CERTIFICATE

This is to certify that the dissertation entitled

"Characterization of Blue Ballpoint Pens Using UV-Visible Spectroscopy and Pattern Recognition Techniques"

the bonafide record of research work done by

NUR ATIQAH SALWA BINTI BAHMAN

During the period of February 2015 to June 2015
Under my supervision

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and the Most Merciful.

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ABSTRACT

Analysis of ink is important particularly in the investigation of forgery cases involving handwriting and signatures. Characterisation of ballpoint pen inks among different brands becomes of great interest in questioned document since most of documents are written using ballpoint pens. The purpose of this study was to evaluate the possibility of discriminating 24 blue ballpoint pen inks from four different brands namely Kilometrico, G'Soft, Faber Castell and Stabilo.

The blue ballpoint pen inks components were first separated using Thin Layer Chromatography (TLC) before the ink extracts were subjected to UltraViolet-Visible (UV-Vis) spectroscopy. The colour of the spots developed on the TLC plate and their Rf values as well as the UV-Vis spectra were examined manually by direct visual examination in attempt to classify the ink into their respective group. However, this approach had failed to differentiate the ink. The UV-Vis spectra of the ink which could not be differentiated were introduced to chemometrics techniques of Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). PCA had successfully differentiated and grouped all the blue ballpoint pen inks according to their brands manifested in the score plot. Using the Euclidean distance as the proximity measure and complete linkage as the linkage strategy to link cluster, HCA had also successfully differentiate the blue ballpoint pen inks under study according to the brand. The results proved that the UV–Vis spectra combine with chemometrics technique of PCA and HCA can be effective tool to differentiate blue ballpoint pen inks of different brands

ABSTRAK

Analisa dakwat pen penting terutamanya dalam penyiasatan kes penipuan yang melibatkan tulisan tangan dan tandatangan. Pencirian dakwat pen mata bulat di antara jenama yang berbeza menjadi tumpuan dalam dokumen dipersoal kerana kebanyakan dokumen ditulis menggunakan pen mata bulat. Tujuan kajian ini dijalankan adalah untuk menilai kemungkinan untuk mendiskriminasi 24 sampel dakwat pen biru bermata bulat daripada empat jenama yang berbeza iaitu Kilometrico, G'Soft, Faber Castell and Stabilo.

Komponen dakwat pen biru bermata bulat pada mulanya di dipisahkan oleh TLC sebelum ekstrak dakwat tertakluk kepada UV-Vis spektroskopi. Warna titik yang terhasil di atas plat TLC dan nilai Rf juga spektrum telah diperiksa secara manual melalui pemeriksaan penglihatan secara langsung dalam percubaan untuk mengklasifikasikan dakwat ke dalam kumpulan masing-masing. Walaubagaimanapun, pendekatan ini telah gagal untuk membezakan dakwat. Spektrum UV-Vis dakwat telah diperkenalkan kepada teknik kemometrik iaitu Analisis Komponen Prinsipal (AKP) dan Analisis Kelompok Hairaki (AKH). AKP telah berjaya membezakan dan mengumpulkan semua sampel mengikut jenama yang dinyatakan pada plot skor. Menggunakan ukuran jarak 'Euclidean' sebagai langkah pendekatan dan penghubung lengkap sebagai strategi hubungan untuk menghubungkan kelompok, AKH juga berjaya membezakan dakwat pen biru bermata bulat mengikut jenama. Keputusan membuktikan bahawa UV-Vis spektrum digabung dengan kemometrik terutama teknik AKP dan AKH boleh menjadi kaedah yang berkesan untuk memisahkan dakwat pen jenama berbeza.

CHAPTER 1

INTRODUCTION

1.1 Ink Analysis in Forensic Science

Questioned document examination is a part of forensic science related to documents that are dubious in nature. It is one of the oldest branches of forensic science. Other than forgery cases, documents also involved in cases such as homicides, suicides, burglaries and robberies. By definition, questioned document involved suspicious origin or fake nature of document by observing at any signature, handwriting, typewriting, or other mark.

Crime related to forgery which involves alterations in the content of document. For instance, deletion, obliteration and addition are increasing. The alteration of content of documents with significant financial value for instances cheques, insurance claims and wills usually use various writing instrument such as pen and pencil. Therefore, analysis of inks is very crucial to detect any kind of these frauds. From the writing instrument, ink analysis that is obtained can help in comparing, identifying, characterising and discriminating the ink. (Ismail *et al.*, 2014)

Forensic examination of question document routinely involves physical and chemical examinations. Ink examinations can be classified into destructive and non-destructive where non-destructive is more preferable as it gives sufficient information without altering the document. (Braz *et al.*, 2013). As time passes by, technique such as UV-Visible Spectroscopy (UV-Vis), Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) were developed which is more

informative and effective for ink analyses. (Braz *et al.*, 2013). However, sample preparation is required.

As pen ink is found difficult to distinguish and error occur when analyzed data manually, chemometrics method is use for further analysis. This technique is used because it gives result that can be expressed more objectively rather than subjectively. The result based on similar and different among pen inks sample in data set. For this technique, Principle Components Analysis (PCA) and Hierarchical Clustering Analysis (HCA) is use to interpret the data.

1.2 History of Ballpoint

The idea and development of ballpoint as modern writing instrument took almost 60 years by three different inventors. In 1888, John J. Loud, a leather tanner invented a ballpoint consisted of a tiny rotating ball bearing. Ink continuously coats the ball bearing. However, there is a limitation about the first invention where the ink was leaking resulting in smearing on paper.

The first commercially notable ballpoint pen was introduced by Ladislas Biro and his brother Georg. They produced their own model by improving Loud's ballpoint pen. The idea was similar but the pen did not leak as bad as before. The leakage problem was solved by Baron Marcel Bich in the following year. He studied the detailed construction of every ballpoint pen on the market, often working with a microscope. He then manufactured the pen to the market. The technology and quality parts of the pen had been improved over the years. (Romanowski, 2015)

1.3 Inks and Its Main Component

The development of ballpoint pens meets greatest challenge when it comes to build up the composition of the ink. Ink which is used in writing instrument for writing, drawing, and printing is in liquid or semi-liquid. In order to get thick liquid which can flow down the barrel of the pen and get quick drying ink, the viscosity of ballpoint pen ink must be precisely controlled. (Allen, 2015). Ink is basically made up of pigments and dyes, vehicle, solvent, additives and surfactant. (Matt, 2004)

1.3.1 Pigments or Dyes

The colour of ink comes from either pigments or dyes, dissolved or suspended in an organic or inorganic solvent. Dyes are soluble in water while pigments, coloured particles are insoluble in water. Pigment can be in the form of organic or inorganic. However, it is more expensive and less colour range. Usually dyes are used for ballpoint pen inks as pigment can cause the ball of the pen to clog due to its insolubility in water. Other than that, dye can produce ranges of colour more than pigments.

1.3.2 Vehicle

Ink vehicle helps to transport the pigment or dyes onto substrate. It is faint bluish-black solution. It can be either in plant-based or solvent-based where plant-based can be dry by penetration and oxidation while solvent-based can be dried by evaporation. Vehicle must have drying characteristics to prepare right "curing" effect of the ink on several surfaces. Vehicle is also known as a binder. It binds ink components together and allows them to remain on writing surface.

1.3.3 Solvent

Solvent that is commonly used for ballpoint pen ink is usually oil-based. Oil can give thickness to the ink beside it dries quickly. Other than that, oil is permanent and also water-fast. Solvent functions as diluent which dilute the colurants and it changes the ink viscosity. Examples of solvents are toluene, mineral oil, alcohols and water.

1.3.4 Additives

Additives such as resins and preservatives are used to alter the final properties of ink which gives improvement to ink's basic qualities. Additives help ink to glide more smoothly over paper besides prevent ink becoming so acidic that cause damages to the pens. Additives have the smallest percentage in the composition of ink.

1.3.5 Surfactants

Surfactants control the surface of properties. It acts as wetting agent which makes the ink wets towards the surface by lowering the surface tension of the ink. In general, the ink will wet more when the surface tension of the liquid. Ethoxylated and propoxylated silicon based surfactants are examples of surfactants used in ink.

1.4 Problem statement

Crime related to questioned document has been a serious problem facing not only to our country but also worldwide. Crime involves forgery and fraud in document rapidly increases since last decade. Statistic by Royal Malaysian Police (RMP) showed they received complaints due to fraud and forgery cases about 730 complaints from year 2005 to 2012. (Berita Harian, 2013). Several analytical techniques used by question document examiner in ink analysis have been proposed in order to combat this problem.

Perhaps the most popular of these is Thin Layer Chromatography. Others have proposed using UltraViolet (UV)-Visible Spectroscopy, High Performance Thin Layer Chromatography, Infrared Spectroscopy and many others. However, the technique not enough to identify and classify information regarding ink component. Another powerful technique is needed to help in ink analysis done by experts.

1.5 Objectives

The objectives for this study are:

I. To analyse blue ballpoint pen inks of four different brands using UV-Visible spectroscopy

II. To analyse the UV-Visible spectroscopic data of the ballpoint pen inks using pattern recognition techniques of Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA)

III. To compare and discriminate ball point pen inks using PCA score plot and HCA dendrogram

1.6 Significant of the study

Analytical techniques used by question document examiner in ink analysis are not enough to identify and classify information regarding ink component because the interpretation of result from spectrum and chromatogram tend to bias as it depends on the examiner experience and knowledge. Pen ink can be difficult to distinguish and error will occur when analyzed data manually. Therefore, comparison and discrimination of ball point pens can be expressed more objectively using chemometric techniques. Analytical technique when combine with chemometric techniques can become a powerful tool regarding ink analysis in document.

CHAPTER 2

LITERATURE REVIEW

2.1 Forensic Ink Analysis

Pen, is one the most common writing implement used in preparing a document. Although it is almost impossible to determine or pint-point which pen had been used to prepare a document, ink analysis can at least helps to narrow down the type and brand of pen used to prepare a document. Ink analysis is one of the key areas in forensic questioned document (QD) examination. It primary purpose as being mentioned earlier is to detect any forms of modifications and alteration in a document in relation to the writing ink used to prepare the document.

There are many different types of pens introduced to the market namely fountain pen, roller-ball, ballpoint, felt-tip and gel pen. (Thanasoulias *et al.*, 2003). The chemical composition for each type of pen is differently designed in order to suit the intended use. For example, ballpoint pen ink which normally contains high proportion of organic dyes is more thick and viscous compared to ink for roller-ball and felt tip marker pen .(Zlotnick and Smith, 1999) therefore the ability to differentiate the physical appearance of ink is one of important steps in determining the type of pen.

Although ink is made up of a very complex formulation, ink analysis mainly focused on the colourant components of an ink i.e. the dye and the pigment. This is due to the uniqueness of these components which differ significantly between manufacturers. Although pigment requires more specialised technique to analyse, dye on the other hand can be easily analysed using readily available techniques in most forensic laboratory for examples ultraviolet-visible (UV-Vis) spectrometry and also chromatography (Fanali and Schudel 1991; Rhode *et al.* 1997; Tebbett 1991; Xu *et al*. 1997; Zlotnick and Smith 1998). In general, ink analysis can be performed either non-destructively using non-destructive techniques or destructively using destructive techniques.

2.2 Non Destructive Techniques

One of the important aspects of nondestructive techniques is that, they do not contribute to physical changes of a document therefore they are extremely valuable for analysis of documents with high historical and financial values (Morsy *et al.*, 2005). The most commonly used non-destructive techniques for forensic ink analysis are Video Spectral Comparator (VSC), Raman Spectroscopy and Fourier Transform Infrared Spectroscopy (FTIR).

2.2.1 Video Spectral Comparator (VSC)

Any changes in the document that are invisible to human eye often can be detected through the device that utilize ultraviolet and infrared wavelength of light. The absorption level of red and infrared used from 455 to 1000 nm while luminescence in visible and infrared light from 455 to 1000 nm. Using radiation filtered at different wavelength, Video Spectral Comparator (VSC) able to analyze inks, visualize hidden security features, and reveal alterations on a document. The desired image can be captured after select the filter and light combinations.

Roux *et al.* (1999) had performed optical examination of ink using VSC. The study was conducted using 49 blue and 42 black ballpoint pen inks from different brands, models and batches, representative ballpoint pens available in the Australian market. The inks were differentiated by grading the similar luminescence or reflectance to the paper as strong, weak, or none. They had found that the VSC can successfully differentiate inks with similar colour and appearances. On the other hand, the discriminating power for the black ballpoint inks was considerably higher rather than blue ballpoint inks. Since document can be analysed *in-situ* and no sample preparation steps are required, analysis time in VSC is relatively quick.

2.2.2 Raman Spectroscopy

Due to the technological advancements, Raman spectroscopy has become one of the instruments of choice for ink analysis. The latest generation of Raman spectroscopy offers advantages such as measurement can be done in short time and offers low power laser intensity. Examination of document using low power laser gives good signal-to-noise ratio and does not destroy the document through burning. (Fabiañska and Trzciñska, 2001).

Raman spectroscopy analysis is sometimes performed when the VSC analysis failed to discriminate ink samples. Information obtained from Raman spectroscopy is particularly useful for cases that are impossible, if not difficult to solve by direct visual observation and optical techniques. According to Fabianska *et al.* (2001), Raman spectroscopy can be supplemental to both visual and optical techniques.

The Surface Enhanced Resonance Raman Scattering (SERRS), a variant of Raman spectroscopy has been routinely used to analyse writing inks especially the ballpoint pen inks. SERRS is discriminative, sensitive and able to identify dyes in mixture (White *et al.*, 1998). It also has high discrimination power and requires literally no sample preparation and pre-treatment step except application of small amount of colloidal emulsion to reduce the effect of fluorescence. The advantages of SERSS make this technique favourable over other non-destructive techniques. Study conducted by Morsy *et al* (2005) revealed that SERSS can be used to successfully differentiate blue and black inks when other non-destructive techniques had failed to achieve that.

Four blue inks and four black inks were collected from various Egyptian sources for ink identification. The result obtained from non-destructive methods such as filtered light examination (FLE) and microspectrophotometry (MSP) were compared with Raman spectroscopy and SERSS result. The result showed any of all physical methods used only one blue ink could be differentiate while the other three blue inks failed to be differentiating by any of the method used. Two black inks were successfully differentiated by SERSS. Therefore, it was concluded that SERRS is a very useful and supportive non-destructive technique.(Morsy *et al.*, 2005).

2.2.3 Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared (FTIR) Spectroscopy had been used by Abdul Halim *et al.*, (2012) to discriminate 24 ballpoint pen inks of six different brands which were Carera, Pilot, G-soft, Papermate, Faber Castle and Stabilo. The first three ballpoint pen inks were successfully discriminated however it was not the case for the last three ballpoint pen inks. Close examination of their FTIR spectra revealed similar resemblance. The study concluded that full discrimination of inks can only be achieved if their spectra are not identical.

2.3 Destructive Techniques

Destructive techniques as its name suggests involve removal of a small portion of a document bearing the ink line which causes irreversible damage to the document under examination. The most commonly used destructive techniques for forensic ink analysis is the chromatographic techniques mainly Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Gas Chromatography. The main reason these techniques is chosen is due to their superior ability in separating ink components into its individual constituent which is required for positive identification (Zlotnick and Smith, 1999).

2.3.1 Thin Layer Chromatography

TLC is well known technique used for analysis ballpoint pen ink and even law enforcement agencies use this technique for ink-source comparisons during investigation (Tebbett 1991; Xu *et al.* 1997) even though not much information is gathered. The principle of TLC based on movement of mobile phase that travel up through the glass plate. The mobile phase carries along the component mixture in stationary phase. Solubility rules "like dissolves like" is followed. Compound with less similar properties to mobile phase will stay behind while compound will travel longest up the TLC plate when it has more physical properties alike to mobile phase due to longer of time stay in mobile phase.

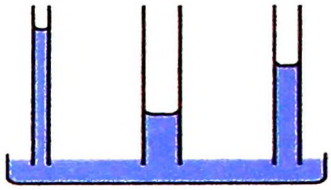

Figure 2.1 : Capillary action
(http://hyperphysics.phy-astr.gsu.edu/hbase/surten2.html)
Accessed on 2nd April 2015)

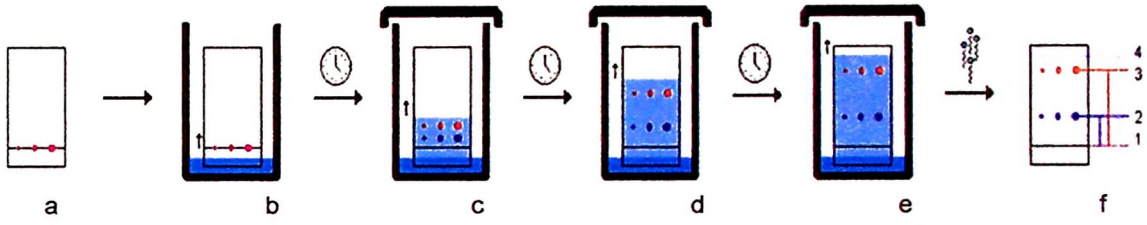

Figure 2.2: Schematic diagram of TLC process
(https://www.highperformance liquidchromatography.org/thin-layer-chromatography/)
(Accessed on 2nd April 2015)

The TLC processes are: a) The mixture will be spotted in a row just above (about 1 cm) from the bottom of the TLC plate. b) TLC plate is then place in developing chamber consist of shallow solvent which the spotted area must be above the solvent. c) The solvent moves up through plate by capillary action. d) Some of mixture component remain with stationary phase while some will dissolve by mobile phase and moving along the plate. e) Solvent front is mark when the plate is removed from tank where the solvent travel close to the top of the plate. f) Distance travelled by component observed using naked eyes or under UV light.

Present of individual component of mixture in TLC is characterized by Retardation factor (Rf) value where it is calculated by measuring the distance travelled by component is divided with distance travel by the solvent (individual spot).

$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

Rf value can only consistent if condition such as solvent system, adsorbent, thickness of adsorbent and amount material spotted are consistent throughout experiment. However, TLC is commonly used for presumptive test and further test is needed for confirmation

Despite the fact that TLC is easy to use, it still has some limitation. For example, it is not typically automated which the ink must be spotted manually onto TLC plate. Retardation factor (Rf) values largely depending on the spotting. Other than that, spots may not clearly be seen due to fading effect. TLC reference slides should be stored in control environment such as humidity to solve the problem or the ink needed to be spotted for a few times. However, size of ink spotted can be too large as the ink may overlap with each other resulting Rf value cannot easily be measured. (Brewer *et al.*, 2005)

There are some studies by researcher on examination of ballpoint pen ink using chromatography technique. According to Williams and colleagues (2009), in 1998, initial preparation of standard TLC library was introduced by Tsutsumi & Ohga. They use black, red and blue inks with total 35 ballpoint pens. They found out that developing solvent using ethyl acetate, ethanol and water (14:7:6) had higher degree of dye separation rather than use ethyl acetate, methanol, 28% aqueous ammonia (5:2:1) or tricholoethylene, 1,1,1-trichloroethane, ethyl acetate (10:1:1). Study by Roux *et al.* (1999) conducted in Australia showed that TLC capable to distinguish blue ballpoint pen inks between and within brands, models, and batches.

2.3.2 High Performance Thin layer Chromatography (HPTLC)

Because of some problems (i.e limited resolution and difficulties with quantification) found on TLC technique, High performance thin layer chromatography (HPTLC) was formed to improved TLC technique (Senior *et al.*, 2012). It was introduced in early 2010s. With the aid of statistical technique, quantitative data obtained from HPTLC chromatogram will gave better interpretation which accuracy of identification and discrimination of pen inks can be greatly improve.

According to Loong and colleagues (2014), HPTLC is an effective tool to separate blue ballpoint pen inks. His study used 12 blue ballpoint pens were performed using ethyl acetate, ethanol and distilled water (7:3:2). Discrimination power of 89.40% was achieved, which confirm that HPTLC was able to differentiate a significant number of pen-pair samples.

2.3.3 High Performance Layer Chromatography

High performance layer chromatography (HPLC) was found way better than TLC because it has distinguished many ink samples which were failed by TLC. However, HPLC requires larger ink sample, cost consuming and high skill than TLC. Thus, it is not popular routine analysis used by questioned document examiner as much as others. Lyter in his study stated that HPLC was able to differentiate ink between batches when he used 10 ballpoint pen inks that unable to distinguish by TLC. (Lyter *et al.*, 1983).

2.3.4 Ultraviolet-Visible Spectroscopy

The principle for UV-Vis spectroscopy is based on electronic transition. The amount of ultraviolet (UV) or visible (Vis) radiation absorbed by a molecular species in solution is measured where every different molecule absorb radiation at different wavelength. The result will be in the form of spectra include wavelength and maximum absorbance of compounds.

UV-Vis spectrometer is one of the popular instruments used in laboratory. It is used to measure the amount of UV-Vis light absorption of compound in the range UV and Vis regions of the electromagnetic spectrum. UV-Vis spectrometer have three basic components; a light source (tungsten lamp) with specific wavelength of light in the UV-Visible region of spectrum, a chamber to place the cuvettes into light path, and a photocell or detector that measure amount of light absorbed by the sample.

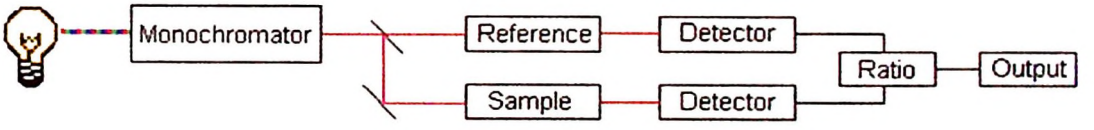

Figure 2.3: Schematic diagram of UV-Vis spectrometer.
(http://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspec/uvvisab3.htm)
(Accessed 3 April 2015)

Cuvette containing sample and blank solution are place together in chamber. Quartz or fused silica cuvettes are used because they are transparent to the UV-Vis radiation. Appropriate condition for instrument is setup which the wavelength 200 nm – 800 nm is used to scan the wavelength based on range of electromagnetic spectrum of ultraviolet and visible light. The intensities of the light beams are then measured by electronic detectors. The photomultiplier tube is the common detector used for UV-Vis spectroscopy.

Analysis fountain pen inks using Ultra Violet-Visible (UV-Vis) spectroscopy showed that UV-Vis gave effective result in characterize ink of different brands.(Sharma and Agarwal, 2014). UV-Vis spectra tend to separate blue ballpoint ink better than IR and HPTLC (Senior *et al.*, 2012). Another study was conducted by Ismail *et al.* (2012) using 36 ballpoint pens from 2 different brands involve blue, red and black ink. The analysis showed UV-Visible spectroscopy able to differentiate ballpoint pen of different color and brand. However, the result which in qualitative information is highly subjective as it depends on experience and knowledge of the questioned document examiner.

2.4 Chemometrics Techniques

In essence, chemometrics techniques use both mathematical and statistical methods to analyses data that are very difficult to measure directly. (Lavine, 2000). Through appropriate mathematical computations, values of interest are obtained from the measurements of the chemical components of samples or objects. Chemometrics, in specific, multivariate analysis has only recently caught the attention of forensic scientists however its impact is quite profound and it has been successfully used to aid ink examination. (Senior *et al.*, 2012). Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) are the two most commonly consulted chemometrics techniques in forensic science.

2.4.1 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a dimensionality reduction technique, in other words, it reduces the number of variables (inter-correlated) in original dataset into a much smaller number of variables (uncorrelated) known as principal components with minimal loss of information. This technique will help to identify an association between sample which will assists in classification and characterisation and possibly individualisation of sample data set in an objective and reproducible fashion.

Raw data obtained from analysis such as from gas chromatography and UV-Vis spectra are described using new variables known as principal component (PCs). The new variables or the PCs are arranged in descending order of importance with the first PC (PC1) describe the largest variation in the dataset followed by the second PC (PC2), the third PC (PC3) and so on. PCA decomposes data matrix into two smaller matrices which are loading and score matrices. The groupings or clusterings within a

given dataset are gathered through or by examining the score plot. (Ismail *et al.*, 2014).

2.4.2 Hierarchical Cluster Analysis (HCA)

Hierarchical Cluster Analysis (HCA) is a clustering technique which produces a dendrogram or a tree like structure in its final outcome. In essence, HCA involves a process of splitting a set of objects into subset with similar objects are grouped closely together and vice-versa. (Abdul Halim *et al.*, 2012). Clustering of a dataset is accomplished either using agglomerative and divisive method. (Ismail *et al.*, 2014). Clustering will be form in a series of step.

In the agglomerative method, the method starts with single cluster then decomposed into many cluster comprising of single object. All samples will be separated and joined back until all the dataset are joined together and produce one big group or cluster. The divisive method works the opposite of the agglomerative technique. The method starts with one or single object and finally formed a single cluster The similarity and dissimilarity between object in dataset are computed most commonly using the Euclidean distance function, although other functions for examples squared Euclidean distance and Manhattan distances are also employed. Linkages functions to link clusters exist in a dataset are single, average and complete linkage.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sample Collection

Four (4) different brands of blue ballpoint pen namely Faber Castell (FC), Kilometrico (K), Stabilo (S) and G'Soft (GS) were considered in this study. Six pens were purchased for each brand therefore in total; twenty four (24) pens were used. The pens were brought from stationery store in Kubang Kerian, Kelantan area.

3.2 Sample Preparation and Ink Extraction

Each pen was scribbled separately onto 1 cm x 1 cm area of a filter paper (Whatman). The scribbling was done until the whole 1 cm x 1 cm area of the filter paper was covered with ink. Using a pair of clean scissors, the scribbled area was cut out and then transferred into a bijou bottle containing 4 mL of absolute ethanol (Merck, Germany) to extract out the ink. To facilitate the extraction of the ink, the bottle was shaken vigorously using a vortex for approximately 5 minutes. Figures 3.1 shows the ballpoint pen ink extracts.

Figure 3.1: Prepared extracted ink of blue ballpoint pen from each pen of brands G-Soft in bijou bottle. From left-right : G1, G2, G3, G4, G5 and G6

3.3 Thin Layer Chromatography (TLC) Analysis

The ink extract was individually applied onto a TLC (Merck, silica gel 60 F254) plate using a capillary tube. After the application, the prepared TLC plate was then dipped into a TLC chamber containing 1 cm layer of a mixture of ethyl acetate: absolute ethanol: water (70:35:30 v/v/v). The plate was developed using the single development technique i.e. the solvent head was allowed to traverse to a pre-determined distance from the origin before it was removed from the TLC chamber. The developed plate was allowed to dry and then observed for any spots using naked eyes and with the aid of ultra-violet light. The colour(s) of the spot(s) was recorded and the retention or retardation factor (R_f) of the developed spot was calculated using the Equation 3.1 below:

$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

Equation 3.1 : Formula calculation for Rf value

Figure 3.2 shows the TLC set-up used in this study.

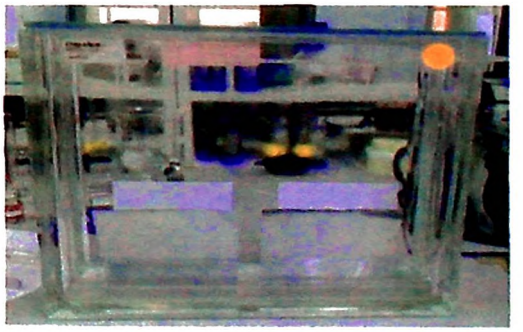

Figure 3.2 : The TLC chamber used in the study

3.3.1 TLC Repeatability and Reproducibility Studies

For the repeatability study, six individual spot from the same ink extract was made on one TLC plate whilst for the reproducibility study, six individual spot from six different extracts of six different ballpoint pens of the same brand was made on one TLC plate. The plates were developed, analysed and recorded according to the conditions previously described.

3.4 UV-Visible Spectroscopy

The UV-Vis spectrometer used to analyse the ink extract was a Varian's Cary® 100 UV-Vis Bio spectrometer (Varian, Inc.) as shown in Figure 3.3 below. The UV-Vis spectrometer was interfaced to a personal computer by a Varian Cary WinUV Analysis package to enable spectrum and data collection. To obtain the UV-Vis spectra, the ink extract was first transferred into a quartz cuvette using a plastic dropper. The cuvette was wiped clean using tissue paper to remove any dirt attached on its faces and then placed into the spectrometer chamber. The extract was scanned from 200 nm to 800 nm wavelength range. A blank which was prepared by extracting the filter paper using the absolute ethanol was also analysed in order to rule out any interferences from the filter paper.

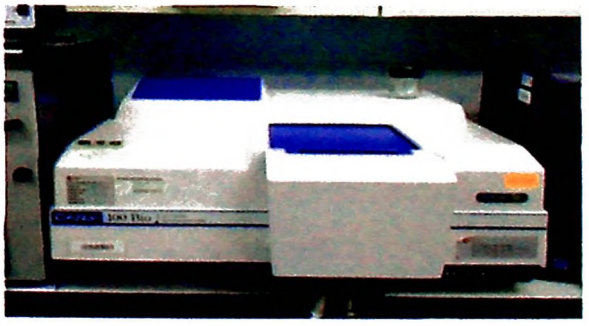

Figure 3.3: Varian's Cary® 100 UV-Vis Bio spectrometer (Varian, Inc.)