Application of Multivariate Chemometry for Discrimination of Black Ballpoint Pen Inks Based on the IR Spectrum

L. C. Lee [a,b*], Mohamed Rozali Othman[a], H. Pua[b] and Abdul Aziz Ishak[b]

[a] School of Chemistry Sciences and Food Technology, Faculty of Science & Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor D.E. Malaysia; [b] Program of Forensic Science, Faculty of Health Science, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

Abstract: This preliminary work investigates whether FTIR, in combination with multivariate analysis can be used to differentiate 24 black ballpoint pen inks from six different varieties. Ink entries from black ballpoint pens were analyzed directly on paper using micro ATR-FTIR spectroscopy technique and the data obtained was processed and evaluated by a series of multivariate chemometrics. Absorbance value from wavenumbers of 2000-675 cm^{-1} were first grouped into eight different clusters by cluster analysis (CA), followed by principal component analysis (PCA) to form a set of new variables. After that, discriminant analysis (DA) and one-way ANOVA were conducted using the new variables set. Results showed that six black ballpoint pen varieties could be classified into three main groups via discriminant analysis (DA). Differentiation analyses of six different pen varieties performed using one-way ANOVA indicated only two pairs of varieties cannot be differentiated at 95% confidence interval. It can be concluded that micro-ATR FTIR spectroscopy coupled with chemometric techniques could make a powerful non-destructive discriminating tool for analysis of inks based on their infrared spectrum.

Keywords: ATR-FTIR spectroscopy; ink analysis; multivariate analysis; discriminant analysis

Introduction

In general, forensic document examination comprised of handwriting identification and ink analysis. Ink analysis aims at revealing information of chemical components inside inks. It can be of destructive or non-destructive in nature. Nowadays, most of the techniques used by forensic ink analyst posses some disadvantages. In brief, ink analysis techniques available nowadays possed three main weaknesses. Firstly, techniques like high performance liquid chromatography (HPLC) and gas-chromatography mass spectrometry (GCMS) can give better results in term of resolution and also able to detect more components of inks [1-3]. However, those techniques are destructive where part of the sample need to be destroyed or dissolved to prepare the sample in the form that suitable to be analysed by aforementioned techniques. During court procedure, integrity of evidence is the main issue to be discussed. Secondly, if the quantity of the available sample is too little, desctructive techniques cannot be applied on it. Thirdly, non-destructive techniques such as Raman and FTIR spectroscopy usually do not give much information about inks and the interpretation of the spectrum tended to be biased as it depends on human being eye to do the evaluation [4, 5].

As such, this study has been conducted to develop an ink analysis method that is able to give result comparable to destructive techniques while ensuring the integrity of sample. In this study, feasibility of ATR-FTIR spectroscopy coupled with multivariate techniques for ink analysis has been explored. The chief advantages of micro-ATR FTIR spectroscopy are that it is non-destructive, does not require any prior preparation of the sample, requires only a small amount of sample for examination, not time-consuming due to its short analysis time, eco-friendly and cost-effective as it does not involve any reagents.

On the other hand, multivariate analysis is useful in handling high dimensional spectra data and in assessing quantitatively the minor differences of a particular ink relative to other inks that otherwise could not be noticed easily by visual inspection. In this study, principal component analysis (PCA) and discriminant analysis (DA) have been used to process the data. PCA is an unsupervised multivariate statistical method which reduced a large number of raw data to a smaller number of principal components. Each of the principal components formed is a linear combination of the raw variables that represents significant variability in the data but which are uncorrelated with each other [1]. Here, the raw variables refer to the list of wavenumbers. Whereas, DA is a supervised multivariate statistical method that specifically attempts to describe, model and explain the differences between known classes (category variable) based on list of response variables [6]. In this study, the six different varieties of pens act as category variables and response variables would be the newly formed list of principal components.

Experimental

Samples

A total of 24 black ballpoint pens of six different varieties were purchased from a stationary shop at Subang Jaya, Selangor, Malaysia. All of these pens were obtained in multiple packs of the same product to ensure all of them were from the same production batches as the variation between batches will not be considered in this study. Each pen variety was allocated a sample ID for the purpose of this study. Detailed descriptions of collected samples used in this study are presented in Table 1. Double A™, (Thailand) was the white paper used throughout the study. Each of the 24 pens was used to draw three different small circles with a 3 mm diameter on a piece of paper. Experimental work was carried out on the ink deposited on the white paper not later than one day after drawing to minimize changes due to ink aging.

Micro-ATR-FTIR Analysis

All experimental spectroscopy was carried out on a Thermo Scientific Nicolet iN10 MX FT-IR microscope with mercury cadmium telluride (MCT) detector. A *Ge* crystal tip ATR objective was used as micro-sampling accessory. The background spectrum was reacquired after every analysis to reduce variation in the spectra due to instrumental drift. Each spectrum was the result of an average 16 scans at 4 cm^{-1} resolution over a spectral range of 4000 to 675 wavenumbers (cm^{-1}). Three spectrums were collected from each of the 24 pens. The IR spectral data was stored in a data matrix in Microsoft Excel® spreadsheet.

Software

Data collected was processed and analyzed using statistical package SPSS (Statistical Package for the Social Sciences, Windows version 15.0, SPSS Inc., Chicago, USA).

Normalization of Selected IR Region

All three spectra of each sample were included to assess the material reproducibility and homogeneity while ensuring representativeness of each sample due to the minimal size of inks contacted by ATR *Ge* tip and the potential heterogeneity of the inks [7]. Triplicate analysis of 24 pens gave 72 spectra. As such, full data set comprised 72 spectra, consisting of absorbance values as a function of wavenumber, were normalized prior to carrying out the multivariate analysis to reduce variation in the data due to different thickness of the pen inks deposited on paper samples. The absorbance values for each spectrum were divided by the total absorbance resulting from each spectrum across wavenumbers 2000-675 cm^{-1}.

Transformation of Raw Data to New Set of Variables

In this study, the selected IR region (2000-675 cm^{-1}) composed of 688 raw data. The normalized absorbance values were transformed into a new set of variables. This is an important step as discriminant analysis (DA) is restrictive with regard to the number of predictor variables versus the number of samples. In addition, a smaller number of input variables also means results can be obtained with reduced computational and economical expense while improving the accuracy and efficiency of the classification tasks [8]. Variable reduction was achieved by conducting cluster analysis and principal component analysis sequentially. Initially, cluster analysis was performed on the raw variables (688 data points) over the objects (ink samples) by using Ward's method and squared Euclidean distance to form eight different clusters. Each cluster shall contain the same variables describing the ink composition variables that carry similar information about the objects. Subsequently, correlation PCA was performed on each of the eight clusters, separately. The number of components to be extracted was decided based on the Kaiser criterion [9]. The new set of variables comprised all selected principal components that were labeled accordingly, e.g. PCA 1_2 refers to the first component produced by PCA conducted on the second cluster of data points.

Results & Discussion

Infrared Spectrum

Although all the spectra were scanned from 4000-675 cm^{-1}, only the regions from 2000-675 cm^{-1} were analyzed. The IR absorption peaks at the region between 4000 and 2000 cm^{-1} and this was mainly due to water vapour and carbon dioxide from the atmosphere and only a few weak and broad bands attributable to ink [8]. The respective spectra of six different varieties of black ballpoint pen inks analyzed by micro-ATR-FTIR spectroscopy in the region of 2000-675 cm^{-1} are shown in Figure 1.

Table 1: Sample identification for all six studied black ballpoint pen varieties

Sample ID	Sample Name	Quantity
19	G-soft gs 66	3
20	G-soft gs 77	3
21	G-soft r 100	4
22	MGM BP 713	6
23	MGM e-Rite	4
24	PILOT Super GP	4

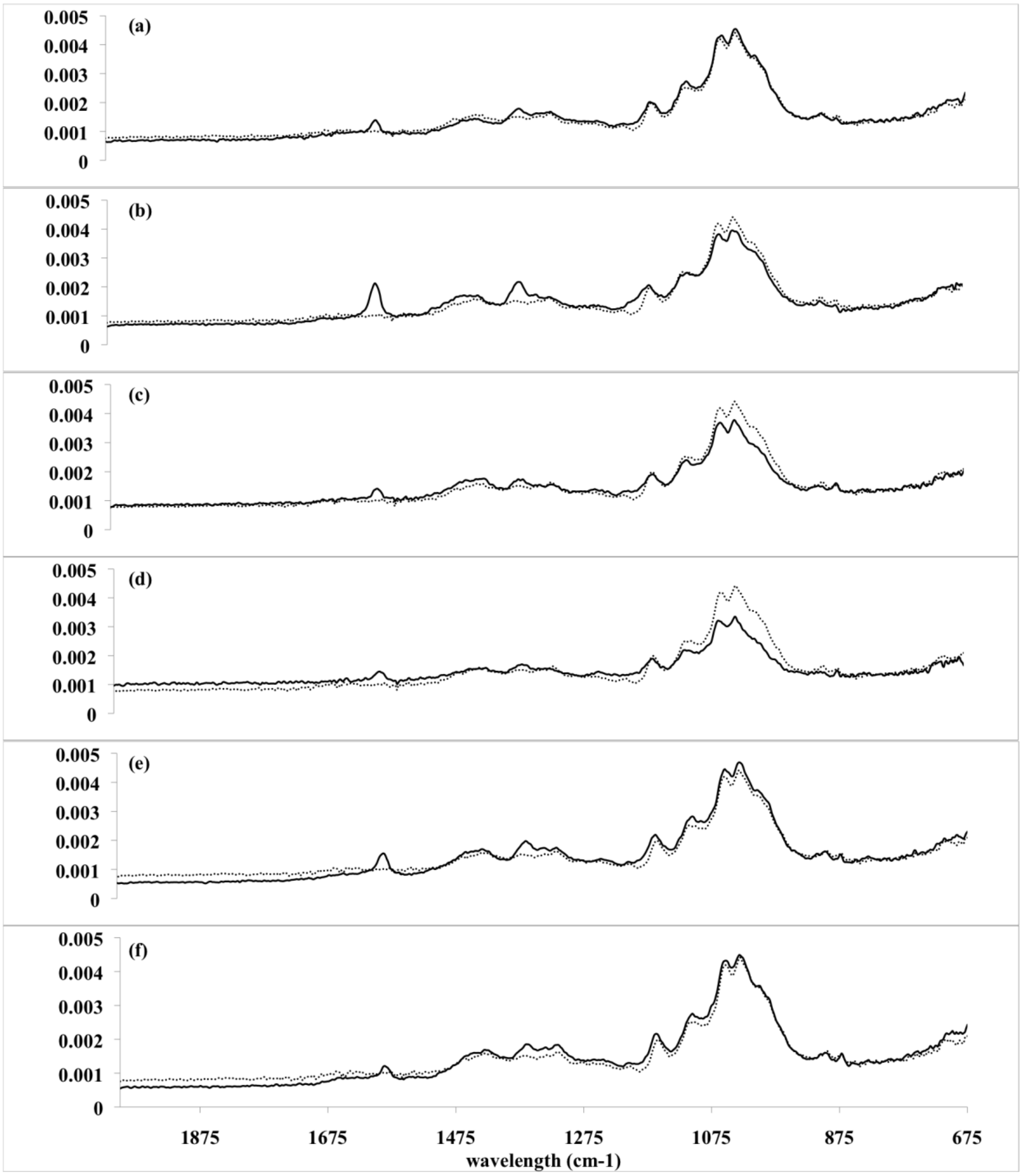

Figure 1: The respective spectra of six black ballpoint pen varieties (–) and blank paper (…) analyzed by micro-ATR-FTIR spectroscopy in the region of 2000-675 cm^{-1}: (a)19; (b) 20; (c) 21; (d) 22; (e) 23; and (f) 24.

In general, all spectra from six different varieties were showing similar spectral patterns. All exhibit a prominent peak at approximately 1584 cm^{-1} but with differences in the form of relative peak height and its shape (Figure. 1) in which 19, 21, 22 and 24 gave lowest absorption intensity while 20 gave the highest absorption intensity. Besides, 20 also showed few minor peaks at region between 1500-1400 cm^{-1}. Peak at 1584 cm^{-1} corresponding to a skeletal vibration of triarylmethane dye and the C=C stretch vibration of epoxy resin (about 1581 cm^{-1}) [10]. As such, the quantity of crystal violet may play an important role in the differentiation of those six pen models. The region between wavenumbers 1100-1000 cm^{-1} is of less concern as it contains peaks mostly from paper substrate [11]. The vibrational assignment for other peaks has been explained elsewhere [10].

Discriminant Analysis (DA)

Stepwise DA was conducted on the new set of variables to see whether all 72 IR spectra could be discriminated according to their pen varieties (category variables). Stepwise DA was performed by using the Mahalanobis distance method and probability-*F* as the criterion. Prior probabilities were equal for all groups (pen varieties) as the representativeness of population was unknown [12]. DA was used with jack-knife classification in the training set. Jack-knife classification is a type of cross-validation techniques that enables all of the available data to be utilized for training while still giving an unbiased estimate of the generalization capabilities of the resulting classifier [8].

Thirteen of 39 principal components that reduced from the selected IR region were selected by DA as predictor variables to form five linear discriminant functions (LDF). LDF 1 explains 62.5% of the total variance in the original data and was highly correlated (r=0.947) to category variable (pen varieties). LDF 2-5 accounted for less than 20% of the total variance. This means LDF 1 play the most important role in explaning differences among six different pen varieties.

Figure 2 shows the projections of the 72 spectra into the space of the first two discriminant functions, displaying 79.10% of the between-to-within group variation in the data. Based on Figure 2, three main clusters were detected. LDF 1 was found to be responsible for separation ||19 20 21||22 23||24||. In fact, each of the three clusters was represented by three different brands. Varieties 19-21; 22-23 and 24 belonging to the brands of G-soft, MGM and Pilot, respectively.

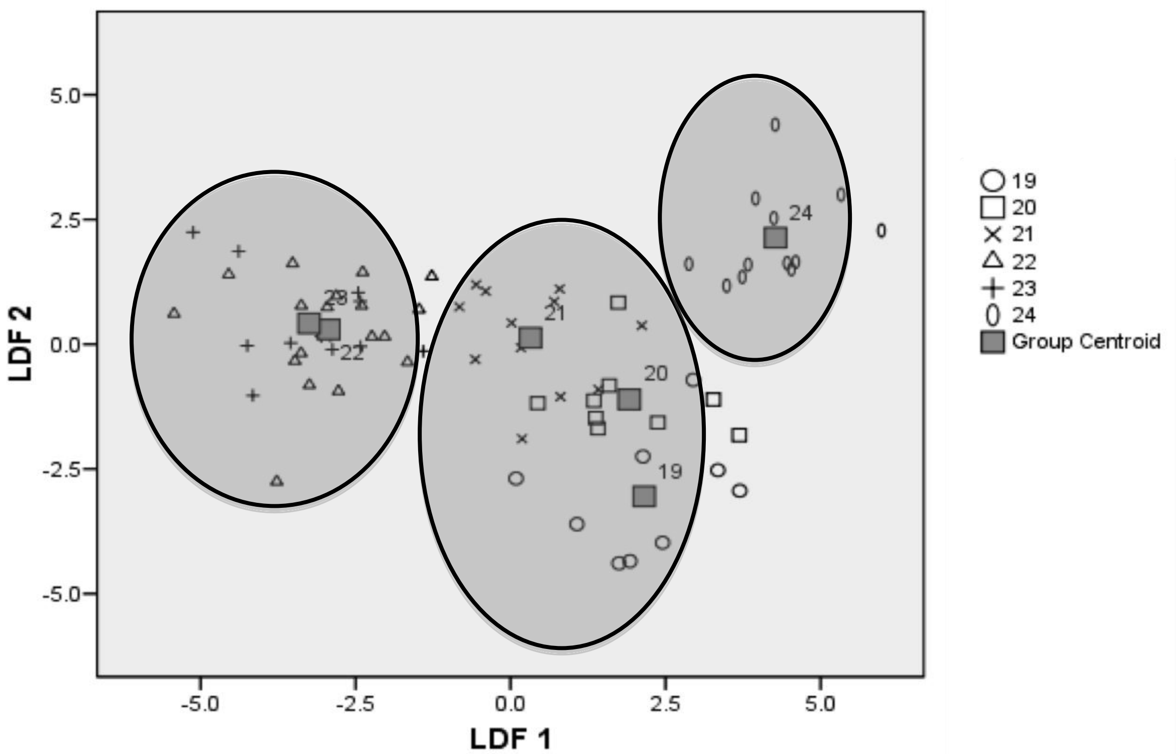

Figure 2: Scores plot of first two linear discriminant functions showing the separation of group centroids for six black ballpoint pen varieties.

Table 2 showed jack-knife classification results for the six groups (pen varieties) discriminant analysis. Out of the 72 spectra, 77.80% of IR spectral are classified correctly. The classification results also show overlapping groups. All the misidentification occured between varieties of a single brand, except for variety of 24. As such, if the DA was conducted to differentiate 72 spectra based on brands, higher correct identification rate could be expected.

Table 2: Jack-knife classification results for each variety.

Variety	Predicted group membership (%)						Overall hit rate (%)
	19	20	21	22	23	24	
19	78	11	0	0	0	11	77.80
20	0	100	0	0	0	0	
21	8	17	75	0	0	0	
22	0	0	0	72	28	0	
23	0	0	0	42	58	0	
24	0	8	0	0	0	92	

Differentiation Analyses

Pair-wise comparison was conducted to determine whether all six different pen varieties might be discriminated through the 39 predictor variables. With six different pen varieties, 15 ((1/2)K(K-1)) possible pairs have been created. One-way ANOVA was applied to find out whether the means of each predictor variable differed between pen varieties, to a 5 % significance level. Subsequently, appropriate post-hoc test was also conducted to determine the pairs of pen that differed significantly at particular variables. With the utilisation of the 95% confidence interval, there was a 5% probability of committing a type I error (false exclusion). When the confidence interval was increased to 99%, only a 1% chance (or less) of committing a type I error was allowed, however at the same time the probability of committing a type II error (false inclusion) increased.

With respect to forensic casework, type II errors (indicating that two samples originated from the same source when they in actual fact do not) should be reduced or eliminated, if possible. In other words, although both types of errors pose problems for the forensic examiner, arguably from a forensic point of view data that presents a higher percentage of type II errors is preferred over the contrary [13].

Figure 3 shows the summary of results for 15 pen pairs. A discrimination power of 86.67% (13/15 X 100% = 86.67%) was achieved. Out of 15 pairs, only two pairs (20:21 and 22:23) were statistically indistinguishable suggesting that they share very similar (statistically the same) organic profile and were likely to have from the same source of origin. In fact, 20 and 21 as well as 22 and 23 were from brands of G-soft and MGM, respectively.

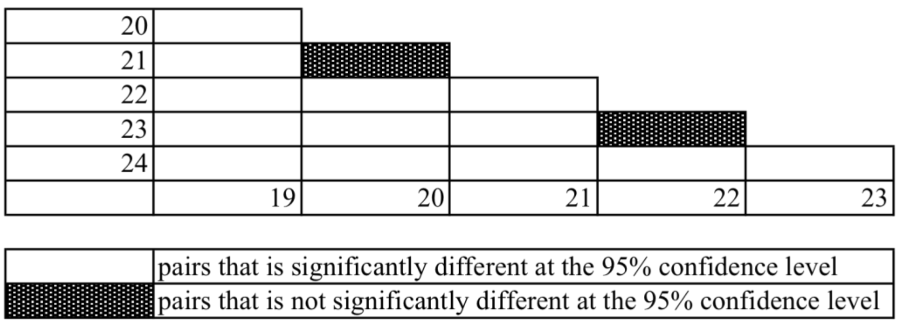

Figure 3. Univariate pair-wise group comparison of six black ballpoint pen varieties.

Conclusion

In conclusion, all six different pen varieties could be grouped into three clusters according to their brands via DA. Discrimination power of the proposed methods was 86.67%.

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Additional information and reprint requests:
L. C. Lee
(Email: *dkcindy@gmail.com*)
Forensic Science Programme,
Faculty of Health Science,
Universiti Kebangsaan Malaysia,
Selangor, Malaysia