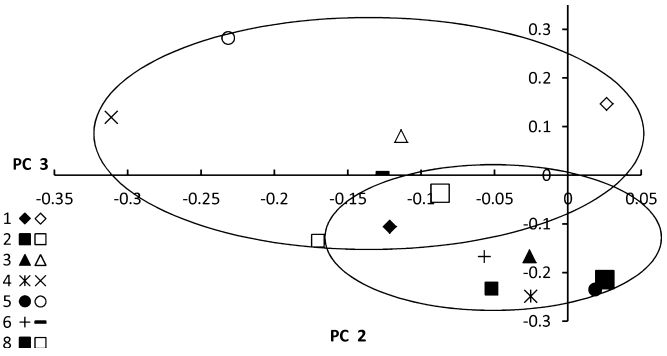

FIG. 4—*Loadings of group C from the PCA for the pen ink and extracted ink lines of the full pen set. (Legend shows number of pen, 1st marker for pen ink, and 2nd marker for ink line, respectively).*

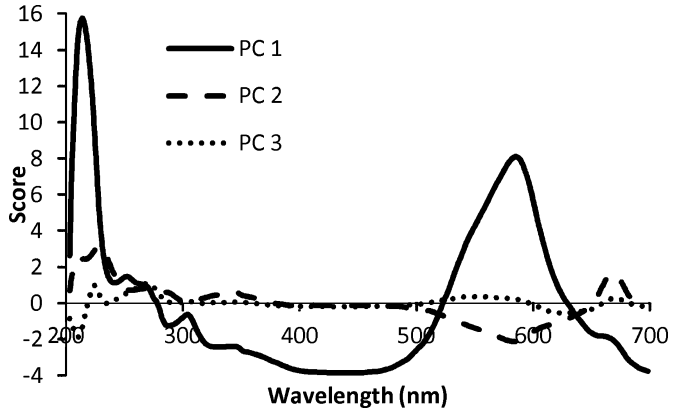

FIG. 5—*Scores from the PCA for the first three principal components.*

sample, these are, of course, not absolute numerical parameters. They depend on the complete set of spectra included in such an analysis. However, if a reference set (contains 10 pen inks) of spectra plus one questioned spectrum are subjected to PC analysis, inks which are spectrally similar to the questioned sample will be has quite the same loadings which are numerically close to the sample itself. This means that the result of such calculation will be a list of reference inks whose dye composition is identical to that of the questioned ink. To characterize a questioned sample is the apple of PCA calculation on a data set compressing a total of 19 spectra. These data set comprising the spectra of 10 ink lines and the 9 spectral measurement of a given three extracted ink lines 1, 7, and 10 each extracted three times. The results of this calculation are shown as loadings plot of PC1 versus PC3 (Fig. 6), while the loadings for the first three principal components are shown in Table 1.

First, all of standard pens (1, 7, and 10) are lies or present in its group. This is owing to the dominance of the MV absorption peak envelope in these spectra. As the mix of MV components varies between inks, the movement of this peak on the wavelength axis (about the mean for the full data set) is reflected in a very small change in the PC1 loading and a consequent change in the PC2 and PC3 loading, in either the positive or negative sign.

Second, the data points on these graphs show that, for the three inks in question, the matching of these loadings between the multiple measurements (standards) and the single reference measurement is good in these cases. Matching between the multiple, standard inks, and the reference is best achieved by examining a full set of loadings.

Effect of time on PCA for ink lines extracted from the full pen set after time in the dark and in the normal condition: (i) In the

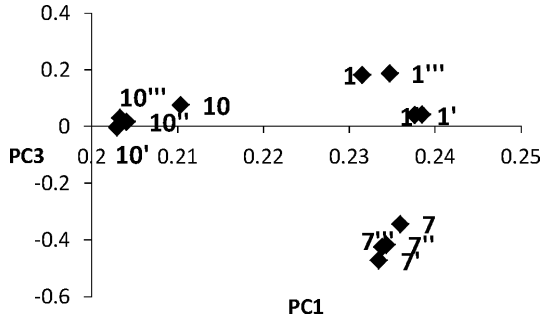

FIG. 6—*Loadings for the ink lines of the pens 1, 7, and 10 from the full pen set plus the multiple measurements on three extracted ink lines standards.*

dark, the PCA was applied to UV–Vis absorption spectra of the 10 ink lines extracted at interval times. The samples were analyzed at 2, 6, 12, and 18 months and showed only the first three PCs modeled 99.03, 99.04, 99.65, and 99.1% of the spectral data, respectively. The results are best described by loadings of PC3 versus PC2. The comparison of these results and with that of the ink lines extracted at zero time showing an observable difference between them. However, the three PC's eigenvalues are completely different. It was observed that the PC's loading plots are scattered in a different way indicating that the time variable lead to change in the UV–Vis absorption spectra of the compounds making pens. Furthermore, that change is increased by increasing the time interval. This postulation is confirmed by another PC analysis using the full set of ink lines extracted at zero time, 6, 12, and 18 months. The results of this analysis are best described by a loadings plot of PC3 versus PC2 (Fig. 7). The analysis of pens 1, 3–9 can be well discriminated from 1′, 3′–9′ on the basis of PC2 with the PC3 loadings while the pen 10 inks which extracted at 0 and 6 months cannot be discriminated. (ii) Similarly in the normal condition, the PCA was applied also to UV–Vis absorption spectra for the ink lines extracted at interval times. The samples were analyzed at 2, 6, 12, and 18 months and showed that only the first three PCs modeled 99.38, 99.23, 99.30, and 99.1% of the spectral data, respectively. The results of these analyses are best described by loadings of PC3 versus PC2. Comparing these results and with that of the ink lines extracted at zero time showing there is difference between them. However, the three PC's eigenvalues are completely different. The PC's loading plots are scattered in a different way indicating that the time variable lead to change in the UV–Vis absorption spectra of the compounds making pens and that change is increased by increasing the time interval. To get a general comment to confirm or not that point, another PC analysis was performed using the full set of ink lines extracted at zero time, after 6 months, 12 months, and 18 months. The results of this analysis are best described by a loadings plot of PC3 versus PC2 (Fig. 8). The analysis of pens 1–10 at different times can be discriminated on the basis of PC2 with the PC3 loadings.

The major aim of the forensic chemistry is determining the time that questioned document written. Using the time effect on the UV spectra of the 10 pens, we can get a relation between the time and the PC loadings. This can be performed as follows; each pen spectra were subjected to PCA with the two references MV and CV at each time studied and then the PC1 loading was correlated with the time using multiple linear correlation analysis. Good correlation was obtained when using the third order equation of time. The correlation coefficient R^2 was found to range from 0.887 to 1.000. The correlation parameters are given in (Table 2). For any questioned document, spectra of extracted ink line can be subjected to PCA

TABLE 1—*Loadings of the principal components for reference samples and standards.*

Ink Line Sample	Loadings		
	PC1	PC2	PC3
Pen 1 reference	0.183	−0.222	0.231
Pen 1 standard	0.040	0.052	0.238
Pen 1 standard	0.044	0.032	0.238
Pen 1 standard	0.189	−0.112	0.235
Pen 7 reference	−0.342	−0.032	0.236
Pen 7 standard	−0.471	−0.092	0.233
Pen 7 standard	−0.415	−0.098	0.234
Pen 7 standard	−0.423	−0.100	0.234
Pen 10 reference	0.077	0.388	0.210
Pen 10 standard	−0.002	0.455	0.203
Pen 10 standard	0.018	0.447	0.204
Pen 10 standard	0.031	0.454	0.203

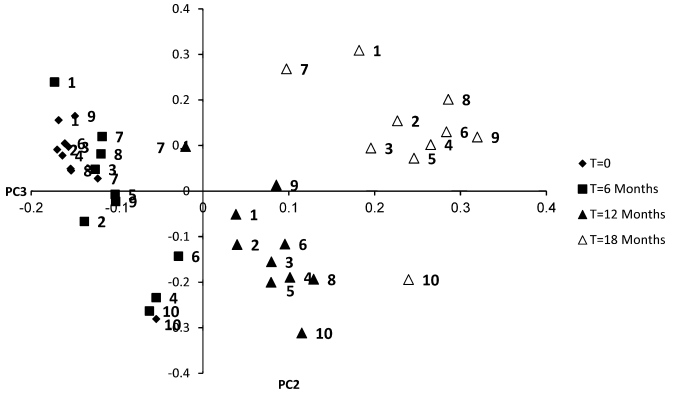

FIG. 7—*Loadings of the PCA for the ink lines extracted at zero time and after 6, 12 and 18 months in the dark.*

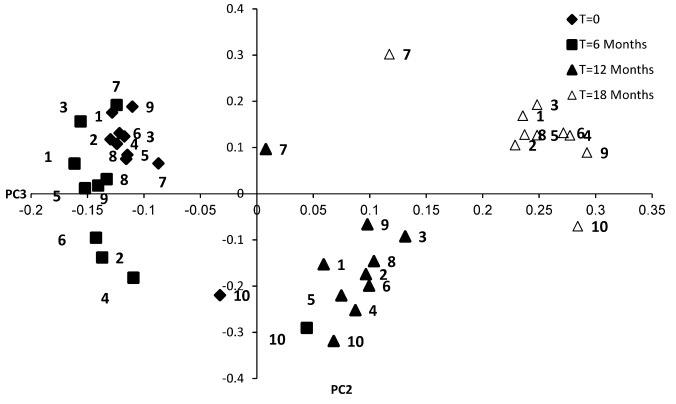

FIG. 8—*Loading of the PCA for the ink lines extracted at zero time and after 6, 12 and 18 months in the normal condition.*

with the two references and the loading of PC1 can be used to solve the third order equation in time analytically to get the time in which that document was written. To confirm this point, spectra of pens 1, 7, and 10 as examples from groups C, B, and A, respectively, were subjected to the PCA with the MV and CV after 4 months. The loading of PC1 was 0.564, 0.565, and 0.487. Using these values in the equations of pens 1, 7, and 10 and solving it for time, the output time was found to be 3.645 months and 3.854 months for pens 1 and 7, respectively. In case of pen 10, owing to it is slightly affected by time especially in the range of 0–6 months (Fig. 8), the correlation equation does not give suitable results in this range.

TABLE 2—*The correlation parameters and the correlation coefficient* R^2 *of the 10 blue ballpoint extracted ink lines at zero time and after 6, 12, and 18 months.*

Pen Number	*a*	*b*	*c*	*d*	R^2
1	0.531018	0.000362	−0.009367	0.038516	0.995
2	0.529471	0.000316	−0.007490	0.014410	0.999
3	0.506407	0.000529	−0.013766	0.064658	0.980
4	0.522203	0.000460	−0.010635	0.020124	1.000
5	0.514025	0.000136	−0.003916	0.026406	0.887
6	0.526291	0.000430	−0.010786	0.034562	0.999
7	0.514025	0.000136	−0.003916	0.026406	0.887
8	0.501237	0.000400	−0.010778	0.048088	0.996
9	0.550431	−0.000004	−0.001879	0.004385	0.999
10	0.273938	−0.000077	0.003018	−0.058264	0.897

IR Spectroscopy Data

PCA for the full blue ballpoint pen set: IR spectra were obtained for all inks directly taken from 10 blue ballpoint pen of different factories and two standard reference dyes, MV and CV. The data, from 4000 per cm to 500 per cm, were subjected to PC analysis using five components. The first three PCs described 98.01% of the data points. Interpretation is most readily achieved here using a plot of the loadings for PC3 versus PC1 and PC3 versus PC2, where the 12 spectra fall into four clusters. A first cluster (labeled A) contains three pens (10, 5, and 8). The second cluster (labeled B) contains three pens (3, 7, and 9). The remaining pens (1, 3, and 6) are fairly closely clustered into one group labeled C. A further cluster (labeled R) contains the two reference dyes, MV and CV.

Similar strategy is performing that a given pens are removed from the set that are readily discriminated, purely on the PC1 loading. Thus, the PCA calculation was repeated on the set of 10 pens except cluster A and the reference dyes MV and CV (Cluster R). The results show that the first three PCs described 98.85% of the data. The pen classification can be seen on the basis of PC1, PC2, and PC3 loadings (Fig. 9), where three groups of pens are identified. A number of conclusions may be drawn from this plot: there are three clusters, labeled F, D, and C, discriminated on the bases of three PCs. In group F, pen 3 is well discriminated from each other groups. In group D, pens 7 and 9 are closely clustered. In group C, pens (1, 2, 4, and 6) are also closely clustered while they cannot be discriminated with the PC3 loadings.

The scores for the first three principal components showed that the first PC reflects the dominance of the peaks arising from the MV dye in lower wave number range (1500–500 per cm). In addition, complementary absorbing dyes are present at the higher wave number region. The second PC shows moving the lower wave number peaks to slightly lower wave number with decay in intensity and it involves a large effect on the peak appears at 2000 per cm. In contrast, the third PC has less effect on the lower wave number peaks. The close link between the first PC and the MV spectrum in lower wave number range, normalized to a mean of zero and unity standard deviation, illustrates how the spectra of each pen ink may be characterized by the relative loadings for each of these principal components.

HPTLC Data

PCA for full blue ballpoint pen set: HPTLC spectra were obtained for all inks directly from the 10 pen inks of different

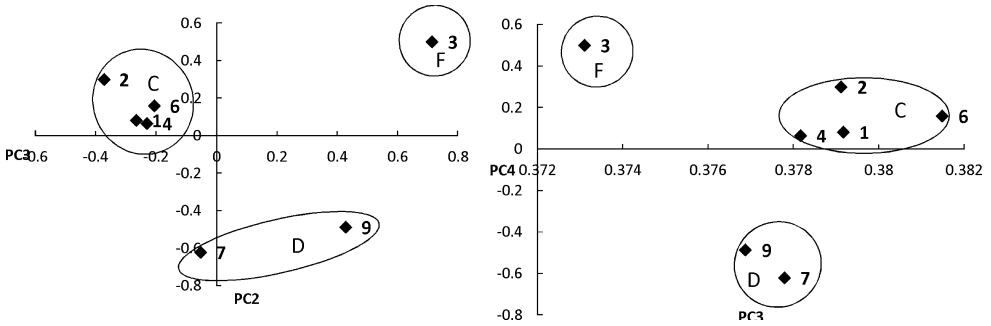

FIG. 9—*Loading for the PCA of IR of the pen ink from the reduced pen set.*

factories and for the two standard reference dyes, MV and CV. The spectra of 10 blue ballpoint pen are measured at 6 wavelengths (215, 305, 347, 547, 585, and 665) and the percentage of the maximum height are considered. The Retention Factor (R_f) values and color tones of the bands of the blue ballpoint inks separated by normal-phase TLC analysis using (Butanol/Ethanol/water) system (5:0.5:1.5) permitted us to classify the writing blue ballpoint inks into three classes (Table 3).

The A, B, and C classes include Bic blue ballpoint pen, ZEBRA, and (ROTO (1), PRIMA FORMA (2), SCHNEIDER (3), REYNOLDS (4), FABER-CASTELL (5), ZA B1000 (6), STICK (8), and GHM501/P (9), respectively. So, applying PCA technique for the third group (group C Reduced set) at nearly constant R_f to separate these pens from each other. The first four PCs described 99.01% of the data points. Interpretation is most readily achieved using a plot of the loadings for PC3 versus PC1. Such plot shows that the spectra of eight pens fall into four clusters of varying compression with individual pens (no. 3: the SCHNEIDER pen, labeled D) and (no. 9: the GHM501/P pen, labeled E). These pens are differentiated mainly on the basis of the PC1 component and the two clusters are separated from each other. A third cluster (labeled F) contains two pens (2 and 8). The last cluster (labeled G) contains four pens (1, 5, 4, and 6).

PCA for ink lines extracted from the full pen set: The spectra of 10 extracted blue ballpoint pen inks are measured at 6 wavelengths (215, 305, 347, 547, 585, and 665) using HPTLC technique and the percentages of the maximum height are considered. The R_f values and color tones of the bands of the blue ballpoint inks separated by normal-phase TLC analysis using (Butanol/Ethanol/water) system (5:0.5:1.5) permitted to classify the writing blue ballpoint inks into various three groups.

The data of the percentage of the maximum height of extracted pen inks of (group C Reduced set) at nearly constant R_f value were subjected to PC analysis. The dyes CV and MV have not been included in this calculation. The first three PCs described 99.34% of the data points. Although the clustering of points is similar in this graph to the corresponding data for the pen inks, the scaling and detail are slightly different. This is simply a consequence of the fact that the principal components are specific to the full data set employed for any particular analysis.

Interpretation is most readily achieved using the loadings for PC3 versus PC2. Such plot shows that the spectra of eight blue ballpoint pens fall into four clusters of varying compression with individual pens (no. 3: the SCHNEIDER pen, labeled D) and (no. 9: the GHM501/P pen, labeled E). The latter pens are differentiated mainly on the basis of the PC2 component, and the two clusters are separated from each other. A third cluster (labeled F) contains two pens (2 and 8). The last cluster (labeled G) contains four pens (1, 5, 4, and 6).

TABLE 3—*Max* R_f *values of HPTLC spots of blue ballpoint pen ink and its extracted lines using Butanol/Ethanol/Water (5:0.5:1.5) system.*

Pen Name	R_fS	R_fE	Color
ROTO	0.57	0.55	Violet
	0.62	0.59	Blue
	0.72	0.71	Violet
	0.81	0.80	Pale violet
PRIMA FORMA	0.57	0.55	Violet
	0.63	0.60	Blue
		0.63	Violet
	0.71	0.72	Bluish-violet
SCHNEIDER	0.52	0.57	Violet
	0.58	0.61	Blue
	0.68	0.73	Bluish-violet
REYNOLDS	0.56	0.57	Violet
		0.62	Blue
	0.62	0.64	Violet
	0.68	0.73	Bluish-violet
FABER-CASTELL	0.58	0.57	Violet
	0.64	0.64	Blue
	0.72	0.73	Bluish-violet
ZA B1000	0.57	0.55	Violet
	0.63	0.62	Blue
	0.72	0.71	Faded Bluish-violet
ZEBRA	0.36		Pale green
	0.57	0.56	Violet
	0.64	0.63	Violet
	0.71	0.72	Violet
	0.82	0.80	Violet
STICK	0.57	0.55	Violet
		0.59	Blue
	0.63	0.62	Violet
	0.72	0.71	Bluish-violet
	0.57	0.55	Violet
GHM 501/P	0.64	0.62	Blue
	0.72	0.71	Violet
	0.81	0.80	Bluish-violet
Bic	0.31	0.27	Pale green
	0.37		Blue
	0.55	0.57	Violet
	0.62	0.65	Faded violet
MV (1×10^{-4})	0.55		Violet
	0.62		Violet
	0.70		Violet
	0.79		Faded violet
CV (1×10^{-4})	0.57		Violet
	0.64		Violet
	0.72		Violet
	0.81		Violet

Comparison Between PCA of IR, UV, and HPTLC

- In the case of the full pen set, the UV spectra of 10 pen inks fall into three clusters of varying compression with an individual pen (no. 10: the Bic pen, labeled A) clearly separated

from the other areas. A second cluster (labeled B) contains two pens (7 and 9). The last group (labeled C) contains seven pens (1, 2, 3, 4, 5, 6, and 8). The IR spectra of the 10 pen inks fall into three clusters with an individual pen A first cluster (labeled A) contains three pens (5, 8, and 10). The second cluster (labeled B) contains three pens (3, 7, and 9). The last group labeled D contains four pens (1, 2, 4, and 6). So, one can say that the IR separates the pens (5, 8) from group C (1, 2, 3, 4, 5, 6, and 8) in the UV. However, the UV spectra separates pen 10 in a separated class which grouped with two pens (5 and 8) in the IR spectra, and separates the two pens (7, 9) from group C (3, 7, and 9) in the IR.

- In case of reduced pen set, the UV of spectra of seven pen inks fall into four clusters of varying compression with an individual pen (no. 1: the ROTO pen, labeled D) is clearly separated from the other areas. A second cluster (labeled E) contains two pens (2 and 8). The third cluster (labeled F) contains two pens (3 and 6). The last group (labeled F) contains two pens (4 and 5). While in case of IR the spectra of the seven pen inks fall into three clusters of varying compression with an individual pen (no. 3: the SCHNEIDER pen, labeled F). The second group G contains two pens (7 and 9) are closely clustered. The third group D, containing four pens (1, 2, 4, and 6), is also closely clustered and cannot be discriminated with any PC loadings. Consequently, the UV separates pen 1 from group D (1, 2, 4, and 6) in the IR, and separates pens (2, 4) into two groups with (5, 8) from group D in the IR. However, the IR spectra separates the spectra of pen 3 from group F (3, 6) in the UV.
- For the HPTLC, it shows again four classes which are (no. 3: the SCHNEIDER pen, labeled D) and (no. 9: the GHM501/P pen, labeled E). A third cluster (labeled F) containing two pens (2 and 8). The last cluster (labeled G) contains the four pens (1, 4, 5, and 6).

From the previous classes, we can conclude that:

- UV analysis separates pen no. (1) in a separated class, which does not separate in case of IR and HPTLC analysis.
- UV and HPTLC analysis separates pen nos. (2, 8) into a separated group.
- IR and HPTLC analysis separates pen no. 3 in a separated class which not separated alone in the UV.
- HPTLC analysis separates pen no. 9 in a separated group which combined with pen no. 7 in case of IR.
- UV separates pens (4, 5) and (3, 6) in separate groups but in case of IR and HPTLC the pens (1, 4, and 6) are combined in one group.

From the previous discussion, one can say that UV spectra are effective and powerful tool to separate blue ballpoint pen ink in most cases rather than IR and HPTLC.

Conclusions

The technique of PCA has been applied to the UV–Vis, IR, and HPTLC of the spectra of inks and the corresponding extracted from 10 blue ballpoint pens available in the Egyptian market. The loadings for the first three or four principal components have been shown to characterize inks, both taken directly from the pen and when extracted from an ink line on paper using ethanol.

Systematic classification of inks has been performed using the signs and magnitudes of the PCA loadings. This approach was found to be useful in classification and individualization of a questioned ink from a database through matching the loadings of principal components, and this approach may be facilitated by a two stage principal component calculation based on first classifying and then individualizing the sample.

The analysis of pens after a short time can be well discriminated from that at zero time based on the PCA loadings in most cases. Although when increase the time intervals, all pens are well discriminated.

Using time effect, good correlation between the loading PC1 and time was obtained. This correlation can be used to estimate the time at which a questioned document was written.

It is clear that PCA method is a good path way to discriminate between extracted ink lines especially with increasing interval time of extraction.

The results proved that the UV–Vis spectra are effective and powerful tool to separate blue ballpoint pen ink in most cases rather than IR and HPTLC.

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