Ultra-Violet and Visible (UV-VIS) Spectroscopy and Chemometrics Techniques for Forensic Analysis of Ballpoint Pen Inks: A Preliminary Study

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ABSTRACT: Ink analysis is one of the areas in forensic questioned document (QD) aims at comparing, identifying, characterising and discriminating ink obtained from writing instrument used to write on a document. Ink becomes an important forensic evidence when it is written on a document suspected to be associated with criminal activities such as threatening letters, insurance frauds and will frauds. This study introduces effective technique that can objectively compare and discriminate ballpoint pens of different colours and brands commonly used in Malaysia. A population of thirty six (36) ballpoint pens comprising of six (6) blue, six (6) red and six (6) black of two different brands *i.e.* Papermate Kilometrico and Faber Castell were analysed by ultra-violet and visible (UV-Vis) spectroscopy at wavelength from 200 – 800 nm. The resulting UV-Vis spectra were first subjected to manual visual examinations and followed by chemometrics techniques of principal component analysis (PCA) and hierachical cluster analysis (HCA). The results of this study demonstrates that conventional and low cost UV-Vis spectroscopy when coupled with chemometrics techniques can become a powerful tool that can be employed for forensic question document (QD) analyses.

Keywords: UV-Vis spectroscopy, chemometrics techniques, ball point pen inks, forensic science

Introduction

The idea of ballpoint pen was introduced in 1888 by John Loud, a leather tanner who invented a roller ball tip marking pen featuring its own ink reservoir. Fifty years later, Loud's invention was improved in term of design and ink formulation and eventually commercialised by Ladislas and George Biro. Ballpoint pen ink consists of a very complex mixture of dyes, pigments, solvents, resins, viscosity adjustors and ball lubricants [1] in appropriate combination so as to prevent the ink from drying out while in the pen reservoir, do not diffuse on both side of paper and fix quickly when applied. Despite the popular use of photocopiers and peripheral associated with computers *i.e.* the printers, ballpoint pens are still the method of choice to write on documents making them frequently encountered in forensic questioned document (QD) analyses.

Current practise in forensic ballpoint pen inks analyses involve both chromatographic and spectroscopic techniques. The latter either employs direct *in-situ* analysis using state-of-the-art Surface Enhanced Raman Scattering (SERS) [2] or the more conventional and low-cost ultra-violet and visible (UV-Vis) spectroscopy [1, 3]. Both techniques provide qualitative and quantitative information where the former is in the form of spectra whilst the latter showing intensities or absorbances ranging over few hundred of wavenumbers or wavelengths. Presenting the qualitative information *i.e.* the spectra seem to be the common practise in the court of law. The problem associated with qualitative information is that it is highly subjective and the success of presenting such information will entirely depend on or subject to the experience and the extent of knowledge of the forensic QD examiners.

Chemometrics is an approach to data analyses that uses both mathematical and statistical methods to determine the properties of substances that otherwise would be very difficult to measure directly [4]. Measurements related to the chemical composition of a substance are usually taken and the values of some properties of interest are inferred from the measurements through appropriate mathematical relationships [4]. Chemometrics techniques particularly principal component analysis (PCA) and hierarchical cluster analysis (HCA) are the

two most commonly used chemometrics techniques for the interpretation of data of forensic science relevance [5-9].

Principal Component Analysis (PCA)

PCA is used to identify patterns in data in such a way as to highlight their similarities and differences [10]. Original data from instrumental analysis such as UV-Vis spectra, gas and liquid chromatograms and microspectrograms) are being described using new variables which are known as principal components (PCs). They are derived from linear combinations of the original variables of the original data with its specific loading. In PCA, the PCs or the new variables are arranged in decreasing order of importance where the first PC accounts for the largest variation in the datatset followed by the second, third and so on. In choosing the number of PCs to describe the original dataset, values in the range of 70 – 90% are suggested [10]. PCA generates two smaller matrices known as loading and score matrices. The latter is of interest as a plot known as score plot can be constructed out of it where groupings or clusterings of the objects are deduced.

Hierarchical Cluster Analysis (HCA)

HCA is a clustering technique that produces dendrogram (similar to a tree diagram) as its final outcomes. The dendrogram shows the linkage between an individual sample or object with groups or clusters of samples that exist within a given dataset. HCA uses either agglomerative or divisive methods to identify clusters in a given dataset [11]. The former is the most common approach where clustering starts with all samples being separate and join to form clusters in a series of steps until all clusters exist within a given dataset are joined together to form one large group. The divisive method, works the opposite way of the agglomerative technique. The closeness or distance between objects in a given dataset are computed using the Euclidean distance function whilst single, average and complete linkage are amongst the linkage functions used to link the clusters that arise in a given dataset.

The aims of this study were to analyse ballpoint pen inks commonly found in Malaysia using UV-Vis spectroscopy followed by manual visual examinations and chemometrics techniques of PCA and HCA and to demonstrate the benefit of chemometrics techniques over manual visual examination for forensic analysis of ballpoint pen inks. UV-Vis spectroscopy was chosen in this study because of its low cost and is widely available in forensic laboratories. As far as this study is concerned, there is no reported article on similar subject in Malaysia.

Materials and Methods

Samples Collection and Samples Preparation

A total of thirty six ballpoint pens comprising of twelve blue, twelve black and twelve red manufactured by Kilometrico and Faber Castell were used in this study. The ballpoint pens were purchased from stationery stores in Kubang Kerian, Kelantan in February 2013. Each pen was scribbled onto a 1 cm x 1 cm square area of unlined 80 g/m^2 A4 office paper (Tesco, Malaysia). The scribbled area was then cut using a pair of clean scissors and placed into a vial containing 2ml absolute ethanol (Merck, Germany) to extract the ink out of the paper. To rule out any interference from the background matrix *i.e.* the A4 paper, a blank extract of the A4 paper was also prepared and analysed. Prior to performing UV-Vis analyses, within-run and between-run precisions were conducted.

UV-Vis Spectroscopy

UV-Vis spectra of the ballpoint pen ink extracts were collected using a Varian's Cary® 100 UV-Vis Bio spectrometer (Varian, Inc.) using quartz cuvettes with pathlength of 1 cm. The UV-Vis spectrometer was interfaced to a personal computer (Dell, Texas) by a Varian Cary WinUV Analysis package sofware (Varian, Inc.) for data collection and processing. The spectral range used in the acquisition of data was from 200 – 800 nm. The entire range consisting of 601 data points was used for the PCA and HCA.

PCA and HCA

PCA and HCA were performed using a Minitab® Version 16.2.3 statistical software (Minitab, Inc.). Prior to introduction to the Minitab environment, all UV-Vis spectra were processed and prepared in Microsoft® Excel (Microsoft, Inc.) worksheet.

Results and Discussion

UV-Vis Spectroscopy

A UV-Vis spectrometer detects the absorption and produces a "chemical fingerprint" of the chemical species. As different chemical species absorb UV and Vis radiation at different intensities and wavelengths, different

ballpoint pen inks formulation may produce different and unique chemical fingerprints that permit discrimination by manual visual examination. However this is not always the case as will be seen in the ensuing sections.

Figure 1 shows the UV-Vis spectra of blue Faber Castell (FCB) and blue Kilometrico (KMB) ballpoint pen inks. The blue ballpoint pen inks can be readily discriminated on the basis of their UV-Vis spectra. The differences between the FCB and KMB ink are in term of the broad absorption band occurring from 410 – 680 nm in the former and from 450 – 720 nm in the latter. Other than that, the presence of minor absorption bands at 240, 300 and 410 nm in FCB and at 305 and 350 nm in KMB support definite discrimination between the two blue ballpoint pen inks.

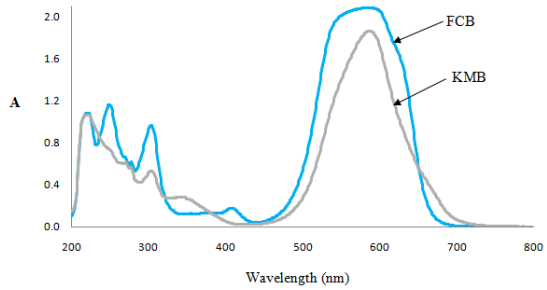

Figure 1: UV-Vis spectra of the blue ballpoint pen inks

Similar to the trend in blue ballpoint pen inks, black Faber Castell (FCH) and black Kilometrico (KMH) ballpoint pen inks can also be readily discriminated on the basis of their UV-Vis spectra as shown in Figure 2. The FCH spectrum shows two broad absorption bands occuring from 320 – 480 nm and from 480 – 660 nm respectively. The KMH although also showing two broad absorption bands, the bands however occur at different wavelengths *i.e.* from 390 – 500 nm and from 500 – 620 nm.

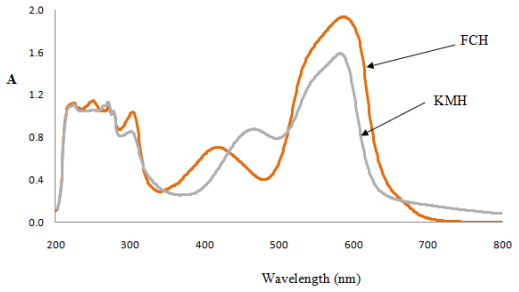

Figure 2: UV-Vis spectra of the black ballpoint pen inks

Unlike the blue and black ballpoint pen inks, red Faber Castell (FCM) and red Kilometrico (KMM) ballpoint pen inks display quite similar UV-Vis spectra as shown in Figure 3. No obvious differences can be seen in the UV-Vis spectra of both ballpoint pens that can permit definite discrimination.

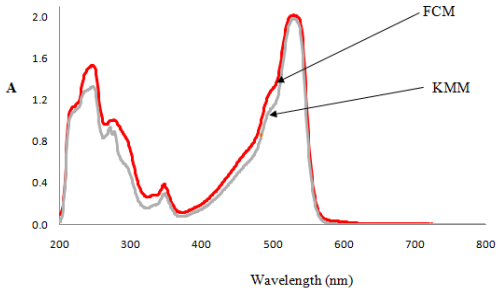

Figure 3: UV-Vis spectra of the red ballpoint pen inks

Prior knowledge of the origin of the two red ballpoint pens is the only information available to claim that the pens are different. In actual forensic cases, forensic QD examiners seldom being provided with prior information of the evidence at hand and it is solely up to the expertise, extent of knowledge and experience of the forensic QD examiners to decide on the origin of the evidence. An example of this degree clearly limit the abilities of a forensic QD examiner and can lead to erroneous decision due to inherent subjectivity therefore a technique that can objectively discrimate FCM from KMM would be of great assistance.

PCA and HCA

PC analysis was performed to the entire 601 data points in the UV-Vis spectra of the ballpoint pens. The variation in the dataset is accounted by the first five PCs which is approximated at 92.8%. The discrimination between all ballpoint pen inks is most readily achieved by plotting a score plot of principal component two (PC2) versus principal component one (PC1) as shown in Figure 4. The plot displays that, the 36 UV-Vis spectra of the ballpoint pens fall into three definite clusters (designated in this study as Cluster A, B and C).

Cluster A which is characterised by negative PC1 and positive PC2 scores is constituted of FCB and KMB ballpoint pen inks whilst Cluster B which is characterised by positive PC1 and PC2 scores is constituted of the FCH and KMH ballpoint pens. Cluster C which is charaterised by positive PC1 and negative

PC2 scores is constituted of FCM and KMM ballpoint pen inks. It is worth to mention that FCM and KMM ballpoint pens that are difficult to discriminate previously on the basis their UV-Vis spectra can now be successfully discriminated after undergoing PC analysis. Within the cluster, the two ballpoint pen inks formed two completely distinct groups that do not overlap.

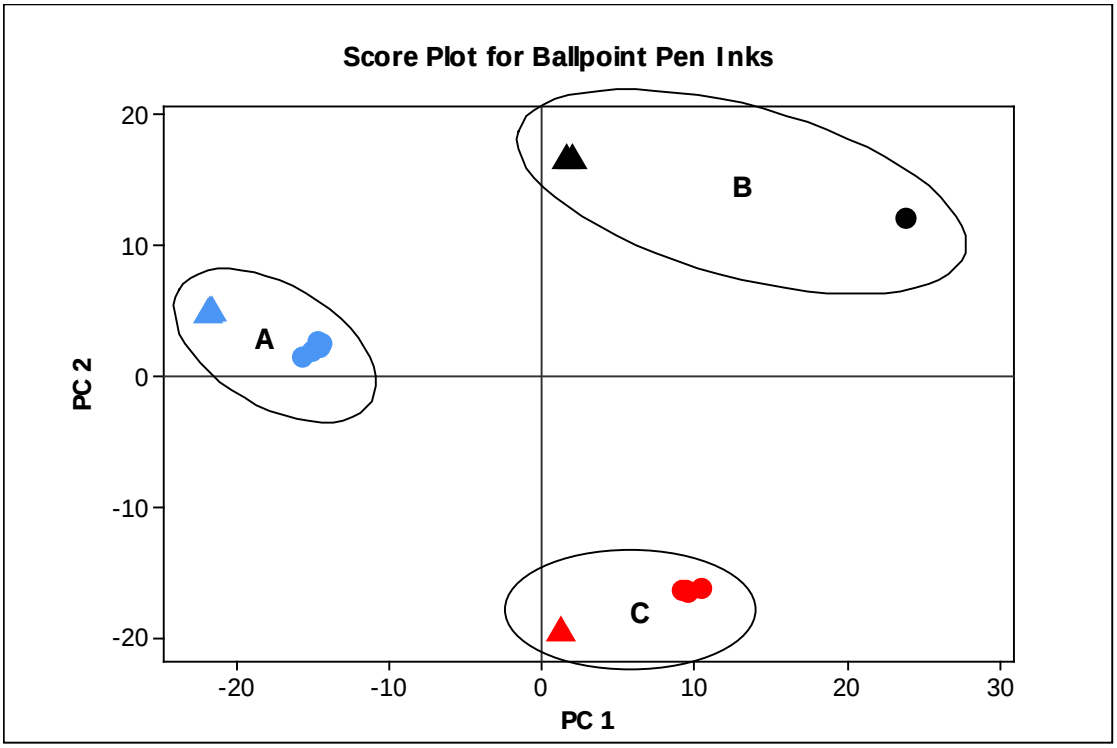

Figure 4: Score plot for the ballpoint pen inks. Triangle = Papermate Kilometrico, Circle = Faber Castell. Colour corresponds to the ballpoint pen ink colour

The dendrogram of the HCA is as shown in Figure 5. Using complete linkage as the linkage strategy, the dendrogram displays six clusters and each cluster corresponds to each ballpoint pen inks under study. The FCB and KMB ballpoint pen inks are linked at 67% similarity level whereas the FCH and KMH are linked at approximately 40% similarity level whilst the FCM and KMM are linked at approximately 90% similarity level. The similarity levels afforded by the HCA would also explain the degree of dissimilarities of the of the ballpoint pen inks. With 40% degree of similarity (hence 60% degree of dissimilarity) shown in the dendrogram, it is not suprising that the FCH and KMH can be easily discriminated on the basis of their UV-Vis spectra. In similar vein, with 90% degree of similarity (hence 10% degree of similarity), it is not suprising that FCM and KMM cannot be discriminated by manual visual examination.

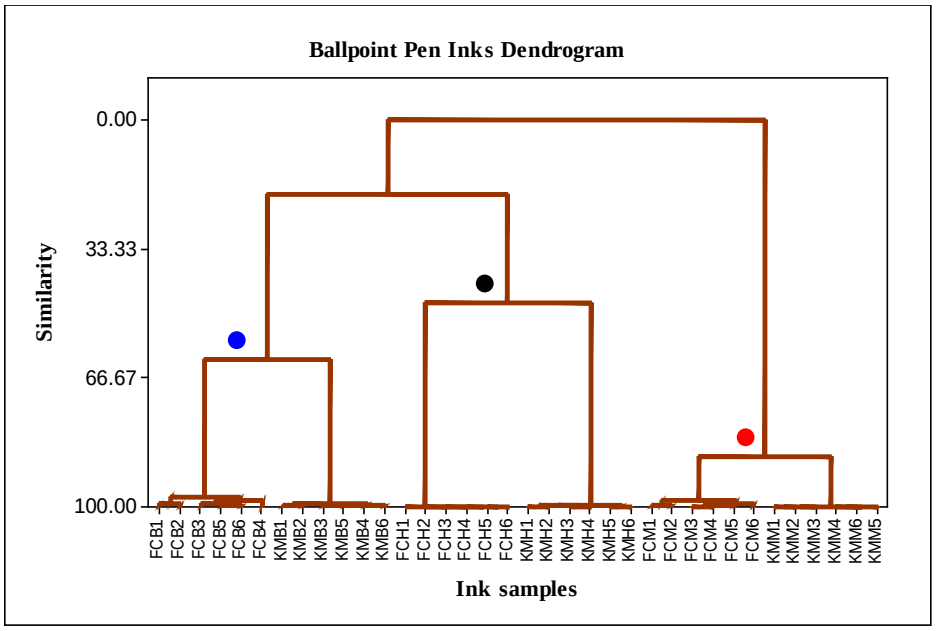

Figure 5: Dendrogram for the ballpoint pen inks constructed using Euclidean Distance and Complete Linkage. The colour dot corresponds to the ballpoint pen ink colour

Conclusions

Manual visual examination of the UV-Vis spectra of ballpoint pen inks although simple and quick to perform, its interpretation is heavily dependent on the expertise, the extent of knowledge and experience of a forensic QD examiner. Moreover the outcomes are very subjective. In contrast, chemometrics techniques although quite complicated to perform, afforded more objective and meaningful outcomes. As shown in this study, ballpoint pen inks that were difficult to discriminate previously on the basis of their UV-Vis spectra alone, were successfully discriminated and resolved after undergoing chemometrics treatments. The potential use and benefits of coupling the low-cost UV-Vis spectroscopy which is widely available in most forensic science laboratories with chemometrics techniques for forensic analysis of ballpoint pen inks had been clearly demonstrated in this study.

Similar study using larger number of ballpoint pen inks gathered from other sources and also the effect of exposing the inks to the environment *i.e.* ageing, to the chemical compositions of the inks will be the subject of our future works. This work has presented a potentially useful approach for forensic ballpoint pen inks analysis that undoubtedly will benefit the forensic QD practices in Malaysia.

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