

CHARACTERISATION OF DIFFERENT BRANDS OF WHITE A4 PAPERS USING MICROSCOPIC AND SPECTROSCOPIC TECHNIQUES

by

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ABSTRAK

Kertas A4 putih digunakan dalam kehidupan seharian kita untuk mendokumentasikan maklumat bercetak dan maklumat yang bertulis tangan. Kertas ini akan mengurai dari semasa ke semasa dan dengan itu secara tidak langsung menyumbang kepada penguraian sebarang teks bercetak dan teks tulisan tangan pada kertas tersebut. Kajian ini menyiasat ciri-ciri mikroskopik dan spektroskopi daripada satu set A4 putih, 70 gram kertas dari lima (5) jenama yang berbeza dengan menggunakan Quanta FEG 450 Imbasan Microskop Elektron dan Bruker Tensor 27 spektroskopi infra-merah Fourier Transform (FTIR) yang dilengkapi dengan jumlah pantulan dilemahkan (ATR) lampiran. Untuk mengkaji kesan pendedahan kepada alam sekitar, kertas-kertas ini telah didedahkan kepada keadaan bilik biasa (28°C) selama 7, 14, 28 dan 60 hari. Setelah tamat setiap tempoh pendedahan, kertas-kertas itu dianalisis secara spektropikal. Dua ujian statistik iaitu Pearson Masa Produk Korelasi (PPMC) dan ujian-t berpasangan telah digunakan untuk menilai kesan pendedahan. PPMC menunjukkan korelasi positif yang kuat (dengan $r > 0.99$) manakala ujian-t berpasangan menunjukkan tiada perbezaan yang signifikan di antara kertas yang terdedah dengan kertas baru apabila didedahkan sehingga 60 hari. Penemuan ini menunjukkan bahawa kertas tidak terjejas, dalam erti kata lain kertas-kertas ini agak stabil dalam tempoh pendedahan dalam kajian ini iaitu sehingga 60 hari.

ABSTRACT

White A4 paper is used in our daily life to document printed and handwritten information. It is believed that through time, this paper will decompose hence indirectly contribute to the decomposition of any printed and handwritten texts stored on it. This study investigated the microscopic and spectroscopic characteristics of a set of white A4, 70 gms papers from five (5) different brands using Quanta FEG 450 Scanning Electron Microscopy and Bruker Tensor 27 Fourier Transform Infrared Spectroscopy (FTIR) equipped with Attenuated Total Reflectance (ATR) attachment. To study the effect of exposure to the environment, the papers were exposed at normal room condition (28°C) for 7, 14, 28 and 60 days. Upon completion of each exposure period, the papers were spectroscopically analysed. Two statistical tests which were Pearson's Product Moment Correlation (PPMC) and Paired t-test were used to assess the effect of the exposure. PPMC showed strong positive correlation (with $r > 0.99$) while the Paired t-test showed no significant different between the exposed papers with their fresh counterparts when exposed up to 60 days. These findings indicated that the papers were unaffected, in other words relatively stable within the studied period of exposure i.e. up to 60 days.

CHAPTER 1

INTRODUCTION

1.1 Document and Questioned Document

A document is defined as any material bearing marks, symbols, or signs that convey meaning or a message. It can be more complex than simply writing on a newspaper. Majority of documents nowadays is written on paper either by using typewriters or by hand using pen and ink or pencil (Lindblom, 2006).

A questioned document is defined as a document that is suspected of being fraudulent or the root or its history can be challenged or dubious. The document may be involved in forgery, blackmail, threats, murder, counterfeiting or a server of other offenses which may have direct or indirect impact on one's property, possessions, reputation, and also wealth. Finding , the actual history and genuinity of a questioned document is of great importance in forensic document examination (FDE) (Lindblom, 2006).

In FDE, forensic document examiners usually examine and analyse items related to the questioned document which include handwritings, signatures, printed materials and inks from the writing implements so as to recognise forgeries, to reveal additions and another important aspect in FDE is the examination of the writing substrate i.e. the paper. Careful analysis could reveal important information among others, distinctive watermark, organic and elemental compositions which may be useful for subsequent investigation. The major issue in forensic ink analysis, particularly pertaining aging is also valid in paper analysis. It is believed that when being exposed to the environment, paper will eventually decompose which will have indirect impact to the printed or handwritten texts on the paper.

In paper analysis, both non-destructive and destructive analytical techniques may be used. Non-destructive technique is a technique that requires no sample preparation, in other words, sample can be analysed *in-situ*, less time consuming and, fast in obtaining the result, whereas destructive technique is a technique that requires sample preparation, in other words, requires removal of certain part of a sample, time consuming and hence relatively slow. In FDE, the former technique is normally preferred.

1.2 Research Statements

The authentication and identification of written or printed documents can be a significant issue in forensic investigations for many criminal and civil events. Those documents such as medical records, and financial documents are usually using paper as a writing surface. In forensic cases, papers can become an important evidence.

In questioned document analysis, study about paper may help a questioned document examiner (QDE) in solving the criminal cases. However, studies focussing on papers are limited. It becomes a problem to the questioned document examiner to make references. This research able to contribute to the analysis of questioned document (paper analysis) that is conducted by a forensic document examiner. This study would contribute some information regarding the characteristics of papers and the effect of exposing (i.e. aging) papers to the environment. The scope of this study was focused on the characteristic of white A4 paper using microscopic and spectroscopic techniques.

The purpose of choosing microscopic technique in paper analysis is because to study about the morphology of paper. The use of spectroscopic technique in this study is because to analyse the aging of the paper in exposed conditions in a particular timeframe. Not many research about these two had done.

The paper choice in this study is because of widely used by community in writing process, easily available in a stationery store and inexpensive. The analysis of the data that was obtained from the spectroscopic technique were Pearson Product Moment Correlation Coefficient (PPMC) and Paired t-test (at 95% CL).

1.3 Objectives

The objectives of this study are to characterise a set of white A4 papers (70 gms) from five different brands using microscopic and spectroscopic techniques of SEM and ATR-FTIR respectively and to assess the effect of exposing the papers to the environment for specified period of time by using statistical tests of PPMC and Paired t-test.

1.4 Research Hypotheses

The research hypotheses in this study consist of null hypothesis and alternative hypothesis. Null hypothesis (H_0) in this study is there was no significance difference between the aged papers with their fresh counterparts. Alternative hypothesis (H_a) in this study is there was significance difference between the aged papers with their fresh counterparts.

1.5 Significance of the Study

The significance of this study is different paper manufacturers will use different formulation. This will be manifested in either the microscopic or spectroscopic profiles of the papers. This will help in the characterisation of the white A4 papers. Besides that, the different components within the papers will age differently under different storage conditions or otherwise. This will give an insight to the chemical properties of aged papers.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Forensic document examination (FDE) is a wide field in forensic science that deals with among others paper analysis, and writing ink composition and characterisation. Although nowadays almost every person frequently uses electronic devices for communication and documentation such as e-mails, messages and etc., papers are still being used to record information therefore, examination and analysis of paper is useful and very important in forensic science. Background history of paper and paper making were described further in section 2.2.

Various instrumental techniques and methods are available for the examination and characterisation of paper. In questioned document examination involving paper, non-destructive techniques are normally preferred so as to maintain the integrity of the paper.

Microscopic technique of Scanning Electron Microscopy (SEM) and spectroscopic techniques of Fourier Transform Infrared (FTIR) and Raman Spectroscopy are among the non-destructive techniques that can be applied for the examination of paper. Destructive techniques, on the other hand require tedious sample preparation, time consuming, and often require large amount of sample.

Microscopic technique is also known as optical examination. Such an examination allows observation in the ultraviolet, visible, separated out, unfiltered artificial light or even the near-infrared parts of the electromagnetic spectrum. This method helps to analyse fibres

in paper. Spectroscopic techniques measure the absorption, emission, or scattering of electromagnetic radiation by atoms or molecules of compounds. The spectra generated are very useful for characterising and identifying compounds (Udristioiu *et al.*, 2012).

2.2 Paper

According to Webster's Ninth New Collegiate Dictionary, paper is defined as a felted sheet of usually vegetable fibres laid down on a fine screen from a water suspension (Meriam-Webster, 1989). In Oxford Dictionary, paper is defined as '*material manufactured in thin sheets from the pulp of wood or other fibrous substances and a material that people usually used for writing, drawing, or printing on, or as wrapping material*' (Press, 2016).

2.2.1 History of Paper

Before paper was invented, many different materials such as rock, clay, bones, wood bark, leaves, bamboo, metal, papyrus, silk, and parchment were used as the writing surfaces. The words paper, papier, and papel are derived from the Greek and Latin words papuros and papyrus. Papyrus is the obvious precursors and an inspiration for the development of true paper (Fortunato, 2006).

Around 3500 B.C., papyrus was the first pre-paper writing material which grew in abundance in Egypt. In the ancient times, Egyptian papyrus was produced from the stalks of the papyrus plants. During that time, the papyrus was used to form a substance that could be written on. It was not considered as paper because the process used was unrelated to the method used to produce modern paper. The process to form the writing substrate using papyrus is the papyrus involves cutting the papyrus into pieces of about 2 feet long and split them down at the centre. Tissue-thin sections were cut from end to

end and laid side by side. Then, the materials were covered with a thin paste of wheat flour and water. Additional strips were laid across in the opposite direction and the two layers were then pressed together (Hunter, 1967).

Paper is made up of cellulose fibres which are collected from their slurry to form a sheet. Cellulose fibres may be found in most plants, in which they constitute the major component of the cellular walls. There are four basic types of cellulose fibres that meet the specifications for papermaking which are seed hair fibres, bast fibres, grass fibres and also wood fibres. Each of these fibre sources has its own limitations such as high cost, low strength, dry storage, accessibility or limited crop yields. Considering all these limitations, the paper industry uses wood as the primary source of paper fibre. It is currently constitutes approximately 95% of all the papermaking in the world (Lindblom, 2006).

The invention of paper was officially reported to have started in China in the 105 A. D. During that period, papers were made from wood bark, hemp waste, old rags and fish nets. The process of papermaking (Figure 2.1) started with the breaking down of the source fibre into individual units. These individual fibres were then poured onto a mould which was a square of coarsely woven cloth held within a four-sided bamboo frame. The mould was dipped into thin slurry and then raised horizontally under the floating fibres. The dipping method was an extremely important step as it determined the thickness of the paper. The matted fibres were then lifted from the water to allow the water to drain and placed under direct sunlight for drying. The purpose of the drying process is to enable detachment of the paper from its mould (Kelly and Lindblom, 2006).



Figure 2.1 The process of paper making (www.paperslurry.com accessed on June, 17, 2016)

The advent in the process of papermaking has enabled wet sheet of paper to be removed from its mould while it is still wet hence allowing the same mould to be reused within a shorter period of time Unlike the traditional mould used in early papermaking process, the so-called "reusable mould" consists of a mould frame and a mould cover which are made from smooth yet very strong material such as wood and steel. The function of the mould frame is to serve as a firm support for the mould cover and to keep it in flat or

horizontal position. In the process of papermaking, both mould cover and its frame are dipped into the vat of pulp fibrous material i.e. the slurry and slowly brought to the surface loaded with the wet fibrous material. The wet fibrous material is removed from the mould by pressing the mould against a board, normally a thick felt (Hunter, 1967).

In the 15th century, papermaking process spread to Italy, France, Germany and England. It was introduced in Spain around the year 1150. The first paper making machine was invented by Nicholas-Louis Robert in 1798 in France to meet the demand for papers (Lindblom, 2006). The paper machine was soon extensively produced by Henry Jr. and Sealy Fourdrinier in England (Hunter, 1967). Unfortunately, the process of papermaking using the machine was complicated because it involved many tedious steps such as sheet forming, wet-end pressing, drying and also calendering. The Fourdrinier paper machine produced paper in the form of a long, continuous sheet or web rather than individual sheets (Fortunato, 2006).

2.2.2 Basic Composition of Paper

A typical paper contains cellulose, hemicelluloses, lignin, resins and extractives.

- **Cellulose**
 - Cellulose is the primary structural component of paper after removal of lignin and various other extractives. It is a semi-crystalline microfibrillar linear polysaccharide of B-1,4-linked D-glucopyranose. It has high molecular weight. Cotton and various algal celluloses (Valonia) are highly crystalline, whereas wood cellulose tends to be less (Roberts, 1996).

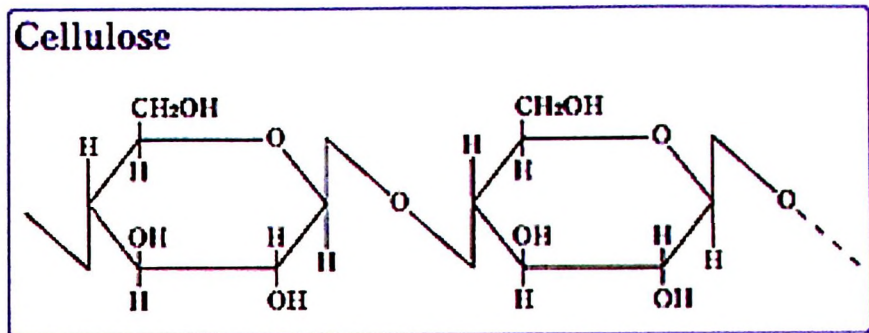
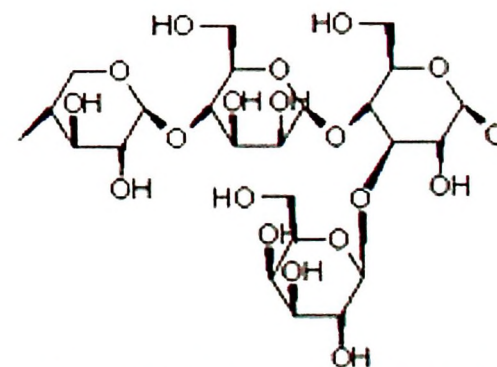


Figure 2.2 Structure of cellulose (www.swicofil.com accessed on June, 15, 2016)

- **Hemicelluloses**

- Hemicelluloses are a group of non-structural, low molecular weight, mostly heterogeneous polysaccharides, which are unrelated to cellulose and are formed biosynthetically by a separate path. They are widely recognised to be beneficial to pulp and paper properties. The hemicellulose content gives positively correlates with the tensile strength of paper (Roberts, 1996).



- Xylose - $\beta(1,4)$ - Mannose - $\beta(1,4)$ - Glucose -
 - $\alpha(1,3)$ - Galactose

Hemicellulose

Figure 2.3 Structure of hemicellulose

(www.upload.wikimedia.org accessed on June, 15, 2016)

- **Lignin**

- Lignin is an aromatic polymer and the structure is extremely complex. Nearly entirely of its properties are undesirable for paper making applications. The properties of lignin are it causes paper to turn brittle and it is also oxidized photochemically to form coloured by-products. It gives results in yellowing and discolouration to the paper. Paper with highest quality are usually made from pulp lignin free (Roberts, 1996).

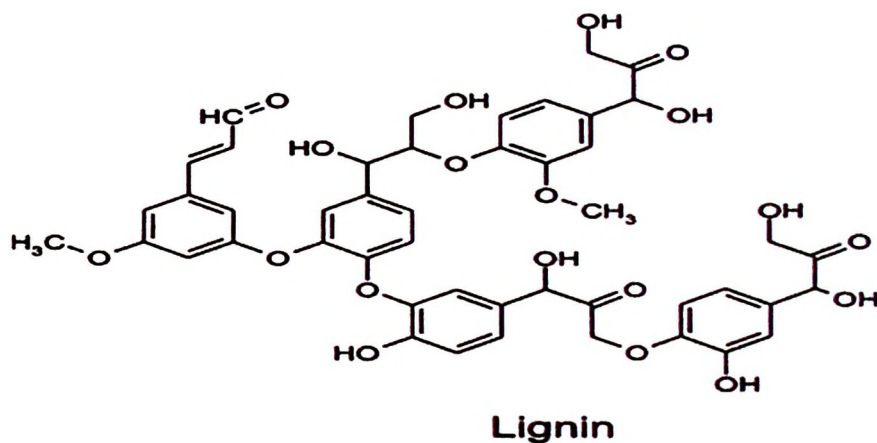


Figure 2.4 Structure of lignin (www.

usmle.biochemistryformedics.com accessed on June, 15, 2016)

- **Resins and Extractives**

- The proportion of extractives varies in hardwoods and softwoods and also between species. These extractives are removed during the chemical pulping process. However, some still remain in the final

sheet of paper. Resin is generally acidic component and easily removed by alkali (Roberts, 1996).

2.3 Non-Destructive Technique (NDT)

Non-destructive technique (NDT) is a technique that requires no sample preparation, in other words, sample can be analysed *in-situ*, less time consuming and, fast in obtaining the result. This technique is preferred for many forensic samples such as questioned document sample because it allow the sample to be preserved and archived for future use. Techniques that are commonly used for NDT employing wide array of instruments such as FTIR, SEM, Raman technique, X-ray diffraction (XRD), energy dispersive X-ray fluorescence (EDXRF) and diffuse reflectance ultraviolet-visible (UV-Vis).

2.3.1 Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) Spectroscopy

Instrumentation for infrared (IR) spectroscopy was revolutionized by the introduction of Fourier Transform Infrared (FTIR) spectrometers. An FTIR instrument in its basic form works by the principle of transmission. A typical FTIR spectrometer consists of IR radiation, a beam splitter, two mirrors and a detector as shown in Figure 2.5.

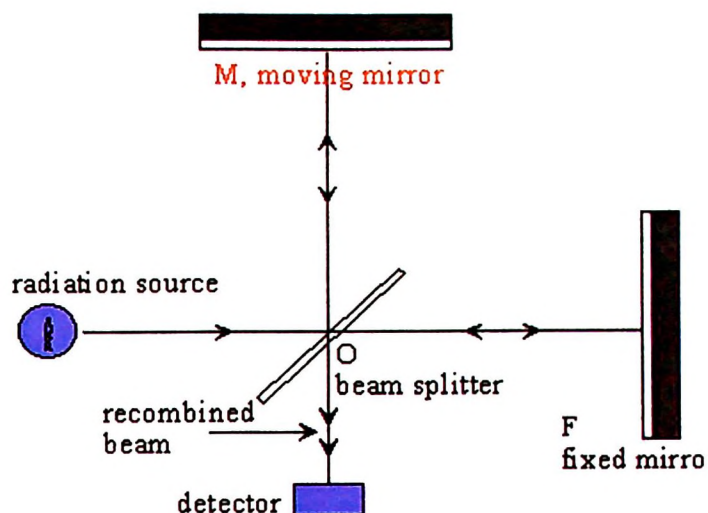


Figure 2.5 FTIR spectrometer (www.ollemo.se accessed on March, 25, 2016)

The measurement process starts when radiation source hits the beam splitter and is partly directed towards the two mirrors. One of them is fixed mirror and the other mirror is a moving mirror, which is moved at constant velocity during data acquisition. The IR beams are then reflected by the mirrors, recombined at the beam splitter, and passed through the sample and reach the detector. The detector records all wavelengths in the IR at the same time. The distances of two beams differ to each other after being reflected by the mirrors. The recombination leads to constructive and destructive interference which resulted in pattern known as an interferogram. A computer is used to process the data recorded by the instrument (Blum and John, 2012).

As dispersive instrument, FTIR spectroscopy requires sample preparation for solid and liquid samples. In the case of solid samples, the samples are ground to a fine powder and disperse in a suitable matrix. Usually, potassium bromide (KBr) is used to mix with the ground material and converted under high pressure into a thin KBr disk with a glass like appearance (Blum and John, 2012). Liquids are traditionally analysed as thin films in cell or cuvette which consists of two IR transparent windows. (Perkin Elmer, 2005).

The problems related with this kind of sample preparations include the time needed for preparing individual samples, the problem of sample-to-sample reproducibility and user-to-user spectral variation (Blum and John, 2012). In order to overcome this problem, the technique of Attenuated Total Reflectance (ATR) has been introduced recently.

An ATR accessory or attachment operates by assessing the changes that take place in a totally internally reflected infrared beam when the beam comes into contact with a sample. An infrared ray is aimed onto an optically dense crystal with a high refractive index at a certain angle. This internal reflectance creates an evanescent wave that continues beyond the surface of the crystal into the sample held in contact with the crystal. It can be easier to think of this evanescent wave as a bubble of infrared that sits along the surface of the crystal. This evanescent wave protrudes only a few microns ($0.5\ \mu - 5\ \mu$) beyond the crystal surface and into the sample. Therefore, there must be good contact between the sample and the crystal surface. In parts of the infrared spectrum where the sample takes up energy, the evanescent wave will be attenuated or altered. The attenuated energy from each evanescent wave is given backwards to the IR beam, which then exits the opposite terminal of the crystal and is extended to the sensor in the IR spectrometer. The system then generates an infrared spectrum (Perkin Elmer, 2005).

The advantages of ATR-FTIR are faster sampling, improving sample-to-sample reproducibility, minimizing user-to-user spectral variation and higher quality spectral databases for more precise material verification and identification.

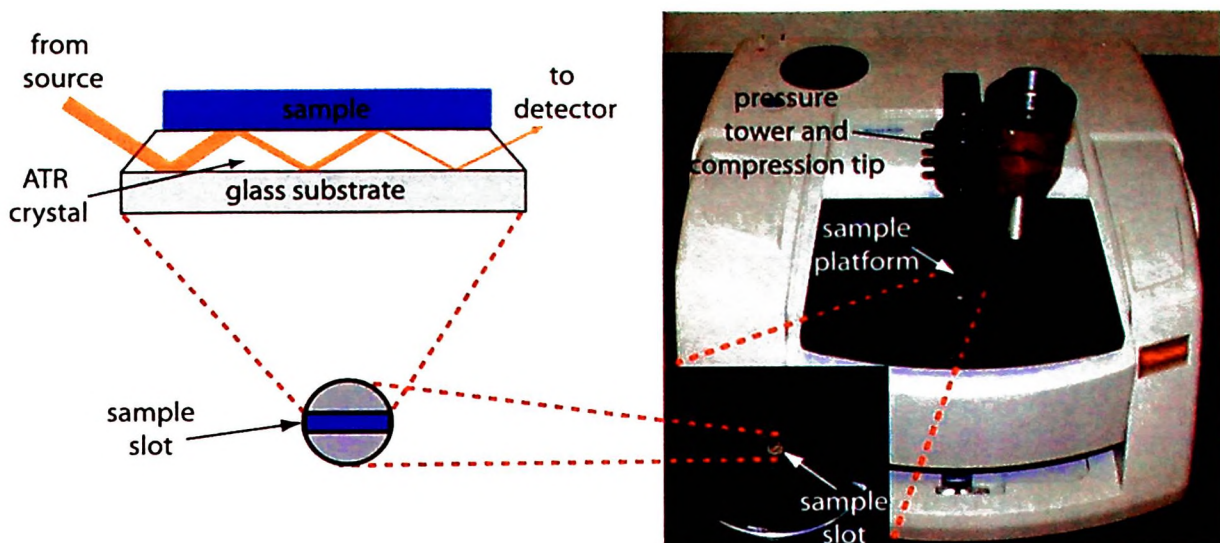


Figure 2.6 ATR-FTIR instrument (www.chemwiki.ucdavis.edu accessed on March, 25, 2016)

ATR-FTIR generates an infrared spectrum as the result from the analysis. The infrared spectrum is formed as a consequence of the absorption of electromagnetic radiation at frequencies that correlate to the vibration of specific sets of chemical bonds from within a molecule (Coates, 2000). There are two regions of organic compounds in a spectrum which are functional group region and fingerprint region.

The functional group region, which is ranging between $4000\text{--}1500\text{ cm}^{-1}$ is a region that peaks are characteristic of specific forms of bonds, and hence can be applied to identify whether a specific functional group is present. The fingerprint region which is ranging between $1500\text{--}400\text{ cm}^{-1}$ is a region that peaks are arise from complex deformations of the molecule. They may be characteristic of molecular symmetry, or combination bands arising from multiple bonds deforming simultaneously (Gable, 2013). Some important IR-active functional groups and their region are stated in Table 2.1.

Table 2.1 IR-active functional groups and their region

Functional group	Region
C-H	<ul style="list-style-type: none"> • 3000-3100 cm^{-1} (sp^2) • 2800-3000 cm^{-1} (sp^3)
C=O	<ul style="list-style-type: none"> • 1600-1800 cm^{-1} • Acids: 1650-1700 • Esters: 1740-1750 • Aldehydes: 1720-1750 • Ketones: 1720-1750 • Amides: 1650-1715
O-H (alcohol)	<ul style="list-style-type: none"> • 3300-3600 cm^{-1} • Monomeric forms: sharp. • H-bonding leads to broadening.
O-H (acids)	<ul style="list-style-type: none"> • 2400-3000 cm^{-1} • Very broad, medium intensity
Single, double, and triple bond of C-C Single, double, and triple bond of C-N	<ul style="list-style-type: none"> • 2200-2100 cm^{-1} • Usually weak; maybe not visible in internal alkynes. • Nitriles are quite strong.
C-O	<ul style="list-style-type: none"> • 1200-1300 cm^{-1} • Often difficult to assign, depending on fingerprint region.
N-H	<ul style="list-style-type: none"> • 3400 cm^{-1} • Usually sharper than O-H.

ATR-FTIR has been applied to study the coating composition in paper. ATR-FTIR is a very appealing method for paper characterisation, particularly because it leaves to perform non-destructive analyses with small sampling depths with the possibility of examining the sheet's surface (Causin *et al.*, 2010). This technique requires no preliminary preparation of the sample. In ATR-FTIR analysis, an optically dense crystal with a high index is compressed against the sample. The depth of penetration of the infrared (IR) beam is inversely proportional to the number of reflections and varies with the type of crystal material (Udristioiu *et al.*, 2012).

Causin *et al.* (2010) used nineteen similar types of office papers and analysed them using ATR-FTIR spectrometer. They found that the IR spectra of the papers were due to two main components which were cellulose and inorganic filler. In fact, cellulose is the polymeric matrix which is the major component in paper, while the inorganic filler is added to give good physical and mechanical attributes to paper. The result showed that the papers contained calcium carbonate (CaCO_3) and no kaolin was detected.

Udristioiu *et al.* (2012) conducted analysis of paper based on the same concept employed by Causin *et al.* (2010) where they used office papers of ten different brands. The result had showed that, out of the ten brands, six contain inorganic filler of calcium carbonate and the rest contain inorganic filler of mixture of kaolin and calcium carbonate, one contained only kaolin, one contained calcium sulfate and the one contained alumina trihydrate.

In a study conducted by Hajji *et al.* (2016), ATR-FTIR was used to determine the chemical structural changes experienced by cellulose during aging and to study its degradation phenomena. Fifty samples (50) of manuscript papers were used where the

samples were kept in two different storage conditions i.e. dry heat and moist heat for 1, 3, 7, 21, and 28 days. The ATR-FTIR results showed that there was alteration to the hydrogen bond networks and desorption of paper residual water content in the papers kept in dry heat condition. Oxidation of cellulose was evident in the paper kept in moist heat condition (Hajji *et al.*, 2016).

2.3.2 Scanning Electron Microscope (SEM)

The first Electron Microscope was invented in 1931 by Ernst Ruska (1906-1988), a German engineer and academic professor. He became interested in the possibility of electron microscopy as a solution to the limitations of optical or light microscopes. Together with Knolls, he developed the first electromagnetic lens, which focused a beam of electrons in lieu of an illuminator onto a source to create a magnified image (Anderson, 2016).

Although this early version did not provide substantial improvements to the optical microscope, Ruska was able to modify the electron lens and get a more powerful version in the late 1930s. He subsequently appointed as an electrical engineer for the Siemens Company, which constructed the first electron microscope. Ruska was awarded the Nobel Prize for Physics in 1986, credited with one of the most influential inventions of the 20th Century (Anderson, 2016).

The scanning electron microscope (SEM) is a microscope that uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. Areas ranging from approximately 1 cm to 5 microns in width can be visualised in a scanning mode using conventional SEM techniques. The interaction of electron-sample produces signal and reveals information about the sample including external

morphology, chemical composition, crystalline structure and the orientation of materials making up the sample. Data is generated in 2-dimensional image that displays spatial variations in the properties (Swapp, 2016).

The component of SEM includes Electron Source ("Gun"), Electron Lenses, Sample Stage, Detectors for all signals of interest, Display / Data output devices, and Infrastructure Requirements (Power Supply, Vacuum System, Cooling system, Vibration-free floor, Room free of ambient magnetic and electric fields) as shown in Figure 2.7.

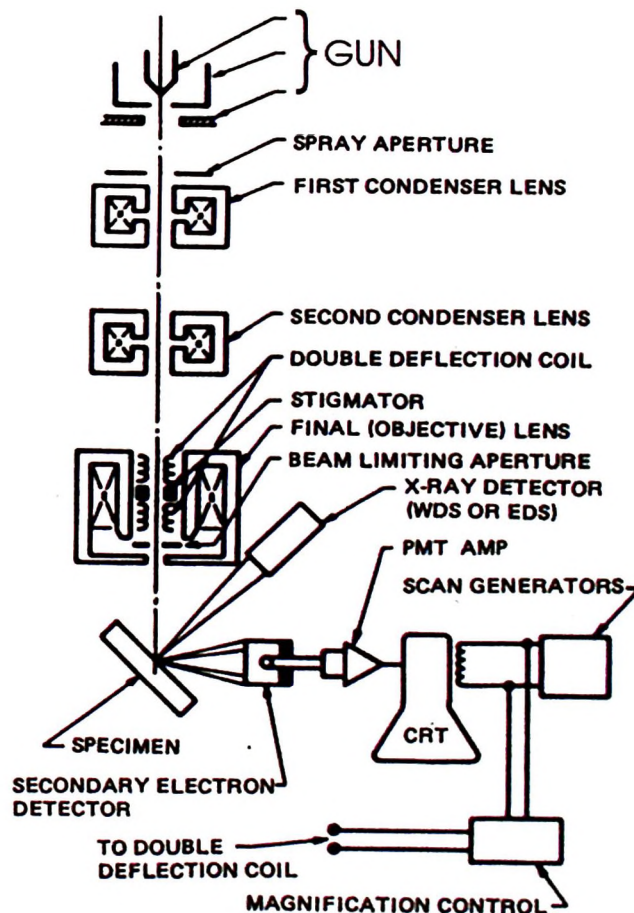


Figure 2.7 Component of SEM (www.cloudfront.net accessed on March, 27, 2016)

SEM analysis is considered to be non-destructive as it does not lead to sample destruction or loss. In SEM, accelerated electrons carry significant amounts of kinetic energy. This energy is dispersed as a variety of signals produced by electron-sample interactions when the incident electrons are decelerated in the solid sample. These signals include secondary electrons, backscattered electrons, diffracted backscattered electrons, photons, visible light, and heat. Secondary electrons and backscattered electrons are usually employed for imaging samples. The use of secondary electrons and backscattered electrons is showing morphology and topography on samples and illustrating contrasts in composition in multiphase samples respectively. X-ray generation is produced by inelastic collisions of the incident electrons by electrons in discrete orbitals (shells) of atoms in the sample. As the excited electrons return to lower energy states, they yield X-rays at a fixed wavelength. Thus, characteristic X-rays are produced for each element in a mineral that is "excited" by the electron beam (Swapp, 2016).

2.3.3 Other instruments

Raman spectroscopic technique also been applied for the analysis of paper. It is a very successful technique for discrimination of filler in papers. A study conducted by Udristioiu *et al.* (2012), that used ten (10) of office papers shown that 60% of the studied papers contained same filler. The study also found that papers with same filler showed some differences at specific spectra due to the different amount of clay present.

X-ray diffraction (XRD) is known as a valuable technique for studying papers. It permits investigation into both the polymeric matrix and the inorganic component of paper composition. Several types of office papers had been examined by using XRD. In the examination, papers were mounted in two different directions: with the X-ray beam parallel to the width and to the height, respectively, of each paper sheet. It was

determined that the repeatability of the measurements was very good and that the obtained diffractograms were superimposable, therefore for subsequent samples just one replicate was done (Causin *et al.*, 2010).

Other than that, XRD was also used to find out the influence of the accelerated aging on the crystallinity of cellulose. XRD was used to compare the crystal form of un-aged and aged papers and the results indicated a noteworthy decline in cellulose crystallinity, as demonstrated by the strong decrease of the crystalline index (CrI%) calculated after undergoing artificial aging (Hajji *et al.*, 2016).

Energy dispersive X-ray fluorescence (EDXRF) is another instrument that can be employed for the analysis of aged paper. EDXRF helped to see the changes within the inorganic content of the fillers added during the papermaking process over time. The method applied is similar to the method applied to in analysis of aged paper using XRD. Energy dispersive X-ray fluorescence demonstrated that the paper elemental composition was slightly affected during both artificial aging tests. Furthermore, EDXRF results showed that when restored papers are presented to both accelerated aging tests, trace elements heavier than calcium remained almost unaffected after 28 days of the aging process compared to the effects received when the treatment holds out only for 1 day (Hajji *et al.*, 2016).

Diffuse reflectance ultraviolet–visible (UV-Vis) spectroscopy is an ideal instrument for examination of surface properties of materials. It is used to characterisation based on the measurements of physical properties such as strength, thickness, mass per unit area (g/m^2), measurement of fibre content, weight, colour, and fluorescence. A quantitative study of UV-Vis absorption is proposed for forensic characterisation of papers collected from the market in Italy in article of the discrimination potential of diffuse-reflectance ultraviolet–visible–near infrared spectrophotometry for the forensic analysis of paper (Causin *et al.*, 2012) and from stationery shops in India in the article of Discrimination of Various Paper Types Using Diffuse Reflectance Ultraviolet–Visible Near-Infrared (UV-Vis-NIR) Spectroscopy: Forensic Application to Questioned Documents (Kumar *et al.*, 2015).

The measurements have been performed in diffuse reflectance mode. The sheets were cut into a 5 cm X 5 cm size for ease of sample handling. In real forensic casework, the sheets can be folded accordingly to focus on the specific area so that non-destructive measurement can be executed. Both positions of the paper show considerable differences on the basis of qualitative as well as quantitative features. These differences are primarily due to the manufacturing process, filler materials, and roughness. After qualitative features, a paired sample t-test was applied for comparison of means of side A and side B. The paired sample t-test brings out that the difference between paper samples of Side A and Side B shows statistically significant effects (Kumar *et al.*, 2015).

2.4 Statistical analysis

Statistical analysis of data obtained from chemical measurements can be very useful for overall interpretation of the samples. It also is useful to support refute experimental hypothesis.

2.4.1 Theory on Product Pearson Moment Correlation (PPMC)

The Pearson's correlation coefficient is a measure of the strength of the linear relation between two continuous variables (Harring and Wasko, 2011). It does not involve the assumption that the relationship between the variables is linear, nor does it involve the variables to be measured on interval scales. It can be used for variables measured at the ordinal level (Hauke and Kossowski, 2011). Pearson's product moment correlation coefficient is denoted as ρ for a population parameter and as r for a sample statistic. It is used when both variables being studied are normally distributed (Mukaka, 2012). For a correlation between variables x and y , the formula for calculating the sample Pearson's correlation coefficient is given by

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2]}}$$

n = number of pair of scores

$\sum xy$ = sum of the products of the paired scores

$\sum x$ = sum of x scores

$\sum y$ = sum of y scores

$\sum x^2$ = sum of squares x scores

$\sum y^2$ = sum of squares y scores

The value of r will always lie between -1 and 1 . If r is zero, then this suggests that there is no linear connection between the variables. Notice that there might be some non-linear relationship, but if r is zero, then there is no consistent linear component of that kinship. If $r = 1$, then there is a perfect positive linear relationship between the variables, and all individuals sampled would lie just along the same straight course with a positive gradient. If $0 < r < 1$ then there is a positive linear trend but sampled individuals would be scattered around this common trend line; the smaller the absolute value of r the less well the data can be characterized by a single linear relationship. If r is positive, then an increment in the value of single variable would lead to our expectation that the other variable will also increase. A r value of -1 suggests a perfect negative relationship with any sampled individual always lying on the same linear trend line which will have a negative slope. If $-1 < r < 0$ then sampled individuals will be scattered around the line; again the smaller the absolute value of r the less well the data can be characterized by a single linear relationship (Puth *et al.*, 2014).

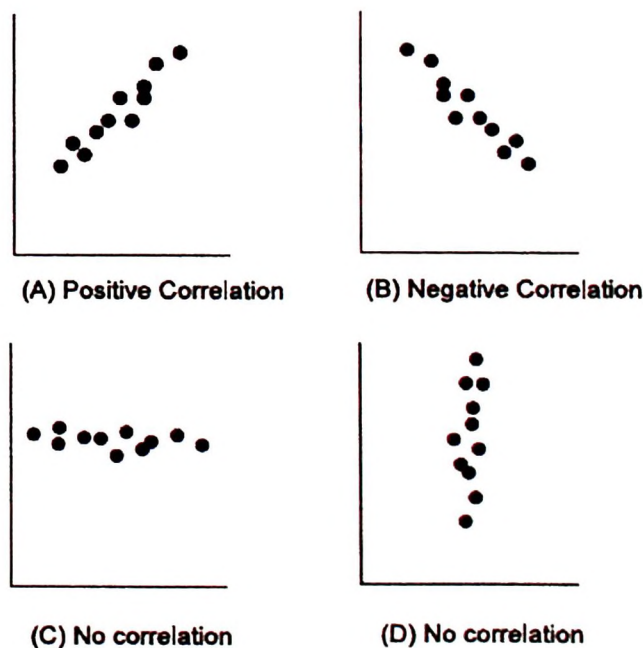


Figure 2.8 Patterns of scatter plot of correlation (Tukey, 1977)

2.4.2 Theory on Paired t-test

The paired t-test is a type of hypothesis testing that is employed when two sets of data are being observed. The data in a paired t-test are dependent, because each value in the first sample is paired with a value in the second sample (Wilkerson, 2008). Paired samples t-test is well known to have more power to detect a difference between the means of two groups as the correlation between the groups becomes larger. That is, as the population correlation coefficient, ρ , increases, the standard error of the difference between the means gets smaller, which in turn increases the magnitude of the t statistic (Fradette *et al.*, 2003). In paired t-test, two hypotheses are set which are null hypothesis and alternative hypothesis. To test the null hypothesis that the true mean difference is zero, the procedure below is followed.

The difference between the two observations on each pair is calculated to make sure the positive and negative differences can be distinguished. The mean difference is calculated. The standard deviation of the differences and the standard error of the mean difference are calculated. The t-statistic is calculated. Under the null hypothesis, this statistic follows a t-distribution with $n - 1$ degrees of freedom. The tables of the t-distribution is used to compare the value for T to the t_{n-1} distribution. This will give the p-value for the paired t-test (Shier, 2004). The equations that involved in paired t-test are stated in Table 2.2.