FORENSIC DISCRIMINATION OF BLACK PERMANENT MARKER PEN INKS USING ATTENUATED TOTAL REFLECTION – FOURIER TRANSFORM INFRARED SPECTROSCOPY (ATR-FTIR) WITH CHEMOMETRICS PROCEDURE

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by

LEVIANA FERAH JOSEPH

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for the degree of
Master of Science (Forensic Science)

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CERTIFICATE

This is to certify that the dissertation entitled **"FORENSIC DISCRIMINATION OF BLACK PERMANENT MARKER PEN INKS USING ATTENUATED TOTAL REFLECTION – FOURIER TRANSFORM INFRARED SPECTROSCOPY (ATR-FTIR) WITH CHEMOMETRICS PROCEDURE"** is the bona fide record of research work done by **LEVIANA FERAH JOSEPH** during the period from February 2020 to September 2020 under my supervision. I have read this dissertation and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Master of Science (Forensic Science).

Supervisor,

……………………………

(DR DZULKIFLEE ISMAIL)

Date: 10/9/2020

DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purposes.

…………………………………….

(LEVIANA FERAH JOSEPH)

Date: 10/9/2020

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LIST OF ABBREVIATIONS

ATR-FTIR	Attenuated Total Reflectance-Fourier Transform Infrared
CE	Capillary Electrophoresis
DP	Discrimination Power
et al.	*et alia – "and others"*
FDE	Forensic Document Examiner
FTIR	Fourier Transform Infrared
GC-MS	Gas Chromatography-Mass Spectrometry
HCA	Hierarchical Cluster Analysis
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
IR	Infrared
LDA	Linear Discriminant Analysis
PC	Principal Component
PCA	Principal Component Analysis
PyGC-MS	Pyrolysis Gas Chromatography-Mass Spectrometry
R_f	Retention factor
SPSS	Statistical Package for the Social Sciences
TLC	Thin Layer Chromatography
UATR	Universal Attenuated Total Reflectance
UV-Vis	Ultra Violet -Visible
UV-Vis-NIR	Ultra Violet -Visible-Near Infrared

ABSTRAK

Analisis dakwat merupakan salah satu bidang yang paling penting dalam pemeriksaan dokumen forensik. Ia melibatkan analisis dakwat alat tulis atau mesin pencetak. Tujuan utama analisis dakwat adalah untuk mengenal pasti asal atau sumber dakwat yang diletakkan di atas sesuatu substrat seperti kertas. Berhubungan dengan analisis dakwat pen, kajian terhadap pen marker kurang mendapat perhatian oleh para penyelidik berbanding dengan analisis dakwat pen mata bulat dan pen gel. Terdapat dua jenis teknik dalam analisis dakwat iaitu teknik merosakkan dan tidak merosakkan. Kajian ini bertujuan untuk membezakan dakwat pen marker kekal hitam dari jenama yang berbeza menggunakan spektroskopi Attenuated Total Reflectance (ATR) - Fourier transform infrared (FTIR) yang digabungkan dengan Analisis Komponen Utama (PCA) dan Analisis diskriminan Linear (LDA). Kuasa diskriminasi untuk pemeriksaan visual hanya memberikan 47.62% dan 28.57%, masing-masing untuk pemeriksaan visual langsung dan pemeriksaan visual spektrum IR. Manakala untuk PCA dan LDA, kuasa diskriminasi jauh lebih tinggi. PCA memberikan 81.14% untuk kedua-dua kumpulan data mentah dan standard dan 95.2% kuasa diskriminasi untuk set data terbitan. LDA memberikan 95.2%, 76.4% dan 100% klasifikasi yang betul bagi sampel dakwat hitam masing-masing untuk set data mentah, standard dan terbitan. Kesimpulannya, teknik kemometrik, iaitu, analisis komponen utama dan analisis diskriminan linear memberikan diskriminasi yang lebih baik berbanding dengan pemeriksaan visual.

ABSTRACT

Ink analysis is one of the most important area in forensic document examinations. It involves the analysis of ink of a writing instrument or printer machine. The main purpose of ink analysis is to identify the origin or source of the ink deposited on a substrate such as a paper. Regarding on the ink analysis of pens, study onto marker pen have been received less attention by researchers compared to ink analysis of ballpoint pens and gel pens. Inks analysis involving two types of techniques namely destructive and non-destructive technique. The aim of the present work is to discriminate black permanent marker pen inks of different brands using non-destructive Attenuated total reflectance (ATR) – Fourier transform infrared (FTIR) spectroscopy coupled with Principal Component analysis (PCA) and Linear discriminant analysis (LDA). The discrimination power for visual examinations provides only 47.62% and 28.57%, for direct visual examination and visual examination of IR spectra, respectively. Whereas for PCA and LDA the discrimination power is much higher. PCA provides 81.14% for both raw and standardised dataset and 95.2% for derivatised dataset of discrimination power. LDA provides 95.2%, 76.4% and 100% of correct classification of black marker ink samples for raw, standardised and derivatised dataset, respectively. It can be concluded that the chemometrics procedures, which are, principal component analysis and linear discriminant analysis provide better discrimination of permanent black marker pen inks as compared to visual examinations.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Forensic document examinations are aimed to verify the originality of questioned documents which is carried out on the whole documents or any part of the document (e Brito *et al.,* 2017). When the sources or the originality of a document being doubted, therefore, it is defined as questioned documents (Claybourn and Ansell, 2000). Hence, in order to solve the crime that is related to questioned documents, forensic document examiners will take action by conducting suitable and systematic examinations. For example, criminal activities that associated with questioned documents are forgery, fraud, threatening letters, illicit drugs trafficking and homicide.

One of the important examinations in forensic document examinations is ink analysis which can help to obtain useful information about a questioned document (Thanasoulias *et al.,* 2003). Ink analysis is conducted to differentiate between inks in order to prove the originality of a questioned document (Halim *et al.*, 2012). Two types of techniques regarding analysis of inks, namely destructive and non-destructive methods (Senior *et al.*, 2012). Destructive technique involves destruction on a sample because a small part of ink will be removed from its support before any further analyses. In contrast, no removal of ink from the document involves in non-destructive technique.

There are three types of ink that were used in a writing instruments namely, liquid, semi-liquid and solid (Awab *et al.*, 2011). Different components of ink were used for different types of writing instruments or printing. The modification of ink formulations from the over the years were done to improve the characteristics of the ink (Vila *et al.*, 2007). Dyes, pigments, solvent and resins are one of the composition in ink and the most important part is the colourants.

There are three well established pen types in the market (Calcerrada and García-Ruiz, 2015), namely fountain pen, ballpoint pen and rollerball pen. Fountain pen uses water-based liquid ink which is delivered through a nib. The ink flows due to capillary action and gravity. It flows from a reservoir through a “feed” to the nib. In ballpoint pen, the viscous oil-based ink was dispenses by rolling a small hard sphere or a metal ball. On contact with paper, the ballpoint pen ink dries almost immediately. Similarly to ballpoint pen, rollerball pen also dispenses water-based liquid or gel ink through a ball tip. It has less-viscous characteristic than oil-based ink make it more easily absorbed by paper.

1.2 Problem statement

Marker pens are now popular in various applications, therefore, recently the identification and comparison of marker pen inks started to rise in questioned document examinations. However, on literature search, studies into permanent marker pens for forensic investigation are quite limited. Sharma *et al.* (2018) stated that most of the study only focused on the characterisation and the differentiation of ballpoint pens ink. This is due to the probability of encountering ballpoint pens in criminal cases may be higher compared to other pens. However, there is still a probability of encountering permanent or whiteboard marker pens in criminal cases, because the use of marker pen as writing instrument is common. For example, as a study material, in art and also documentations in parcel.

Apart from that, it is challenging for Forensic Document Examiners (FDE) to identify and determine source of origin of the inks especially for similar colour of inks. It is hard to distinguish ink of different brands but of similar colour.

1.3 Objectives of the Study

This study was conducted discriminate seven (7) different brands of permanent marker pen inks using ATR-FTIR spectroscopy with chemometrics procedures. Black colours permanent marker pen inks were used in this study.

The specific objectives of this study are:

1. To compare and discriminate black permanent marker pen inks from different brands using ATR-FTIR spectroscopy.
2. To analyse the ATR-FTIR spectroscopic data of the inks using manual visual examination.
3. To analyse the ATR-FTIR spectroscopic data using chemometrics procedure of Principal component analysis (PCA) and linear discriminant analysis (LDA).

1.4 Significance of the Study

Generally, ink is made up of several components such as resins, solvent, dyes and any other materials. Different manufacturers use different formulations for their products.

Thus, it is hard to identify the exact marker pen was used to criminal cases. However, the brand of marker pen still have the possibility to be identified (Awab *et al.*, 2011).

The ability to discriminate ink of similar colours will allow the actual origin or source of the ink to be identified, which is very advantageous in forensic investigation. Discrimination using ATR-FTIR spectroscopy is more efficient, direct and non-destructive. Chemometrics also allowed a more desirable and significant results because it can give more objective result.

CHAPTER 2

LITERATURE REVIEW

2.1 History of marker pen

The usage of marker pen as a medium of communication, documentation and also for teaching purposes is a common sight nowadays, especially in the office or workplace setting. It serves variety of functions and various age groups including children and adults. Most probably felt tip marker was the first marker which is produced in 1940s (Bellis, 2019). Mainly, it was applied for labelling and artistic. Later, a marker named “Magic Marker” was produced by using a wool felt wick and a glass bottle where the ink was held. The marker was open to the market in 1952 by its creator, Sidney Rosenthal. In 1958, the uses of marker becomes famous among public and people started to use it in various applications such as lettering and labelling. Later than in 1962 a stationery company in Japan invents the modern fibre tip pen. It was invented at Tokyo Stationery Company by Yukio Horie (Bellis, 2019).

After the production of permanent markers, then, in 1990s erasable marker and superfine-points started to gained popularity. In 1991 Binney and Smith introduced a redesigned Magic Marker line that included highlighters and permanent markers (Bellis, 2019). In 1996, fine point Magic Marker II DryErase markers were introduced for detailed writing and drawing on whiteboards, dry erase boards, and glass surfaces.

2.2 The manufacturing process of marker pen

There are two parts of processes involved in the manufacturing process of marker pen. The first part is production of the ink (Figure 2.1). The ink mixture is prepared with a preferable ratios 1-10 % water by weight, with the remainder of the weight being made up of a solvent such as alkyl or alkylene carbonate. After that, conventional additives was added, such as alkylpoly-glycol ether, nonylphenylpolyglycol ether, fatty acid polyglycol ester, or fatty alcohol ethoxalates, and preservatives, such as ortho-phenolphenyl and its sodium salt, ortho-hydroxydiphenyl, or 6-acetoxy-2,4-dimethhyl-m-dioxane to the mixture ("How marker is made - material, manufacture, making, history, used, product, machine, History, Raw Materials," 2011).

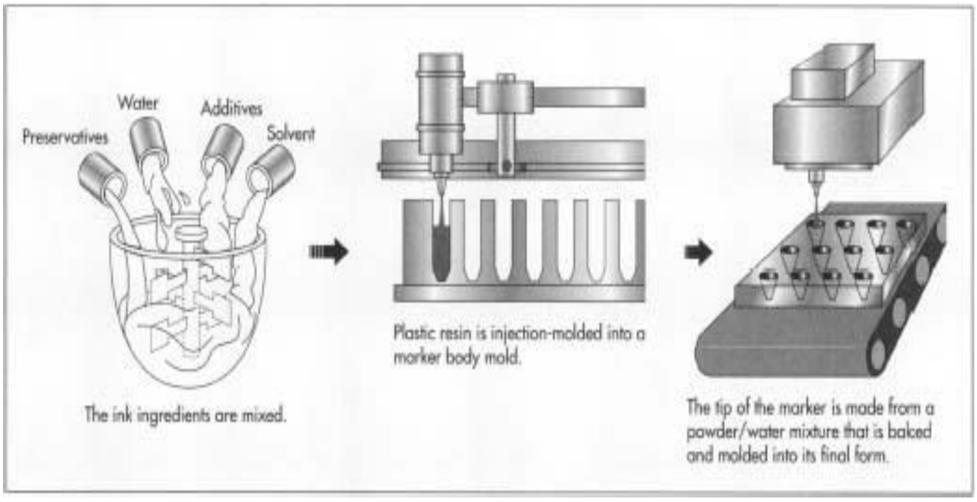

Figure 2. 1 The process of making the marker ink by "How marker is made - material, manufacture, making, history, used, product, machine, History, Raw Materials," retrieved from http://www.madehow.com/Volume-3/Marker.html

The second part of the process named construction of the marker's body (Figure 2.2). In this process a plastic resin was heated until melted then injected into a marker body mold before allowing it to cool and harden. The same manner will be used to form the marker caps and plugs. As for the nib, or tip, of the marker it is made from powder which is mixed with water, moulded, and baked into its pointed or flat form. After that, using one machine for all the following functions, an assembler then places a polyester cylinder inside the marker barrel to form a reservoir for the ink, fills the reservoir with ink, and inserts the nib at the bottom and the cap at the top. Then, the markers are placed into colour assortment. Finally, the marker are packaged for retail marketing ("How marker is made - material, manufacture, making, history, used, product, machine, History, Raw Materials," 2011).

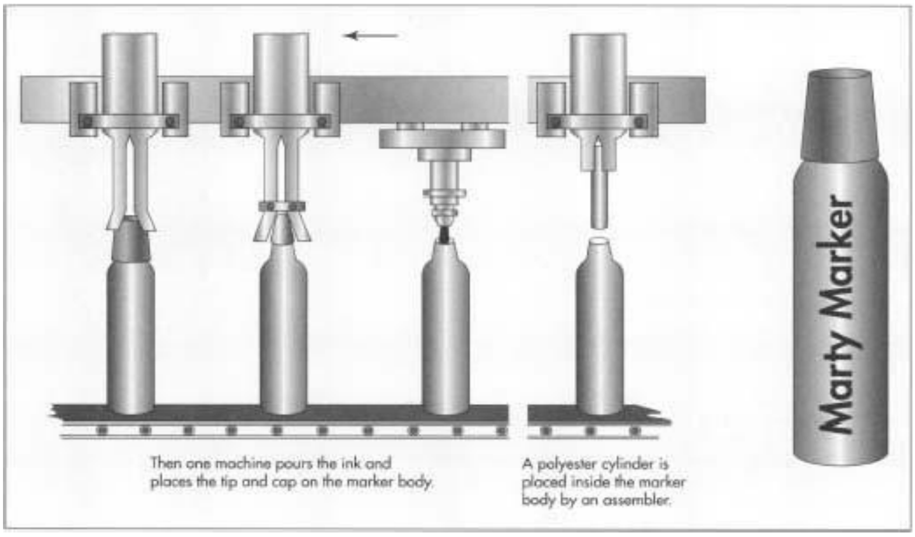

Figure 2. 2 The process of making the marker by "How marker is made - material, manufacture, making, history, used, product, machine, History, Raw Materials," retrieved from http://www.madehow.com/Volume-3/Marker.html

2.3 Method of analyses

Generally, two commonly used approaches in the analysis of inks are destructive and non-destructive with regard to the changes they brought about to the questioned document (Thanasoulias *et al.*, 2003). Destructive methods involve removal a portion of the ink from the document before the analysis. The purpose of this methods are to determine the organic composition in pen inks. Ultra-violet spectrometry (Adam *et al.*, 2008), thin layer chromatography (Djozan *et al.*, 2008) and capillary electrophoresis (Copper *et al.*, 2019) are example of destructive method that commonly used. On the other hand, non-destructive methods utilised reflectance technique for the observation of ink on the document to obtain spectral characteristics of the ink. This type of method is not involving removal of the sample, in other words, the sample is still intact with its support i.e. paper. This methods involved mainly the use of Raman (Asri *et al.*, 2020) and reflectance infrared spectroscopy (Silva *et al.*, 2013).

2.3.1 Non-destructive method

Infrared (IR) spectroscopy has been used in inks analysis to characterise inks of different types of pen such as ballpoint and gel pens (Calcerrada and García-Ruiz, 2015). This particular technique detected absorption peaks for almost all of the components in ink including the solvent, dyes and also additives (Houck and Siegel, 2006). Due to this, IR can be very helpful in comparing two ink samples to assess whether or not they could originate from the similar sources. Minimum samples preparation were involved in this technique and usually does not result in destruction of the sample, however it is still depends on the type of IR mode used. Normally discrimination is conducted based on comparison of spectra at the fingerprint region of each sample because spectra obtained are complex due to the large number of compounds that absorb in the same range of the IR spectrum. Most studies focused on the dye composition, although in some cases chemical groups from other substances contained in inks such as resins or binders have also been identified. In order to achieve a definite and objective, this technique can be coupled with statistical tools (Calcerrada and García-Ruiz, 2015).

Kher *et al.* (2006) utilised high performance liquid chromatography (HPLC) coupled with attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) to classify ballpoint pen inks. This study had compared and calculated the uses of HPLC and IR for the characterisation of blue ink samples of the ballpoint pen. Then, it followed by their subsequent data analysis using principal component analysis (PCA) and linear discriminant analysis (LDA) for classification purposes. Though HPLC offers thorough information about the concentration of different components of inks, however it is destructive. Therefore ATR-FTIR was applied as an alternative method because it is a non-destructive technique. In addition, it is rapid, cost effective and requires less preparation of sample. However, this study suggested that HPLC technique was more effective compared to IR spectroscopy when combined with chemometrics procedure.

Reed *et al.* (2012) suggested an analysis to identify and classify different brands or models of gel pen inks using ATR-FTIR technique. In the analysis, 29 black gel pen inks from different brands were involved. Statistical methodology was used to achieve unbiased discrimination of the gel pen inks. Lee *et al.* (2012) had published a study aimed to explore the potential of micro-ATR-FTIR spectroscopy combined with multivariate analysis for systematic analysis of ballpoint pen inks. This study analysed 155 black inks of ballpoint pen from nine different brands. Out of the 155 inks analysed, only two pairs of inks from two different brands cannot be differentiated using one-way ANOVA at 95% confidence interval. They concluded that this non-destructive approach is straight forward, fast and easy.

Silva *et al.* (2013) proposed an analytical method to classify blue pen inks from different brands based on infrared spectroscopy. Three types of pen involved which were gel, ballpoint and rollerball. Spectrum of each ink was acquired using a Perkin Elmer Spectrum 400 IR spectrometer with universal attenuated total reflectance (UATR) accessory, in the range 4000 to 650 cm^{-1}, with resolution of 4 cm^{-1} and scan time of 16. Linear discriminant analysis (LDA) was used in this study to objectively discriminate the pen inks. The proposed method enable to successfully classify all pens into their respective brands and could be an advantageous tool for detection and confirmation of forgery in documents of legal importance.

Kamil *et al.* (2015) utilised ATR-FTIR spectroscopy to analyse eleven (11) unbranded ballpoint pen inks. Spectral measurements were taken over a spectral range of 4000 cm^{-1} to 650 cm^{-1} with spectral resolution of 4 cm^{-1}. After that, data obtained were evaluated using chemometrics procedures of hierarchical cluster analysis (HCA) and principal component analysis (PCA) to prove either by using PCA or HCA objective discrimination of the ink samples can be achieved. They concluded that neither PCA nor HCA had successfully give a neat clusters or objective discrimination of the ink samples. This was probably due to the fact that the inks of the unbranded pens came from the same sources, in other words, they have same ink formulations.

Asri *et al.* (2015) had characterised fifteen (15) blue and fifteen (15) red ballpoint pen inks using ATR-FTIR spectroscopy. Only the spectral range from 1200 cm^{-1} to 1800 cm^{-1} was used for PCA to analyse the data obtained from ATR-FTIR analysis. PCA was applied to give unbiased and definite discrimination between the inks samples. Based on the findings, they had concluded that ATR-FTIR spectroscopy combined with PCA offers great potential to be used for characterising the pen ink samples.

Sharma and Kumar (2017) presented a work study involving 57 ballpoint pen ink samples of blue colour. The characterisation was conducted using ATR-FTIR spectroscopy and high performance thin layer chromatography (HPTLC) together with multivariate analysis. The overall discriminating power (DP) reported was 99.69% for the non-destructive technique of ATR-FTIR which is more significant compared to visual inspection with DP=97.93% and destructive HPTLC with DP=93.80%. In addition, the combination of ATR-FTIR with multivariate analysis had proven to be an effective tool for objective characterisation of compound ink formulations.

Basinge *et al.* (2019) had compared two techniques, namely ATR-FTIR spectroscopy which is a non-destructive and a destructive thin layer chromatography (TLC) to characterise and discriminate twelve (12) ballpoint pen inks, however, the result shows that by using only ATR-FTIR, it was not able to differentiate ink samples. This is maybe due to the same ink formulations of the samples which is almost the same to each other. On the other hand, TLC had successfully discriminated the ink samples thus demonstrating the usefulness of TLC in the examination of inks.

Yadav and Sharma (2020) had published a study to evaluate the capability of ATR-FTIR spectroscopy in classifying different colours (blue, black, green and red) and brands of fibre-tip pens. The spectrum of each individual colour was first examined visually, which resulted in discriminating power (DP) of 77.20%, 98.40%, 96.60% and 96.60% for black, red, green and blue inks, respectively. This study showed the capability of ATR-FTIR spectroscopy for the analysis of inks on paper, in other words, *in-situ* ink analysis.

Raman spectroscopy provides different analytical information which is a complementing the information given from IR (Calcerrada and García-Ruiz, 2015). IR can detect ink matrices such as binders, organic additives and dyes composition, whereas Raman is capable of identifying pigments present in gel pen and rollerball pen inks (Calcerrada and García-Ruiz, 2015). In a similar manner, Raman is also a non-destructive analytical method which allowing samples to be examined *in-situ* with minimal or without any samples preparation (Mazzella and Buzzini, 2005). In addition, when coupled together with some other methods such as chemometrics procedures, Raman was found to be ideal for forensic application (Khandasammy *et al.*, 2018). Back in 2015, Mazzella and Buzzini demonstrated the use of Raman spectroscopy for gel pen inks analysis. In their studies, fifty five (55) blue gel ink samples were analysed using this technique to test the capability of Raman in discriminating the samples. Based on their results, it showed the interest of utilising such a non-destructive, non-invasive and rapid technique for pigment-based gel pen inks.

Lee *et al.* (2016) employed non-destructive technique to classify and identify ballpoint pen inks. This study conducted to show the feasibility of Raman spectroscopy as simple “green” tool in analysing twenty four (24) different types of ballpoint pen inks without involving any complicated statistical technique or expensive chemicals. In the study, ink samples were prepared by drawing a straight line of 5 cm on the paper template, then they were analysed by using Raman spectrometer equipped with a 685 nm wavelength laser to obtain their spectra. The effect of the paper was also studied. Based on the Raman spectra of the pen inks, all blue and black inks did not showed significant different features. Thus, the differentiation was done based on the presence of particular minor bands within a selected region or absolute positions. The discrimination power (DP) calculated was above 90%.

Recent studies conducted by Asri *et al.* (2019) and Ramanathan and Lakshmanan (2019) demonstrated the uses of Raman spectroscopy. It was used to analyse blue gel pen inks. Both studies were proposed the application of chemometrics procedures to increase the accuracy of distinction of the ink samples. To obtain Raman spectra of the ink samples, laser at 685 nm wavelength were used in Asri *et al.* (2019) study, while 532 nm wavelength were used in Ramanathan and Lakshmanan (2019) study. Both studies reported successful works and proved that Raman analysis coupled with chemometrics procedures are able to produce correct classification and objective discrimination between ink samples.

2.3.2 Destructive method

Adam *et al.* (2008) had used UV-Vis spectroscopy coupled with PCA for grouping and differentiation of ballpoint pen inks of black colours. This work was conducted on UV-Vis spectrophotometry data of the ink samples to determine the capability of PCA in forensic analysis. A thin capillary tube was dipped into the pen's barrel in order to obtain the ink sample, then the ink was deposited into a tube containing ethanol. As a result, the combination of UV-Vis spectroscopy and PCA showed very good repeatability with a small, but statistically measureable, scatter in the loadings which objectively defined each ink.

Causin *et al.* (2008) had performed an analysis on black and blue ballpoint pen inks using UV-Vis, diffuse reflectance FTIR spectroscopy and thin layer chromatography to evaluate the discriminate power of each technique and possibility of integrating the three analytical procedures. A total of thirty three (33) ballpoint pen inks (21 black and 12 blue) from various brands and models were analysed in the study. Two types of ink extraction were performed. First, ink was directly extracted from the pen ink reservoir with ethanol and second, ink was extracted from the deposition of ink on a paper. In this study, UV-Vis technique was used as an initial screening and samples were discriminated based on the comparison of their qualitative appearance of their absorption spectra in the visible range. As a result, discriminating power of 90% and 79% of the black and blue inks, respectively, were reported. However, when further analysis was done using TLC and FTIR, the overall discriminating powers (DP) reported were 100% for black inks and 98% for blue ink.

Two studies performed by Zaharullil and Ahmad (2012) and Senior *et al.* (2012) described the uses of UV-Vis spectroscopy in ballpoint pen inks analysis. Zaharullil and Ahmad's study was aimed to discriminate between the colours of the inks and the brands among the ballpoint pen inks (black and blue). The analysis of the inks was conducted using UV-Vis in the wavelength range from 400 to 700 nm. FTIR spectroscopy was also applied in the study, and IR spectrum of each sample was obtained within the range from 450 cm^{-1} to 4000 cm^{-1}. The outcomes of their study showed that UV-Vis technique can discriminate the ink samples, whereas for IR analysis, it was difficult to discriminate the ink samples because they had similar ink formulations. Hence, the discrimination was accomplished based on the intensity of main peaks as well as the pattern of each spectrum. On the other hand, Senior *et al.*'s study not only aimed at discrimination between different ballpoint pen inks using UV-Vis but also examined the effect of the time on the spectroscopy of the written inks. PCA method also found to be useful for objective classification and individualisation of the ink samples.

Ismail *et al.* (2014) aimed to analyse ballpoint pen inks (black, blue and red) using UV-Vis spectroscopy, then followed by manual visual examinations and chemometrics procedures of PCA and HCA. This to demonstrate the advantageous of chemometrics procedure over manual visual examinations for forensic analysis inks. Data were obtained at the spectral range from 200 to 800 nm then PCA and HCA were performed to the data. They concluded that outcomes from manual visual examinations are subjective. In contrast, chemometrics procedures afforded more objective and meaningful outcomes. The study showed that it was hard to classify pen inks by looking on their UV-Vis spectra only. Hence, chemometrics procedures is useful in pen inks forensic analysis.

Kumar and Sharma (2017) presented a study that utilised both destructive and non-destructive technique on the analysis of writing inks. The techniques involved were UV-Vis spectroscopy and diffuse reflectance UV-Vis-NIR spectroscopy together with chemometrics procedures. A total of 57 blue ballpoint pen inks were analysed. For the destructive technique, i.e. UV-Vis spectroscopy, ink lines were cut and extracted by using 10 ml ethanol. The ethanolic extracts were then analysed using UV-Vis from 200 to 800 nm wavelengths region. On the other hand, the spectra obtained using the non-destructive technique, i.e. UV-Vis-NIR were recorded in the wavelength range between 190 to 800 nm. The non-destructive technique gave higher discriminating power (DP) when combined with PCA which was 99.46% compared to 69.67% and 98.72%, for visual inspection of the spectra and destructive UV-Vis spectroscopy respectively.

Chromatographic techniques have been proven to be an effective and productive method to separate ink into its constituents component dyes, for both ink comparison and ink matching to chromatograms (Djozan *et al.*, 2008). One of the simplest and most widely used chromatographic techniques for analysis of ink is thin layer chromatography (TLC). It is widely used because it is fast, cost effective and cause minimal destruction to documents. According to Adam *et al.* (2008) TLC is a well-known technique for analysis of ballpoint pen inks. In this method, retention factor (R_f) values was used for qualitative evaluation of the chromatograms, however, it is not precise, especially when comparing the ink to a database of chromatograms.

Djozan *et al.* (2008) had used TLC to discriminate forty one (41) ballpoint pen inks of blue colour and compared various writing inks on documents. Other than that, they had proposed a new and fast data acquisition method by designing a new and targeted image specific image analysis software for evaluating thin layer chromatograms (TLC-IA) after scanning using ordinary office scanner. This method comparing two inks based on the R_f colour range and intensity of the separated ink components. The results of this study had shown that all the 41 ink samples of different trademarks were successfully analysed and discriminated using the suggested method. The discrimination power (DP) achieved in this study was 92.8% which confirmed this method is capable in differentiating a significant number of pen-pair samples.

Lee *et al.* (2014) worked on the analysis of ballpoint pen inks using an improved TLC technique, i.e. high performance thin layer chromatography. They used a set of blue ballpoint inks from twelve (12) different brands. The analyses were performed using a solvent system containing ethyl acetate, ethanol and distilled water (7:3:2 v/v/v). The discriminating power (DP) of the different brands and different models were also calculated and the DP was determined to be around 89.40%. Other than that, they had also conducted a study on ink homogeneity on two selected pens. Based on the analysis, the composition of blue pen inks was found to be homogeneous (RSD<2.5%) and the proposed method showed good repeatability and reproducibility (RSD<3.0%). It is concluded that HPTLC is an effective tool to separate blue ballpoint pen inks.

Saini and Rathore (2018) published two separate studies that described the use of advanced TLC which is HPTLC for analysing ink samples. Both study also included the uses of gas chromatography-mass spectrometry (GC-MS). Their first study was aimed at distinguishing blue, green and red gel pen inks based on their chemical constituents using HPTLC and GC-MS(Saini and Rathore, 2018a). Based on the result, they had found that HPTLC was suitable for studying dye-based gel inks whereas GC-MS was useful for both pigment and dye-based gel pen inks.

The second study conducted by Saini and Rathore (2018) was aimed at analysing ballpoint pen inks of black and red colour. They utilised HPTLC and GC-MS technique. HPTLC provides complete discrimination of all the samples. Whereas GC-MS was not able to completely discriminating red (DP=32.85%) and black (DP=63.58%) ballpoint pen inks. Two levels of classification was performed which first is based on major components and the second is based on minor compounds. The second level of classification gave maximum differentiation compared to first level classification.

Sombut *et al.* (2020) had discriminated blue ballpoint pen inks of different brands using UV-Vis spectroscopy and TLC. Prior to chemical analysis, ink was removed from the paper where the ink was deposited. To improve the accuracy and discrimination pen inks, mathematical and statistical methods were used. The results obtained indicate that the

TLC technique was a more effective tool for the analysis of ballpoint pen than the UV-Vis spectroscopy technique.

Capillary electrophoresis (CE) is one of the most important analytical techniques that can provide fast and high-resolution separations of complex mixtures (Burgi and Chien, 1991). CE is a destructive technique due to the requirement of extraction of a sample from a paper or document. The separation in CE is carried out by the two related electrokinetic effects, i.e. electrophoresis and electroosmosis (Chen, 2002). Copper *et al.* (2019) had used CE for the analysis of ballpoint pen inks by comparing two types of sampling method which are conventional method (indirectly) and direct method. They proposed a direct method of analysis of the dyes which involving no sample pre-treatment. The inks involve were extracted from a pen then the paper the dyes were injected directly into the CE instrument. As a result, the method proposed is a viable technique for the analysis of writing inks. This novel technique minimises sample preparation time, uses less sample and produces less waste than conventional method i.e. indirect sampling method.

2.3.3 Analysis of Marker Pen Inks

Until now, only a few studies on marker pen inks were done. Van der Werf *et al.* (2011) had used pyrolysis gas chromatography-mass spectrometry (PyGC-MS) in the analysis of permanent marker inks. Generally, PyGC-MS is a powerful tool for differentiation of several polymeric materials and especially paints, in automotive coats, spray paints and works of art (van der Werf *et al.*, 2011). This study investigated a selection of twenty-four (24) permanent markers from seven (7) different manufacturers and of seven (7) different colours. The result showed the effectiveness of PyGC-MS to differentiate permanent marker inks based on the presence of specific minor and major compounds. In fact, the result show differentiation based on binder identification. Whereas, identification based on pigments may be incomplete. PyGC-MS may be efficiently used as a complement to other techniques for the characterisation of marker inks. However, this technique is destructive which disturb the integrity of the document.

Awab *et al.* (2011) proposed a method with non-destructive manners. They used spectroscopy methods, namely FTIR with a micro-ATR attachment. Four different colours (black, green, blue and red) from three different brands of permanent and non-permanent marker pen inks were used in this study. The study was conducted to analyse marker inks from writing made on solid matrices, especially whiteboard of non-porous surface and to differentiate between the permanent and non-permanent marker using the proposed method. Based on the result, IR spectra were used to discriminate between inks analysed due to the presence of diagnostic peaks in the IR spectra. This proposed method is non-destructive. It is a preferable method in document examinations. Other than that, this method provides minimal sample preparation and cost-effectiveness.

Sodo *et al.* (2012) had utilised Raman spectroscopy on the analysis of marker pen inks. The investigation was aimed at the composition and degradation processes of the marker pen inks. TLC was also employed in this study to identify the multi-components of the dye mixture. Raman measurements had been performed directly on the paper for both original drawings and standard samples, and also on the separated TLC spots, directly on the TLC plates. The outcomes from the TLC had shown that Stabilo, Stabilo-old and Staedler red marker pen inks were much more complex and demonstrate that a mixture of different coloured inks had been used to obtain the desired hue. The outcomes from Raman investigation on the TLC separated spots allowed for the identification of most of the dye used. This study has proven that Raman spectroscopy on marker pen drawings was subtle towards the colouring agents and also to the paper components, whereas it did not evidence features coming from the solvents, bindings and additives and stabilisers used in industrial production. Moreover, Raman spectroscopy can identify precisely the different colouring agents and distinguish between tautomeric forms of the same compounds. Raman spectroscopy together with TLC was found to be ideal for forensic application due to the non-destructive nature of the methodology and lack of sample preparation.

A recent study was conducted by Sharma *et al.* (2018) used twenty four (24) marker pens consisting of 12 whiteboards and 12 permanent containing blue inks from different brands. Both destructive ultraviolet-visible spectroscopy (UV-Vis) and non-destructive diffuse reflectance ultraviolet-visible near-infrared spectroscopy (UV-Vis-NIR) were involved for the discrimination of the samples. In addition, chemometrics tools were applied to provide better results than visual comparison and give higher discrimination power.

In real cases, all the methodology proposed by the previous studies can be used for the analysis of ink. However, a non-destructive technique such as ATR-FTIR, Raman spectroscopy UV-Vis-NIR is preferred over destructive technique because the former does not disturb the physical integrity of the document. These techniques demonstrate a lot of advantages such as easy, fast and precise due to no sample preparation needed, cost-effective, does not involve any reagents, gives information about the chemical compositions of the inks such as solvents and colourants and most importantly non-destructive. On the contrary, it provides insufficient information to discriminate ink, especially ink of similar formulations. However, by introducing chemometrics procedures such as PCA and HCA, the interpretation of data has become more objective and most importantly give meaningful outcomes, in other words, the discrimination power of the ink discrimination is improved by incorporating chemometrics procedures (Asri *et al.*, 2020).

2.4 FTIR Microscope

Advanced chemical analysis is needed to support forensic casework has become more crucial because of the complexity and sophistication of the fraudulent items ("Forensic analysis of paper currency with FTIR microscopy," 2019). High-performance laboratory instruments allow detection and study of anomalies not by visual inspection alone but also for the progression of chemical domain of questioned documents, such as banknotes. Fourier Transform Infrared (FTIR) microscope is outstandingly suitable for the study of the inks, toners and papers of fraudulent documents. This instrument used standard visible light microscopy combined with non-destructive molecular spectroscopy analysis.

The region for FTIR spectroscopy and microscopy are located between 4000 cm^{-1} to 400 cm^{-1} (Doyle, 1992). The introduction of mapping and imaging equipment has improve the performance of FTIR microscopy. This allows the collection of a large number of spectra and to assemble a pattern showing the distribution of the different compound. The transmission of FTIR analyses worked together with IR radiation that passes through the samples. The method produced high energy output and high sensitivity. FTIR microscope consists of FTIR spectrometer combined with an optical microscope. The attachment of Attenuated Total Reflectance (micro ATR) in FTIR microscopy brings benefit to the analysis because the same aperture was maintained during the investigation of smaller areas.

2.5 Chemometrics Procedures

2.5.1 Principal Component Analysis (PCA)

Principal component analysis (PCA) is a statistical procedure which allows a huge number of sample dataset to be described in terms of smaller number of variables called principal components (PCs) (Wold *et al.*, 1987). Among all the statistical techniques, PCA is commonly used by almost all scientific disciplines. In PCA the important information from a dataset is extracted and expressed as a set of new orthogonal variables called principal components (Abdi and Williams, 2010). PCA also represents the pattern similarity of the observations and the variables by displaying them as points in maps.

2.5.2 Linear Discriminant Analysis (LDA)

Discriminant analysis is the most commonly used parametric method for method recognition (Adams, 2004). Linear discriminant analysis (LDA) is a classical statistical method for supervised dimensionality reduction and classification (Ye and Ji, 2010). LDA computes an optimal transformation in order to obtain maximum class discrimination by minimising the variance within sample and maximising the variance between-class simultaneously. The optimal transformation in LDA can be readily computed by applying an eigen decomposition on the scatter matrices. The aim of a discriminant analysis is to group objects into one of two or more mutually exclusive categories by a set of independent variables (Ayinla and Adekunle, 2015).

CHAPTER 3

METHODOLOGY

3.1 Sample collection

A total of seven (7) different brands of permanent marker pens were used in this study. All marker pens were purchased from bookstores within Kubang Kerian and Kota Bahru, Kelantan from March 2020. Each marker and ink sample was allocated specific reference code. Black colour inks was investigated in this study. Table 3.1 lists the marker pens used in this study with their respective model names and reference code.

Table 3. 1 List of permanent marker pens used in the study.

No.	Marker Pen Brands	Model	Code for Marker pen	Code for Black ink
1.	Artline	Artline 70 High Performance (EK-70)	A	HA
2.	Beifa	70 Permanent marker (PY237800) 2-3mm	B	HB
3.	Faster	Faster 70 Permanent Marker (M-F-70)	C	HC
4.	Flex Office	Flex Office (FO-PM03) – 1.5mm	D	HD
5.	Monami	Monami 501 Bullet Type	E	HE
6.	Pentel	Pentel (N850-AO)-Bullet Point	F	HF
7.	Pilot	Permanent Marker 100-Fine/Bullet	G	HG

3.2 Sample Preparation

A straight line of approximately 3 cm was made on a sheet of white 70 gsm A4 paper (IK Yellow) using each of the permanent marker pen. After that, each sample was labelled according to its code and was dried approximately 20 minutes before analysis under room temperatures.

3.3 Direct Visual Examination

Upon analysis the ink samples using ATR-FTIR spectroscopy, the IR spectra obtained were also examined. The samples were also examined visually using naked eyes with the aid of white light. Different brands of a similar colour of the permanent marker pen inks were compared side by side and discriminated based on their colour appearance.

3.4 ATR-FTIR Analysis

LUMOS FTIR-Microscope (Bruker) equipped with Attenuated Total Reflectance (ATR) sampling interface was used for the spectra acquisition as shown in Figure 3.1. Prior to sample analysis, the instrument was undergone several processes to ensure that it was calibrated and ready to be used. After that, the sample was placed on the sample stage and ready for analysis. The scan range was set to be from 4000 cm^{-1} to 600 cm^{-1} with resolutions of 4 cm^{-1} and scan time of 64.