AN INTEGRATED RAMAN SPECTROSCOPY AND SELF-ORGANIZING FEATURE MAP CHEMOMETRICS ANALYSIS FOR THE DISCRIMINATION OF GEL INKS

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by

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-My success is not by me but by Allah-

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LIST OF ABBREVIATIONS

ATR	Attenuated Total-Reflectance
ANN	Artificial Neural Networks
BMU	Best Matching Unit
CCD	Charge Couple Device
D%	Dating percentage
DART-MS	Direct Analysis In Real Time Mass Spectrometry
DSA-MS	Direct Sample Analysis Mass Spectrometry
DP	Discriminating Power
DRIFTS	Diffuse Reflectance Infrared Fourier Transform Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
FT	Fourier Transform
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
HPTLC	High-performance thin-layer chromatography
HCA	Hierarchical Cluster Analysis
HSI	Hyperspectral Imaging
IP-HPLC	Ion-Pairing High-Performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LDA	Linear Discriminant Analysis
LDMS	Laser desorption/ionisation mass spectrometry
LIBS	Laser Induced Breakdown Spectroscopy
MeOH	Methanol

NIR	Near Infrared Spectroscopy
PC	Principal Component
PCA	Principal Component Analysis
PLSDA	Partial Least Squares Discriminant Analysis
PPMC	Pearson Product Moment Correlation
R_f	Retention Factor
RS	Raman Spectroscopy
%RSD	Percentage Relative Standard Deviation
SOFM	Self-Organizing Feature Maps
SWGDOC	Scientific Working Group of Document
SOP	Standard Operating Procedure
S:N	Signal to Noise Ratio
TLC	Thin Layer Chromatography
UV-Vis	Ultra-Violet and Visible Spectroscopy
VSC	Video Spectral Comparator
XRF	X-Ray Fluorescence

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SPEKTROSKOPI RAMAN DAN PETA CIRI MENGATUR DIRI ANALISA KEMOMETRIK BERSEPADU BAGI DISKRIMINASI PEMBEZAAN DAKWAT GEL

ABSTRAK

Salah satu teknik yang sering diguna pakai oleh ahli forensik dokumen dalam analisis dokumen dipertikai adalah spektroskopi Raman. Teknik ini telah terbukti berkesan dalam mencirikan dakwat secara cepat dan tepat dengan memberikan kelebihan menyebabkan kemusnahan kekal terhadap dokumen yang dianalisis. Pen dakwat gel telah diperkenalkan dalam pasaran sekitar tahun 1990an oleh Sakura Colour Products Corporation, Jepun bagi memberikan pilihan alat tulis harian kepada pengguna selain pen mata bola. Dipasarkan dalam pelbagai warna yang menarik dan mesra alam, pen dakwat gel telah menjadi pilihan popular pengguna dan kemunculannya dalam kes-kes melibatkan penipuan dokumen sememangnya dijangka. Walaubagaimapun, kajian melibatkan pen dakwat gel dari perspektif dokumen dipertikai adalah terhad dibandingkan dengan pen mata bola. Dalam penyiasatan forensik, dilaporkan bahawa terdapat 80% dokumen yang ditulis dengan pen mata bola. Oleh itu, disebabkan kekurangan objektiviti dakwat gel, kajian ini dicadangkan menggunakan teknik kemometrik novel untul mendiskriminasi dakwat gel pen. Kajian ini mengetengahkan aliran kerja konsepsi menyeluruh dengan kaedah kemometri lazim iaitu analisis hierarki-gugus (HCA), analisis komponen utama (PCA) dan kaedah kebaharuan peta ciri aturan kendiri (SOFM) digabungkan bersama spektroskopi Raman untuk membezakan dakwat gel tiga warna berbeza iaitu biru, merah dan hitam daripada pelbagai jenama sebelum dan selepas melalui proses penuaan selama hampir tiga tahun. Kajian awaldengan melarutkan dakwat gel di dalam pelbagai pelarut organik menunjukkan dakwat gel berasaskan pigmen kebiasaanya

tidak melarut di dalam pelarut organik manakala Kromatografi Lapisan Nipis hanya berjaya terhadap dakwat gel berasaskan pencelup. Dengan merujuk kepada pengkalan data spektrum Raman yang mengandungi 200 pigmen terhadap spektrum Raman yang dihasilkan daripada dakwat gel biru, merah dan hitam, CI Pigmen Biru 15: 1 dan 15: 3 berkemungkinan pigmen utama dalam dakwat gel biru, Organize Merah DPP BO (23180 – CI Pigment Red) dan XSL Poppy Merah (26308 – CI Pigmen Merah 112) berkemungkinan pigmen utama dalam dakwat gel merah manakala pigmen utama dalam dakwat gel hitam tidak dapat dikenalpasti. Dalam hampir semua keadaan, analisis hierarki-gugus (HCA) telah merekodkan jumlah gugusan yang lebih sedikit berbanding analisis komponen utama (PCA). Secara umumnya kedua-dua teknik kemometri lazim ini tidak dapat membezakan sampel dakwat gel mengikut jenama masing-masing walaubagaimanapun ini tidak berlaku terhadap kaedah kebaharuan peta ciri aturan kendiri (SOFM). Kebaharuan peta ciri aturan kendiri (SOFM) menunjukkan persamaan dengan klasifikasi yang dibuat oleh dakwat Raman visual. Kesalahan klasifikasi kepada sampel (HCA dan PCA) telah didapati berjaya di selesaikan dalam SOFM dan ini menungjukkan keupayaannya dalam diskriminasi dan pengelasan sampel. Pengesahsahihan dengan menggunakan kaedah *pengesahsahihan-k* terhadap model-model peta ciri aturan kendiri (SOFM) telah merekodkan kadar pengkelasan tepat 100% untuk dakwat gel biru, merah dan hitam. Sumber asal dakwat gel yang telah melalui proses penuaan juga telah dapat ditentukan melalui kaedah peta ciri aturan kendiri (SOFM). Dapatan daripada kajian bukan sahaja mengariskan aliran kerja konsespi menyeluruh untuk analisis dakwat gel tetapi yang terpentingnya menandakan potensi keupayaan peta ciri aturan kendiri (SOFM) untuk diguna pakai bersama spektroskopi Raman sebagai alternatif kepada kaedah kemometri lazim yang sangat berguna dalam siasatan penipuan dokumen melibatkan dakwat gel.

AN INTEGRATED RAMAN SPECTROSCOPY AND SELF-ORGANIZING FEAUTURE MAP CHEMOMETRICS ANALYSIS FOR THE DISCRIMINATION OF GEL INKS

ABSTRACT

One of the techniques often used by forensic document examiner in the analysis of questioned documents is Raman spectroscopy. This technique has proven effective in rapidly and accurately characterising ink with the advantage of not causing permanent damage to the analysed document. Gel ink pen was first introduced to the market around 1990s by the Sakura Colour Product Corporation, Japan as an alternative daily writing instrument other than ballpoint-pen. Being marketed in varieties of attractive colours and also environmentally friendly, gel ink pen has become very popular among consumers therefore it appearances in cases involving document fraud is anticipated. Unfortunately, studies involving gel ink pen in the perspective of questioned document are limited compared to ball-point pen. In forensic investigation, it was reported that 80% handwritten documents were written using ballpoint pens. Therefore, due to the lack of objectivity in interpreting gel inks, this study proposed using novel chemometric techniques for discriminating gel-pen inks in forensic investigation. This study show the comprehensive conceptual work flow with conventional chemometrics techniques of Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA) and novel Self-Organising Feature Maps (SOFM) in tandem with Raman spectroscopy to discriminate gel inks of three different colours i.e. blue, red and black before and after undergoing ageing process for nearly three years. Preliminary dissolution studies performed by dissolving gel inks in a varieties of organic solvents show that gel inks of pigment based colourants do not normally dissolve in organic solvents while Thin Layer Chromatography (TLC) is only

successful to dye-based colourant gel inks. Queries made to the acquired blue, red and black gel inks Raman spectra against a database containing Raman spectra of 200 pigments, suggested that CI Pigment Blue 15: 1 and 15: 3, could be the main pigment in blue gel inks conversely Red DPP BO (23180 – CI Pigment Red) and XSL Poppy Red (26308 – CI Pigment 112) could be the main pigment in red gel inks however main pigments in black gel inks remain unidentified. In most cases, Hierarchical Cluster Analyses (HCA) have recorded lesser number of clusterings compared to Principal Component Analyses (PCA). In general, both conventional chemometrics techniques are unable to discriminate the gel inks according to their brands however this is not the case for the novel SOFM. All SOFM maps are in line with the classification made by the visual Raman comparison of inks. The misclassified sample (HCA and PCA) was successfully resolved using the SOFM model signifying its capability for both discrimination and classification purposes. Cross validations employing the *k-validation* strategy have recorded 100% correct classification rates for all the SOFM models, thus signifies the robustness and potential of SOFM for discrimination of gel inks. As for the aged gel inks, SOFM has also successfully sourced the aged inks to their fresh counterparts. This study reported the first use of SOFM for discrimination and classification of gel inks. The findings of this study did not only communicate the comprehensive work flow for gel ink analysis but most importantly signify the potential of SOFM to be employed in tandem with Raman spectroscopy as an alternative pattern recognition technique to the conventional chemometrics techniques that can be highly useful in document fraud investigation involving gel inks.

CHAPTER 1

INTRODUCTION

1.1 Introduction

In this chapter, the general idea of forensic ink analysis in document examination and the gaps identified in this field are briefly discussed. This chapter highlights the aim of this study to enhance the existing technologies, to facilitate new development of new examination techniques for forensic document examiners (FDEs) Forensic document examiners (FDEs) are required to determine (i) whether there is any alteration and obliteration on documents, (ii) to differentiate the inks, and (iii) to identify the origin of the inks on the documents. Indeed, this study navigates the future development of forensic document examination area that may be of assistance in addressing and effectively operational in actual cases.

1.2 Documents

Documents have an integral role in finance, legal, and business domains on a daily basis. Documents refer to materials with symbols or signs that convey meaning or message (Braz *et al.*, 2013). Although documents can be made on almost any type of material, including waxes, stones, plaster of Paris, plastic materials, and glass, most documents that end up in forensic science laboratories are those written or created on papers.

1.3 Gel Pens and the Art of Instrument

In fraudulent or forgery cases, forensic examination determines if a portion of the ink line could have been modified using two or more inks from its original creation, such as altered values in bank cheques. Hence, ink discrimination is an area with crucial contribution to the forensic science segment (LaPorte, 2016). In the 1980s, it was estimated that of all the handwritten documents submitted for examination in forensic casework, 80% were written with ballpoint pens (Florence *et al.,* 2005). Nevertheless, a new type of writing instrument, the gel ink pen – a revolutionary ink - emerged in the early 1980s and has become the fastest growing pen genre available atthe market to date (Gernandt, 1996; Giles, 1997; Mazella *et al.,* 2003; Florence *et al.,* 2005).

The main differences between gel inks and standard ballpoint pen are the compositions of insoluble pigments and the proportion of synthetic dye elements in a water-based solution. By optimising the quantity of pigment composition in gel ink, a permanent fade resistant and smudge proof ink can be created, which is suitable for maintaining important documentary records, hence becoming to known as record ink (Senior *et al.,* 2012). The acidity of these gel pen inks is greatly reduced to hinder corrosive damages unto the metal pen nibs (Neuman *et al.,* 2009; Senior *et al.,* 2012; Herreo *et al.,* 2020), while the use of synthetic dyes provides a range of bright colours, thus making them popular amidst the general public (Senior *et al.,* 2012; Lee *et al*., 2019). Besides, the smooth, fast, and consistent ink flow makes gel ink a more attractive choice of writing instrument than the standard ballpoint pen (Borba *et al.,* 2015). The gel ink pen has continued to grow in popularity and is favoured by both children and artists due to the wide range of ink colours and textures made available (Borba *et al.,* 2015).

The development of destructive and non-destructive techniques to distinguish inks document examination, such as liquid chromatography (Neuman *et al.,* 2009), gas chromatography (Bell *et al.,* 2013), and infrared spectroscopy (Reed *et al.,* 2014), has led to an increasing amount of complex and multidimensional data. In light of such examination, most articles have addressed questions on the use of destructive techniques over non-destructive ones (Borba *et al.,* 2015).

The non-destructive techniques highlighted in a recent journal from the Forensic Science International: Synergy reviewed by Capitaine Marie Deviterne-Lapeyre (2020), the Raman spectrometer emerged as the preferred method to assess ink composition (dye) on paper, as the damages incurred on the document can be kept as minimum as possible. The study further emphasised on several advantages, such as maintaining the physical integrity of the document and more importantly, its non-destructive nature. In the case of Raman Spectroscopy (RS) at least, only a limited number of commercial electronic reference collections for different types of compounds are currently available.

This present study upholds the preference for individual laboratories to build their own Raman reference collection in accordance to their own needs. These computer-based spectral libraries can perform pattern recognition as a relatively more rapid process than attempting to identify a compound by trawling through hard copy reference collections of known spectra (Mazella *et al.,* 2005).

Besides, recent developments have embedded video microscope viewing capabilities to provide images of the sampling area with higher magnification. Such technology ascertains that the Raman spectrum acquired actually derives from the desired sample area, and is of particular value for forensic samples. The use of an

imaging system incorporated into the Raman instrument may resolve the reproducibility issues linked with smaller sample sizes, thus permitting accurate contact between the inks in Attenuated Fourier Transform Infrared (ATR-FTIR) analysis. This is because; although ATR-FTIR and RS are both vibrational spectroscopy, the reproducibility of ATR-FTIR spectra within a sample set needs to be weighed in since the spectra of ink has improper contact with the ATR crystal, which may stem from paper substrate (Silva *et al.,* 2013).

1.4 Chemometrics in Forensic Science

The application of mathematical techniques in the area of science (chemistry) is the called the chemometrics methods. These methods, which involve intricate analytical techniques, are typically applied in the field of sciences to extract chemical information from samples. The combination of analytical and chemometrics methods is elaborated in Chapter 2 (see Section 2.6), which further provides information on the analysis of samples (Sauzier *et al.*, 2021; Asri *et al.*, 2021). As widely acknowledged, the gaps between forensic science and chemometricsians are large. Having that said, this study probed into the application of chemometrics technique within the forensic science segment.

In normal practice, in forensic science, the unsupervised algorithm like PCA/HCA cannot be used to identify substances. In its essence the PCA is a dimensionality reduction technique only, and it is useful for the exploration of data only, and shows the first impression of separation of the samples as the samples can be shown as clusters on the basis of the label information (at the graphic stage), but cannot be used for classification or prediction purposes (Licen *et al*., 2021)

This study investigated the application of novel chemometric technique (SOFM) to the questioned document segment in forensic sciences. Furthermore, SOFM model highlighted and should be used in current era, as its very effective and to obtain the best possible results for the multiclass problem for classification purpose. SOFM has limited applications in forensic science let alone in forensic ink analysis. To best, in forensic, the unsupervised of self-organizing feature maps was used only for the clustering of ignitable liquid (Mat Desa *et al.*, 2010). In PCA and HCA are basically techniques that are used for data exploration only, it gives some errors and less accuracy, therefore the novel SOFM can be used for classification in forensic document examination, with the high number of samples, with the aims to obtain the perfect classification with minimum validation errors.

1.5 Problem Statements

Analytical chemistry finds the application in forensic ink analysis. In the examination of ink, it will help to identify the differences between forged and genuine documents. The challenge in forensic scientist has to give an opinion in the case on identification of the origin of composition of inks in which the physical identical colour impression same. When the pen is the writing material of questioned documents, evidence of the existence of two or more inks in the document can be valuable information for the court because this can indicate that the document was modified by addition or replacement of elements. Distinct pens are commonly used to change the context of different kind of handwriting documents, for example, values of bank checks; data and medicines on medical prescriptions, rights on powers of attorney or beneficiaries in wills. The developed statistical model could potentially allow identification of the pen type from which a questioned ink entry was made, or exclude

dissimilar ink formulations from further examination. Second, one major challenges faced by FDEs to determine the authenticity of documents is to examine documents that contain ink, especially when the suspected document has been exposed to the harsh environment for a long period of time. Both the origin of ink source and the duration of ink lines present in a document are of immense forensic values in solving crime cases. The legitimacy of an ink entry is often an important question during a forensic examination of questioned documents (insurance claims, wills, contracts or tax returns). In this sense, the ability to determine the approximate age of an ink stroke would help to solve this problem. The developed non-destructive technique with statistical model could potentially allow identification of the pen type from which a questioned ink entry was made, or exclude dissimilar ink formulations from further examination. As far as this study is concerned, no researchers have attempted PCA for source determination of aged inks, let alone using other pattern recognition technique such as Self-Organizing Feature Maps (SOFM) therefore this study gained its forensic importance.

1.6 Significance of the Study

The main significance of this study lies in its attempt to develop the systematic standard operating procedure on discrimination and classification of gel inks. As nowadays, that in real case scenario, the gel inks commonly used as an instrument to alter the documents for profit and fraud. Therefore, the diversity of gel inks used in the population and hence likely to encountered in real cases can be give significant value in forensic investigation. In Malaysia, the Department of Chemistry and Royal Malaysia Police (PDRM)are amongst the bodies that carried out questioned document

examination including ink analysis. This shows that it is extremely imperative to have accurate and discriminating ink analysis protocol.

The second significance of this study lies in its attempt to discriminate inks according to brand and origin of ink after undergoing an ageing process in open environment, mainly because the altered spectra due to exposure to harsh environment poses difficulties for examination. Ink aging is still a major challenge in forensic document examination field. Not only the variety of inks and papers, but also the mechanisms of degradation are some of the issues that make the study of the aging process a very complex topic. The rate of degradation of ink on paper starts when the solvent vaporises, while the dyes and pigments start to fade. The complexity of chemical composition in inks on paper may be modified due to several factors, such as temperature, humidity, and exposure to light. The fact that source determination of ink origin is a contemporary problem in this area, this study prescribes a novel idea on forensic investigations using unsupervised methods of principal component analysis (PCA), hierarchical cluster analysis (HCA) (conventional), and Self-Organising Feature Maps (SOFM) to link the inks with their fresh counterpart. This study motivates future research endeavours to focus on the search for more objective and automated investigations.

1.7 Aims and Objectives

The main aim of this study was to analyse gel inks of three different colours i.e. blue, red and black from various brands primarily using chemometrics techniques in tandem with Raman Spectroscopy.

The specific objectives of this study are as follows:

i. To determine the types of colourants in blue, red and black gel inks by mean of dissolution study and Thin Layer Chromatography (TLC).
ii. To determine probable pigments in blue, red and black gel inks by comparing against a *built-in* house Raman spectral library database.
iii. To discriminate the blue, red and black gel inks using the conventionHCA, PCA and novel SOFM and evaluate their ink discriminating performance.
iv. To develop classification models for blue, red and black gel inks using novel SOFM and potential of SOFM for source determination of aged inks.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter briefly describes the chemistry of gel pen inks with a narrowed focus on the instrumentations used to analyse the inks. The description about gel pen as a writing instrument, as well as the instrumentation and the chemometrics in the forensic science domain, particularly in document examination area were described in these section. It places more attention on the theory of destructive technique, particularly Thin Layer Chromatography (TLC), and the non-destructive Raman Spectroscopy (RS), along with justification of selecting these techniques over the other existing ones such as ATR-FTIR. The theoretical background of the chemometrics techniques of Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) are also discussed, and lastly, the general idea of Self-Organizing Feature Maps (SOFM), an unsupervised Artificial Neural Networks (ANNs), is elaborated pertaining to its application in the forensic sciences domain.

2.2 Chemistry of Gel Pen Inks

Many pen manufacturers do not possess the capabilities to manufacture their own ink, thus outsource their requirements to ink manufacturers, such as Documental (Germany) and National Ink (US) (Brunelle & Crawford, 2003). Both pen and ink manufacturers work closely together to ensure that a suitable ink is chosen for a particular class of writing instrument being produced, and it is unlikely that the pen manufacturers are aware of the exact formulation applied (Brunelle & Crawford,

2003). Batch-to-batch, or within intra brand variation, may result in a range of possibilities (Brunelle & Crawford, 2003), as listed in the following:

- During ink manufacturing, the emphasis is on achieving "consistent colour and viscosity" rather than ensuring "precisely the same proportion of ingredients" are added.
- Leftover batches arising from discontinued orders or incorrect ink formulations are generally stored and may be added to batches of ink with the same or similar formulation to reduce losses from manufacturing costs.
- The quality of raw materials used in ink formulations, i.e. dyestuffs, may vary over time between or within suppliers.
- The same reaction vessels are used continually to produce batches of ink with different formulations, which may contaminate a batch if not cleaned properly between use.
- Mistakes made in the measurement of ingredients to be used in an ink formulation.

These batch variations, although apparently unusual, are of great interest to forensic scientists who can detect such subtle variations, thus enhancing the evidential value of the analytical outcomes. The advantages of gel ink pen have been widely highlighted and appear to far outweigh its potential disadvantages. The vast selection of brilliant colours available and the creative textures are of great appeal to the artistic imagination of the general public. Insoluble pigment compositions provide a permanent writing medium suitable for maintaining important documentary records;

resistant to light, water, and chemical degradation; as well as acid free to prevent damages to the paper substrate. The high water-based content of gel ink, as opposed to the solvent-based nature of ballpoint pen ink, coupled with the use of primarily organic pigments and other non-toxic ingredients (i.e., resins and additives), makes gel ink a great appeal to environment-friendly consumers. Besides, the smooth, fast, and consistent ink flow makes gel ink a more attractive choice of writing instrument than the standard ballpoint pen ink (Brunelle & Crawford *et al.*, 2003).

Despite these advantages, the gel ink pen is not perfect. It has been claimed that gel ink pens do not last as long as their ballpoint pen counterparts, and that eight gel ink pens are required to fill the same number of written pages as a single ballpoint pen would do (Brunelle & Crawford *et al.,* 2003). If accurate, the comparatively short writing life, combined with the escalating demand for disposable gel ink pens, counterbalances the ecological benefits of their ink formulations. Others have asserted that gel ink pens possess a long writing span and with the availability of gel ink refills, this apparent disadvantage may not be as detrimental as suggested. Another drawback lies in the specialised pen design that adds to its production cost and ultimately increases its retail price. In 2012, a standard ballpoint pen was typically retailed below 60 pence (RM3) in the UK, when compared to between £1(RM5) and £7(RM35) for a gel ink pen dependent on brand and model (Brunelle & Crawford *et al.,* 2003). This additional expense, combined with shorter writing life, may deter some from choosing the gel ink pen over other types of writing instruments, although for others the additional benefits these gel ink pens offer maybe considered worth the extra cost. Perhaps, the most significant shortcoming is the tendency of gel ink to dry out quickly on the metal ball nib, which can affect the quality of the writing line in terms of ink starting, hesitation, and skipping. However, manufacturers have taken steps to address

these problems by producing capped models and retractable models with removable protective seals for the ball nib (Brunelle & Crawford *et al.,* 2003). In this work, different brands were chosen to reflect a mixture of well-known stationary brands (i.e. Pilot, Faber Castell, etc.) and some lesser known and/or discount brands (i.e. Cross and Gel writer).

There are thousands of different gel pen ink formulations commercially available in the market. In general, a gel pen ink consists of three main components; colouring materials, vehicle, and resins (Barker, 2016). Table 2.1 presents the compositions of gel ink in detail, along with their functions and examples.

Table 2.1 Types of chemicals found in gel ink formulations, their functions, and some examples

Compositions	Functions	Example
Dyes	Provide colour to ink	• Basic blue 11 Victoria blue R • Solvent violet 49 Rhodamine base B
Pigments	Provide colour to ink	• Pigment blue 15 Phthalocyanine blue • Pigment blue 17
Resins	Adjust the density of ink	• Styrene • Allyl Alcohol
Solvents and Vehicles	Adjust the drying kinetic of ink	• Water • Alcohol
Lubricants	Allow the socket of ink instruments to rotate freely	• Acid oleic
Corrosion inhibitors	Preserve the socket point of the writing ink	• Tertiary-butyl hydroperoxide
Additives	Stabilise the mixture and provide ink its desired characteristics	• Glycerides
Surfactants	Adjust the surface tension ink mixture	• Non-Ionic: Polyoxyalkylene Higher Fatty Acid Esters; Higher Fatty Acid Esters of Saccharide • Anionic: Alkylated Sulfonates of Higher Fatty Acid Amides and Alkylallysulfonates

Table 2-1. Continued

Rust Preventatives	Prevent rust from forming on the metal ball	• Benzotriazole and Derivatives; Dicyclohexylammonium Nitrate
Sequestrants	Maintain the ingredients in the solution	• Tetrasodium EDTA or Versene 100 RTM of Dow Chemical; Trisodium Phosphate; Sodium Hexametaphosphate; Sodium Glucoheptanate

Source: (Brunelle & Crawford, 2003)

2.2.1 Colouring Materials

Colouring materials consist of either dyes and pigments or both. A typical gel ink consists of 25% dyes and pigments (Kumar *et al.*, 2017). Dyes are soluble in vehicle (solvent). Pigments are solid particles manufactured from chemicals and are insoluble in water, but slightly soluble in solvent. Coloured pigments are produced from inorganic pigments, such as chromium (yellow, orange, and green), molybdenum (orange), cadmium (red), and iron (blue). Organic pigment, such as carbon (black), is commonly used in the manufacturing of black ink. An example of colouring material is Pigment Blue 16 (Figure 2.1).

Therefore, the uniqueness of the pigment based gel that distinguishes the gel pen from other classes of writing instrument (ballpoint pen), and which poses a problem for forensic scientists, thus forming the basis of this research.

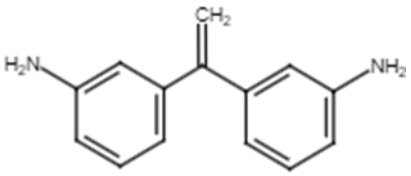

Figure 2.1 Example of Pigment Blue 16 in blue inks
Source: (Kealey et al., 2002; Asri et al., 2020)

2.2.2 Evolution of Forensic Ink Analysis

Ink analysis was first reported in the early 19th century using light of different wavelengths and micro-chemical spot tests (Lucas, 1935; Harrison 1958; Jackson 1962). The physical examination of different lights was based on infrared (IR) photography or photocopy to detect obliterations and mechanical erasures, while the ultraviolet (UV) fluorescence photography to detect chemical erasures (Reed *et al.,* 2014). By the 1950s, the first step of ink examination applied a stereomicroscope at low power magnification (10-100x) to examine the morphology and the general appearance of the ink line, which determined the type of pen used and the colour (shade and depth) on the assessed document (Reed *et al.,* 2014). Clearly, microscopy alone is insufficient to distinguish between the different pen types and the characteristics of a particular writing instrument. During the 1960s and the 1970s, ink analysis underwent a revolution on its composition due to the increasing popularity of writing inks containing synthetic dyestuffs available on the consumer market.

Some studies have looked into paper chromatography (Somerford *et al.,* 1952; Brackett *et al.,* 1952; Brown *et al.,* 1954; Crown *et al.,* 1961) and electrophoresis (Brown *et al.,* 1954; Wilson *et al.,* 2004) to separate the individual dye components of an ink formulation for comparison with other ink samples that enables identification of brand formulation. Initially, electrophoresis was favoured over paper chromatography due to problems arising with poorly resolved dye bands in the latter technique (Somerford *et al.,* 1952). However, in 1966, Tholl (cited in Brunelle *et al.,* 1984) described the efficient separation of dye and non-visible ink components from microquantities of ballpoint ink using the technique of Thin Layer Chromatography (TLC). It was this significant work, combined with developments in the use of dichroic filters, IR luminescence, and UV photography, that has since formed the foundation of modern day ink analysis (Brunelle *et al.* (1984),. Around this time, chromatographic (Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC) and spectroscopic analyses (IR and RS) were still at their infancy stage for the analysis of inks.

Many studies focused on ballpoint pen inks as they contain dyes, solvents, resins, and others. However, the special components in gel inks that contribute to long-lasting colour maintenance on a document refer to the combination of pigment and dyes. Many studies have assessed the use of destructive techniques adopted by FDEs to characterise forensic ink samples, which are vastly reported in the literature from 2004 up to the present time (Table 2.2). One critical element in forensic analysis is maintaining the integrity of evidence on document. As presented in Table 2.2, the combinations of many statistical methods with analytical techniques or the combinations of two analytical techniques have been consistently reported in the field of forensic ink analysis since the past decades. The application of Raman spectrometer

in forensic ink analysis has become increasingly popular as it confirms the identity of specific colorants (pigments) composed in the ink. Although these methods are less time consuming without damaging the evidence of ink on the documents, they are expensive and only limited laboratories can access these facilities to characterise and differentiate samples (Borba *et al.,* 2015: Asri *et al.,* 2022)

Most of the studies conducted from 2004 until 2014 employed destructive techniques and mainly focused on the basic, simple and straightforward statistic indicator such as discriminating power (DP). In 2008, Causin *et al.*, evaluated the discriminating power of ultraviolet-visible (UV-Vis) spectrophotometry, thin layer chromatography (TLC), and diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) in discriminating 21 black and 12 blue ballpoint inks. Based on their findings, the obtained discriminating power using combination of the techniques were 100% and 98% for black and blue inks, respectively. However, due to subjectivity of the results obtained, the authors had proposed on the exploration of other statistical techniques.

Weyermann *et al.,* (2011) had investigated the potential use of laser desorption/ionisation mass spectrometry (LDI-MS) for discriminating blue gel inks. Ink lines were made using 33 gel pen inks of various brands and the Pearson correlation coefficient was used to calculate the discriminating power of the technique. A discriminating power of up to 92% was reported indicating the potential use of LDI-MS for gel ink analysis however analyst's subjectivity remain unsolved.

Biao Li *et al.,* (2014) estimated the age of gel ink deposited on a paper using gas chromatography (GC) technique. A calibration model of the concentration of the volatile component in the gel ink versus the aging period or time was constructed and

was then used to estimate the age of gel ink deposited on paper. Despite the authors emphasised on the usefulness and several advantages of using GC for determining the age of inks on paper, this technique is rather destructive, in other words, it compromises the integrity of document.

Reed *et al.,* (2014) analysed blue, red, and black gel inks using the hyperspectral imaging (HSI) technique. The ink sets containing 42 gel pen inks from different brands were analysed at 400–1000 nm. Results revealed that the discriminating power obtained were 1.00, 0.90, and 0.40 for red, blue, and black gel pen inks respectively. Although the results demonstrated the superiority of HSI especially for discrimination of red and blue gel inks, analyst's subjectivity still remains one of the problematic issues. To tackle this, the authors proposed the use of chemometrics techniques. Liu *et al.,* (2018) investigated pigmented blue, red and black gel inks using laser desorption laser post-ionizationtime-of-flight mass spectrometry (L2MS) and laser desorption mass spectrometry (LD-MS) where the authors concluded that better discrimination of samples were achieved with L2MS analysis than LDMS technique. This technique did not fully explore on chemometrics technique, and proposed RS as the alternative for future work.

From 2014 onwards, most studies involving pen inks employed chemometrics technique of Principal Component Analysis (PCA) (Sharma *et al.,* (2017), Asri *et al.,* (2017), Lee *et al.,* (2018), and Ismail *et al.* (2014). This dimensionality reduction technique enables dataset to be visualised in two or three dimensional space hence allowing objective comparison and discrimination to be made to inks. Although PCA has gained considerable popularity among researchers interested in forensic ink analysis, most of the studies are heavily focussed on discrimination and classification

of fresh or neat inks ballpoint pen inks, in other words are limited towards gel pen inks.

One of the questions often raised in the court of law involving ink is what writing instrument was used to prepare the dubious document. This question although sounds straight forward, it however requires careful considerations since writing inks are susceptible to compositional changes, decomposition and degradation immediately upon exposure to the environment.

Many researchers have been focusing on the use of destructive techniques such as HPLC (Samanidou *et al.,* 2004) and GC (Biao Li *et al.,* 2014) for analysis of ink. Nevertheless, there are also growing interest on non-destructive techniques for ink analysis particularly using ATR-FTIR Spectroscopy (Chophi *et al.,* 2020; Sharma *et al.,* 2020). Due to the advantage of maintaining the integrity of document, non-destructive technique should always be the technique of choice for the analysis of document including ink and this requirement has also been outlined by the Scientific Working Group of Document (SWGDOC).

Table 2.2 Characterisation of inks using analytical techniques (2004–2020)

No.	Instrumentation	Nature of study	Data Analysis Used	Reference
1	GC	Destructive	Calibration curves were used to compare the relationship of the age of gel ink	Li *et al.* (2014)
2	HPLC	Destructive	Identification of the compounds was performed (compare retention time values)	Samanidou *et al.,* (2004)
3	UV–Vis, TLC, and FTIR	Destructive/ Non-destructive	DP	Causin *et al.,* (2008)

Table 2-2. Continued

4	UV-Vis Spectroscopy	Destructive	PCA	Ismail *et al.,* (2014)
5	LDI MS	Destructive	Relative Peak Height	Gallidabino et al., (2011)
6	LDI–MS	Destructive	PPMC	Weyermann *et al.,* (2012)
7	DART-MS and DSA-MS	Destructive	Better discrimination of samples with DSA-MS compared to DART-MS	Drury *et al.,* (2018)
8	L2MS and LD-MS.	Non-destructive	Better discrimination of samples with L2MS than LDMS	Liu *et al.,* (2018)
9	HPTLC and FTIR	Destructive/ Non-destructive	DP and PCA	Sharma *et al.,* (2017)
10	RS	Non-destructive	PCA	Asri *et al.,* (2017)
11	RS	Non-destructive	PCA, PPMC	Asri *et al.,* (2018)
12	FTIR	Non-destructive	PLS-DA	Lee *et al.,* (2018)
13	HSI	Non-destructive	DP only	Reed *et al.,* (2014)
14	RS and TLC (530 samples) (20 blue, 19 red and 14 black ink samples) (Larger number samples)	Destructive/ Non-destructive	PCA, HCA, SOFM Unsupervised SOFM first time reported Perfect match according to developed library	Present study

2.3 Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is a type of planar chromatography, and is a simple technique that can be used to separate dyes from other non-visible organic components present in an ink formulation (Brunelle *et al.,* 1984, 2003; Ellen *et al.,* 2006). A qualitative comparison of the dye components present in two or more ink samples chromatographed together under the same conditions can be made to determine if they could be of the same formulation. Furthermore, it can also be used to identify the ink formulation by comparing it with a known standard. A quantitative

assessment of the relative concentration of each dye component may also be made via Densitometry (Brunelle *et al.,* 1984, 2003; Ellen *et al.,* 2006). Numerous standardised procedures that describe the TLC analysis of different writing inks are reported in the literature, but they all involved the same general procedure (Kealey *et al.,* 2002).

2.3.1 Stationary and Mobile Phases

The stationary phase of a TLC is a thin layer of micro particulate sorbent material (~250 µm), such as silica, which is held on the surface of a flat medium, such as aluminium. Typically, a soluble fluorescent reagent is included in the stationary phase material to combine with the solutes to make component bands visible under the UV light. This is particularly useful to visualise components that are invisible or colourless under the white light. A wide array of TLC plates is commercially available with varied sizes (5-20 cm square) and stationary phases, from which the most suitable can be chosen as desired.

The solvent system or mobile phase can either be a single solvent or, more commonly, a mixture. The latter is preferred because it is possible to vary the proportions of individual solvents within the mixture to influence the overall eluting power of the mobile phase, apart from maximising the resolution of the component bands. The R_f values of the component bands are influenced by the overall polarity of the mobile phase, which can determine the rate at which component solutes migrate. A typical mobile phase for inks is n-butanol or ethanol with water (Barker *et al.,* 2016).

For some forensic examinations of ink, the combination of visual assessment by eye with the aid of a low-power microscope and filtered light examination using a Video Spectral Comparator (VSC) is not sufficient to distinguish between two inks of similar appearance, thus need further examination. Even after these non-destructive

examinations are performed, the two entries in question remain indistinguishable, hence the need to perform further destructive chemical analysis of the ink via certain techniques, such as TLC (Brunelle *et al.,* 1984, 2003; Ellen *et al*., 2006).

2.3.2 Retention Factor (R_f) Values

The term 'retention' denotes the "interaction of solutes with stationary phase slows down the migration relative to the velocity of the mobile phase" (Kealey *et al.,* 2002). After separation, a numerical value (R_f value) for each component band can be calculated using Equation 1.1. An instance of a TLC plate with associated R_f values for separated component spots is displayed in Figure 2.2.

$$R_f = \frac{\text{Distance spot travelled from baseline}}{\text{Distance between solvent front and baseline}} \qquad \textbf{Equation 1.1}$$

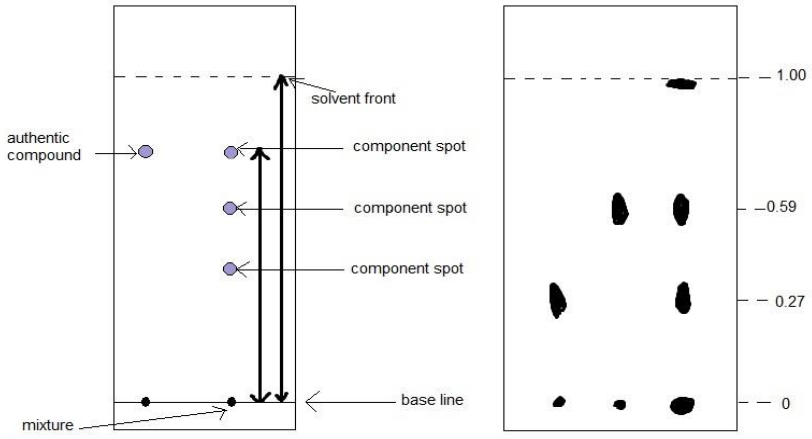

Figure 2.2 A TLC plate with R_f values for component spots
Source: (Dean et al., 2002; Kealey et al., 2002)

Reported literatures on TLC analysis of gel pen inks for forensic investigation as discriminating technique are quite limited. Jasuja *et al.,* (2005) performed analysis on large number (n=112) of gel ink samples (using 13 different solvent systems, two of which that had successfully chromatographed soluble gel inks were butanol: ethanol: water: acetic acid (60:20:20:0.5) and butanol: ethanol: water (50:25:25). The number and coloured spots visible under the white light and UV light (short and long wavelengths) as well as the R_f values for both solvent systems were recorded. Four brands exhibited different R_f values for the same spot when chromatographed using the two different solvent systems. Therefore, the authors proposed the use of spectroscopic techniques for further analysis.

Reed *et al.,* (2014) had conducted TLC analysis to 42 gel pen inks of various brands and colours in three different solvent systems: butanol:ethanol:water:acetic acid(60:20:20:10),ethyl acetate:butanol:water (75:35:30) and butanol:ethanol:water (50:25:25). Prior to the TLC analysis, solubility of the gel inks in various organic solvents was performed to determine the most suitable organic solvent for extraction of the gel inks. Acetic acid, acetone, and methanol were found to be the most suitable solvents for the extraction of blue, red, and black gel inks respectively, with successful extraction to some brands confirming their dye based nature. They had also reported that heating the samples with the aid of a sand bath for 5 minutes greatly assisted the extraction of gel inks especially for those inks that were not readily soluble in organic solvents.

Three solvent systems were used in this study. The first solvent system comprised ofbutanol: ethanol: water (50: 35: 25 v/v/v) and the second solvent system (designated as solvent system 2) comprised of butanol: ethanol: water: acetic acid (60:

20: 20: 0.5 v/v/v/v) whilst the third solvent system (designated as solvent system 3) comprised of ethyl acetate: ethanol: water (75: 35: 30 v/v/v) were used for a larger number (n=530) of samples (blue, red and black). Prior to TLC analysis, acetone, acetic acid, methanol (MeOH), chloroform, hydrochloric acid (HCl), butanol, distilled water (H_2O), ethanol, ethyl acetate (EtAc) and xylene had been tested to determine the most suitable organic solvent for extraction of the gel inks.

2.4 Spectroscopic techniques

Analytical chemistry is related to forensic science**.** There are several types of spectroscopic techniques available and the most commonly applied in forensic science are Infrared (IR) spectroscopy, Raman spectroscopy (RS), Ultra Violet and Visible (UV-Vis) spectroscopy and Nuclear Magnetic Resonance (NMR).

The type of spectroscopy to be employed depends on the physical quantity to be measured. Normally, the quantity that is measured is intensity through absorption, emission and scattering. Spectroscopy can be classified on the nature of their interactions which are absorption, emission and scattering spectroscopy.

2.4.1 Absorption Spectroscopy

Absorption spectroscopy uses the range of the electromagnetic radiations in which a substance absorbs. An example of absorption spectroscopy is the atomic absorption spectroscopy (AAS). There are various molecular techniques that involved absorption such as infra-red and nuclear magnetic resonance (NMR)(Skoog *et al.,* 2020).

2.4.2 Emission Spectroscopy

Emission spectroscopy use the range of electromagnetic spectra in which a substance is emits. The level and quantities of energy emitted by excited electrons, as they return to their ground state, can be measured & studied by means of the emission spectroscopy (Skoog *et al.*, 2020).

2.4.3 Scattering Spectroscopy

Scattering spectroscopy measures the amount of light that a substance scatters at certain wavelengths, incident angles and polarisation angles. The scattering process is much faster than the absorption or emission process. One of the most useful applications of light scattering spectroscopy is Raman spectroscopy (RS).

The IR absorption spectroscopy and RS are two different analytical techniques that use electromagnetic radiation to excite vibrational transitions within a molecule in providing information concerning the chemical structures of a compound during the analysis of forensic exhibits. Elkins *et al.*, (2018) stated that the outcome of this analysis can be presented in the form of an IR absorption spectrum or Raman spectrum respectively. Therefore, those spectra with significant characteristics can be used for identification and comparison purposes as well as quantitative determination of the chemical components present. The two techniques provide different, but complimentary, information about the molecular structures of the compound studied (Elkins *et al.*, 2018; Smith *et al.*, 2019).

There are distinct differences between RS and IR absorption spectroscopy. In RS, the sample is exposed to a single wavelength of high energy radiation and the resulting scattered radiation is used to determine structural information about the