AGE ESTIMATION OF BLOOD STAINS USING DIFFERENT ANALYTICAL TECHNIQUES AND THE IN-SITU IDENTIFICATION OF PROTEINS USING BCA

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I

Abstract

The issue of establishing a correct timeline in terms of body fluids has always been an important aspect in any forensic investigation. The techniques currently being used have all proved to work quite well but have certain limitations. One of the main problems with these techniques is their invasiveness to the sample in question. This is a major problem as, in most cases, the sample analysed is quite small and the destruction of it via the use of one technique can limit it being used for other key experiments, all of which are important for an investigation.

In this project, the aim was to present a collection of techniques that have been proven to be non-invasive on samples and at the same time, provide key information regarding the identity of the sample in estimating the age. Techniques such as Raman Spectroscopy, Colorimetry and Small Angle X-Ray Scattering were used to establish the identity and estimate the age of a stain. Results were obtained which showed that Raman Spectroscopy and Colorimetry provided the most useful information regarding the body fluid used. Another aspect of this project was to develop a technique that could be used to identify the presence of proteins on crime scenes. Bicinchoninic Acid (BCA) was used for this part of the project along with copper sulphate. The results obtained showed that the presence of proteins could be identified but the results were not specific enough to describe what type of protein was being detected. More work needs to be done on this part as it has potential to be modified and become human specific by adding fluorescent probes that are human protein (skin keratin) specific and therefore can be used as an identifying tool in terms of evidence collection and processing it for DNA profiling.

1.0 Introduction and Background

1.1 Body Fluids

Body fluids are fluids that are organic compounds (solutions) found only in the bodies of living organisms. In humans, most of the body fluids are made mostly of water and ions that are important in carrying out different processes of the body[1]. These fluids are present both inside and outside the cells of an organism and are referred to as being intercellular and extracellular. Intercellular fluids contained much more specified compounds which are mostly present in the cytoplasm of cells whereas extracellular fluids contain a solution responsible for sustaining the balance (osmosity, pH, nutrition etc.) of these cells[1].

1.1.1 Types of Body Fluids commonly found:

Blood

Blood is considered as the largest medium for the transport of nutrients, proteins, ions and a number of other components throughout the body. The general appearance of blood is that of a dense red liquid. In humans, blood is mainly made up of plasma, red blood cells, white blood cells and platelets. However the content and levels of different compounds vary as some specific proteins and ions are different thorough out the different types of organism. Blood is mainly made up of four different components which are as follows:

- Red Blood Cells

Red blood cells, or erythrocytes, are biconcave discs which are of most importance for gaseous exchange. Their major product is haemoglobin, the carrier for oxygen and carbon dioxode throughout the body. In humans, the RBC is bioconcave disc which does not possess any nucleus and has the ability to change its shape and form in order to pass though the different channels.Mature RBCs do not contain subcellular particles and rely on anaerobic respiration.Deviations from the normal size, shape, haemoglobin concentration and number of RBCs are important indicators of disease processes[1].

Mature RBCs develop from reticulocytes, in which form RBCs are normally released from the bone marrow. Reticulocytes are young, immature RBCs which are released from the bone marrow into the blood stream in large numbers. These cells do contain some nuclear material and therefore can be referred to as being anucleate. They retain some mitochondria and the RNA required for the formation of globin, but are later removed. About 1% of RBCs are lost each day and replaced by a similar number of reticulocytes. Reticulocytes circulate for 2–3 days before becoming fully mature; thus, in the normal state, the proportion of reticulocytes is 1–2%[2].

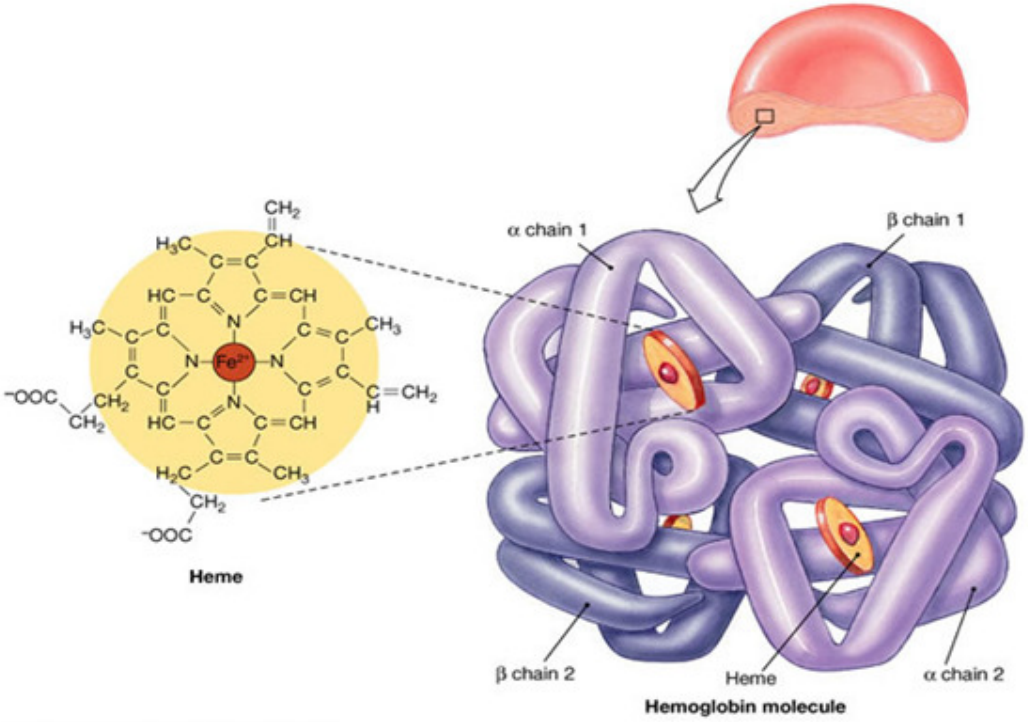


Figure 1: Structure of Haemoglobin [2]

Haemoglobin is a tetramer of two α and two non-α globin chains. The normal adult RBC contains 27–34 pg of HbA, which comprises two α and two β chains [3]. The minor haemoglobin HbA2 is present in adults at levels of <3.0%[3]. Each tetramer contains four haem groups within so-called 'haem pockets'; the amino acids in the globin chains are arranged such that the iron in the haem is maintained in the ferrous state in which it can

associate reversibly with molecular oxygen. An important feature of the haem pocket is that hydrophobic amino acids exclude water molecules, which convert functional deoxyhaemoglobin to methaemoglobin (non oxygen binding). Haemoglobin has two slightly different tertiary structures:

• In the absence of oxygen on the haem group, haemoglobin has a tense structure with a low affinity for oxygen[4].

• When oxygen combines with the haem group, it initiates a conformational change such that the molecule becomes keen for more oxygen (high-affinity, relaxed form)[4].

Haemoglobin has a very complex degradation process in which numerous different by-products can be formed. The chart below represents the degradation routes that can be taken up by haemoglobin provided what types of conditions are provided [4].

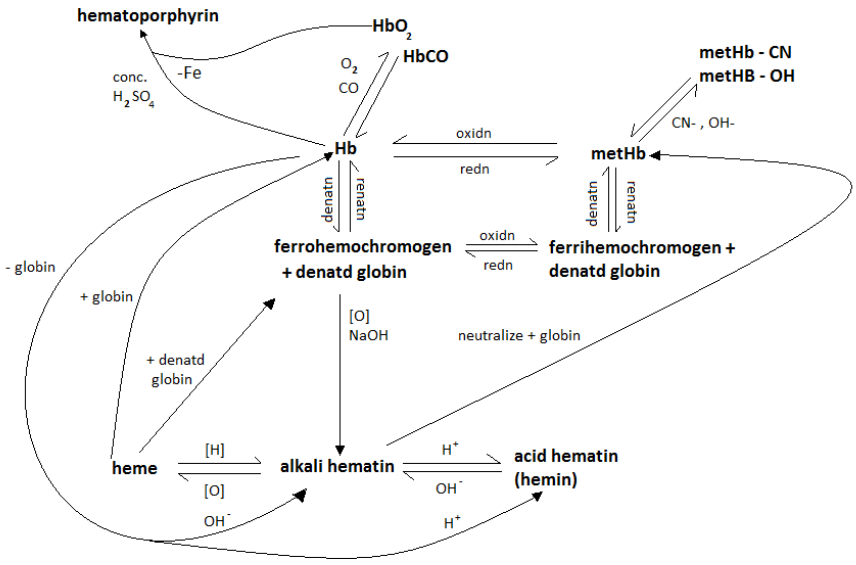


Figure 2 Degradation of Haemoglobin[4]

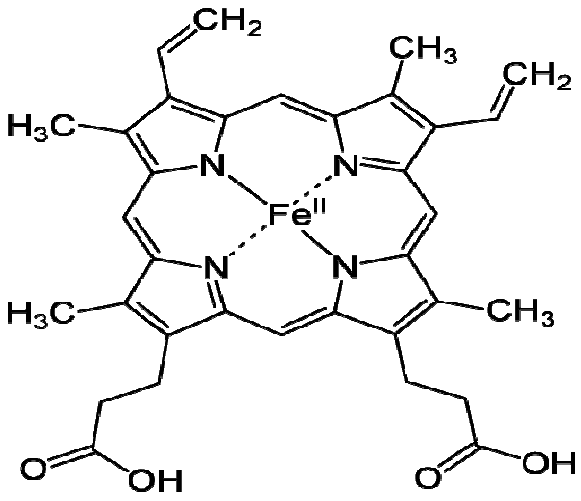


Figure 3: Heme b Group[4]

- White Blood Cells

The major role of human white blood cells is defence against microbial attackers. The different classes of white blood cells have different functions and roles.Neutrophils are primarily involved in the first line of defence against bacteria by directly killing microorganisms, whereas lymphocytes are involved in the group of specific immune responses and have a more strategic role. Unlike red blood cells, white blood cells need to cross the vessel wall to perform their functions in the extravascular space[6].

Neutrophils circulate in the blood for approximately 6 to 10 hours after which they migrate across the vascular wall and undergo apoptosis. Neutrophil number increase in response to any inflammation that maybe caused due to external physical stress or internal problems. The primary role of these cells is to engulf and kill invading organisms which may be found in the extravascular tissues [5].

Lymphocytes comprise the second largest group of white blood cells; 20 to 40%of white blood cells are lymphocytes[5]. The main function of lymphocytes is the production of

antibodies to the response of external antigens. There are three types of lymphocytes which are T cells, B cells, and natural killer (NK) cells. All of these three cells originate in the bone marrow [6].

Eosinophils and basophils are active during allergic reactions. They are in low numbers in the blood media but can be abundantly found in tissues. The number of eosinophils is increased when the body is undergoing an allergic reaction or when there is a parasitic infection. They provide significant ant parasitic activity by de-granulating the protein components and by applying an eosinophil specific peroxidase onto the surface of invading parasites[7]. Basophils and mast cells contain a high affinity IgE receptor. When this binds to an antigen, it causes de-granulation and at the same time synthesizes leukotriene-D_4. In terms of an allergic reaction, this process results in vasodilation, mucus production and muscle contraction[7 8].

- Platelets and Blood Clotting

Platelets are the smallest of blood cells. Even though these cells are fragments of another type of cell, they still play and extremely important role in homeostasis and are important contributors to the Coagulation Cascade.

On activation, platelets change from their normal concave disc shape to a compact sphere with long dendritic extensions which facilitate adhesion [10].

When a vessel is damaged, collagen fibres are exposed which in turn activates the platelets. It swells and becomes irregularly shaped and sticky and releases a number of different chemicals that activate other platelets and initiate the clotting sequence. The clotting of blood involves numerous different steps and clotting factors. The absence of any of these factors can cause excessive bleeding and thus might be lethal in extreme cases. Because the liver is responsible for producing most of the clotting factors in a body, diseases such as hepatitis and cirrhosis can result in a poor clotting of damaged sites. The

various clotting factors are involved in a cascade of steps that activate other substances circulating in the blood. This is referred to as the Coagulation Cascade [11 12 13].

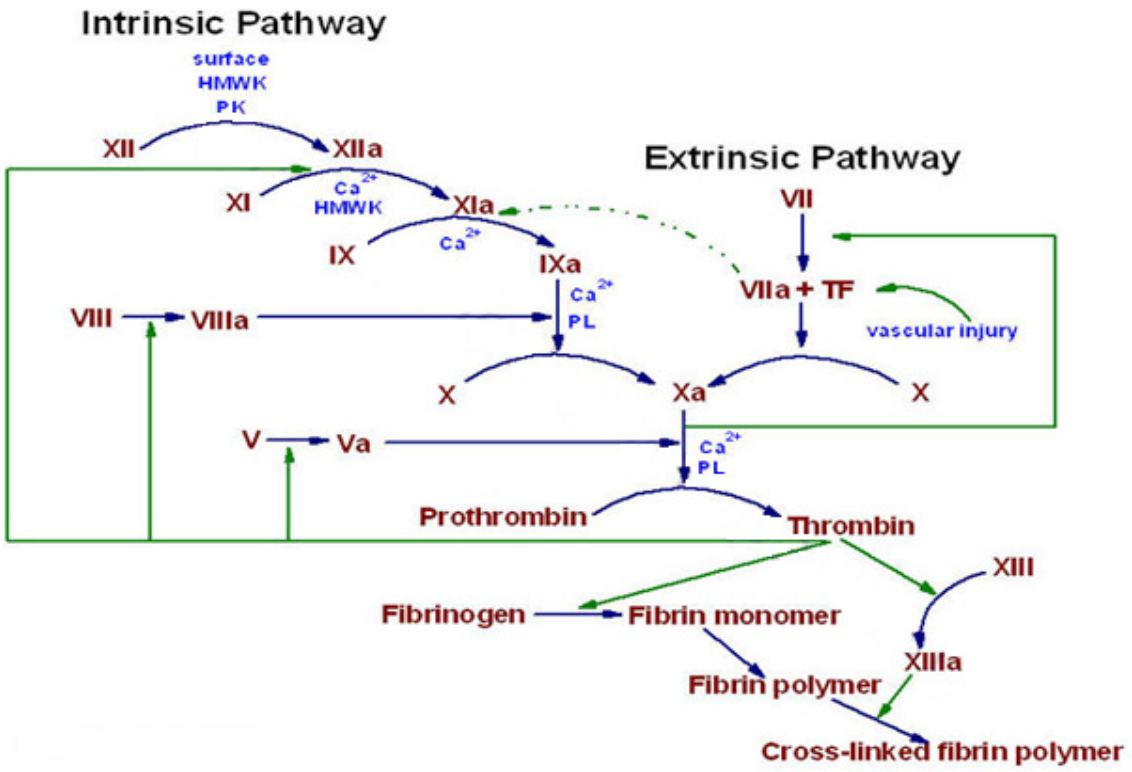


Figure 4: The Coagulation Cascade[11 12]

The intrinsic pathway is initiated when there is a contact between bloods and exposed negatively charged surfaces[14]. The extrinsic pathway is initiated upon vascular injury which leads to exposure of tissue factor, TF. The green dotted arrow represents a point of cross-over between the extrinsic and intrinsic pathways. The two pathways converge at the activation of factor X to Xa. Factor Xa has a role in the further activation of factor VII to VIIa as depicted by the green arrow[15]. Active factor Xa hydrolyses and activates prothrombin to thrombin. Thrombin can then activate factors XI, VIII and V furthering the cascade. Ultimately the role of thrombin is to convert fibrinogens to fibrin and to activate factor XIII to XIIIa. Factor XIIIa cross-links fibrin polymers solidifying the clot[16].

Plasma

Plasma is the other component of blood that makes up a very large percentage of the whole protein. In humans, blood plasma consists of mostly water along with some proteins ions, gases and nutrients. Nutrient molecules in plasma include glucose, amino acids, lipids, cholesterol and lactic acid. Ions that are present include mostly of Na^+ and Cl^- ion[17]. Keeping in view all the components present in plasma, the role of this compound can be quite clearly established as the basic means of transport for all these nutrients and ions throughout the body. A very major protein found in blood plasma is albumin which is responsible for the osmotic potential in capillaries that prevents a massive loss of water from plasma to interstitial spaces. Another protein such as transferrin is also abundantly present that has a role of carrying iron form the gut to where it is stored or used [18].

Overall, there are quite similar characteristics between interstitial fluid and blood plasma and the only difference lies in the concentration levels of proteins in these fluids, which is considerably higher in plasma in comparison to interstitial fluid[18].

Saliva and Seminal Fluid

Saliva is a watery organic fluid produced in the salivary glands in the mouth. Saliva consists mainly of water (approximately 95%) [19] and the rest is made up of proteins, enzymes and other organic compounds. These compounds mentioned play some vital roles in the body.

The mucus in the saliva is extremely effective in binding food into a wet and slippery mass known as a bolus. The wet and slippery nature allows this bolus to travel down the oesophagus without causing any damage due to friction as it passes.Saliva contains a number of enzymes that are responsible for the primary breakdown of food substances. Salivary glands secrete amylase that have the ability to break down starch in to simple sugars and also secretes salivary lipase which breaks down fats. Saliva also contains

certain compounds that play a vital role in defending the body from diseases that may enter. Antibacterial agents such as lactoferrin and peroxidases are known to be present in saliva. The continuous flushing of the oral cavity with saliva also cleans the area away from food debris.

Seminal fluid usually called semen is made up of the male reproductive cells that are also known as spermatozoa and the fluid in which they are suspended. Before ejaculation takes place, a mixture of sperm and semen come out through the ejaculatory ducts through the prostate gland and out through the urethra. Semen is a white/grey fluid and can sometimes appear yellowish, pink or red. Semen contains both sperm and secretions from the various glands in the male reproductive tract. Seminal fluid contains fructose and nutrients that provide energy to the sperm as well as an enzyme called hyaluronidase[15].

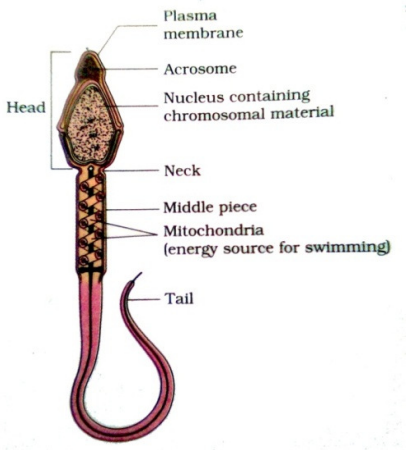


Figure 5: Spermatozoa

1.1.2 Importance of Body Fluids in Forensic Investigations

Biological samples found at the crime scenes play an important role in forensic investigations by providing valuable evidence. Since it is considered that every human has a specific and unique DNA profile, techniques used to analyse and present an individual profile for investigative purposes are of great significance. These can be helpful in convicting a suspect or at the same time, exonerating someone who is wrongly

accused.Apart from this, biological evidence that can be gathered from crime scenes is extremely useful in trying to re-create events that occurred at a specific crime scene. For example, blood stains can indicate some form of struggle and detection of semen or vaginal fluid can indicate the involvement of some form of sexual encounter or assault[20].

When a potential body fluid is found at a crime scene, there may be a need for some external influences which need to be introduced to the sample to make it visible or more prominent for identification purposes. Chemical tests are initially used to give some indication as to the identity of the substance, and further confirmatory tests are then conducted to confirm the origin of the sample. Over the past several years, and thanks to extensive research into forensic investigation techniques, numerous methods and protocols have been devised that can be used to confirm the identity and origin of different biological samples. Techniques which are currently used for forensic body fluid identification include chemical tests, immunological tests, protein catalytic activity tests, spectroscopic methods, and microscopy[20]. Some of these methods are presumptive tests which are used for screening purposes, such as the Leucomalachite green (LMG) and Kastle-Meyer test for blood. Others are confirmatory test that have the ability to prove without reasonable doubt the identity of the questioned fluid. A common example is the microscopic analysis of sperm cells for the identification of semen.However, there are still some limitations to both the chemical presumptive tests as well as the confirmatory tests mentioned.These include low specificity, lack of sensitivity, sample destruction, instability of biomolecule assayed, or incompatibility with downstream individual identification assays [21].

1.1.3 Body Fluid Identification

Blood

- Crystal Tests

The crystal tests, which are now rarely used, are all based on the formation of haemoglobin derivative crystals such as haematin, haemin and haemochromogen. The test is carried outon a microscope slide, with the reagents being added to the stain under a cover slip, and crystal formation observed microscopically. The Takayama crystal test for blood is probably the best known. An alkaline solution of pyridine is added tothe stain and, if blood is present, pink crystals of a complex between pyridine and haem form as the slide is warmed. As well as pyridine, a number of other nitrogenous bases, including nicotine, methylamine, histidine and glycine have been used in variations of this test[22].

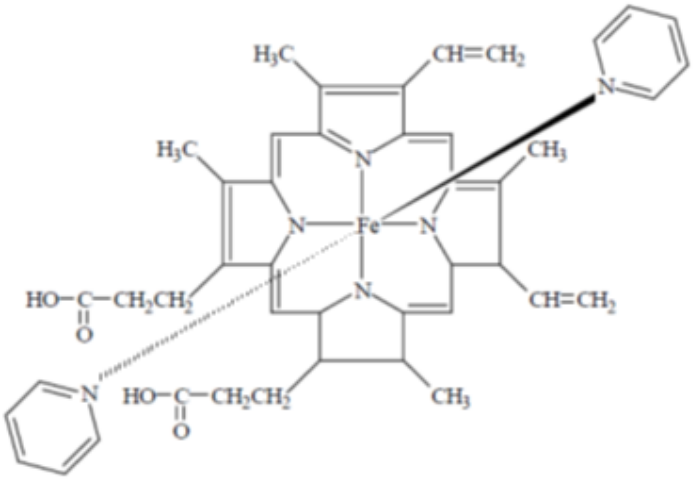


Figure 6: Pyridine ferroprotoporphyrin (Complex formed in Takayama's Test)[16]

It is generally accepted with the crystal tests that a positive result confirms the presence of blood. A negative result does not necessarily show that blood is absent it may, for example, indicate faulty technique - and a positive control should always be run for comparison. Bloodstains up to 20 years old have seen to give a positive result when this crystal test is performed[22].

Another example of a crystal test for blood is of the Teichmann's Crystal Test. This test aims at identifying the haemochromogen derivative of haemoglobin degradation and the test is generally regarded as a confirmatory procedure in determining the sample as being or not being blood. One of the main advantages of using this particular test is that after being treated with the necessary reagents, the sample can still be used in spectroscopic analysis[22].

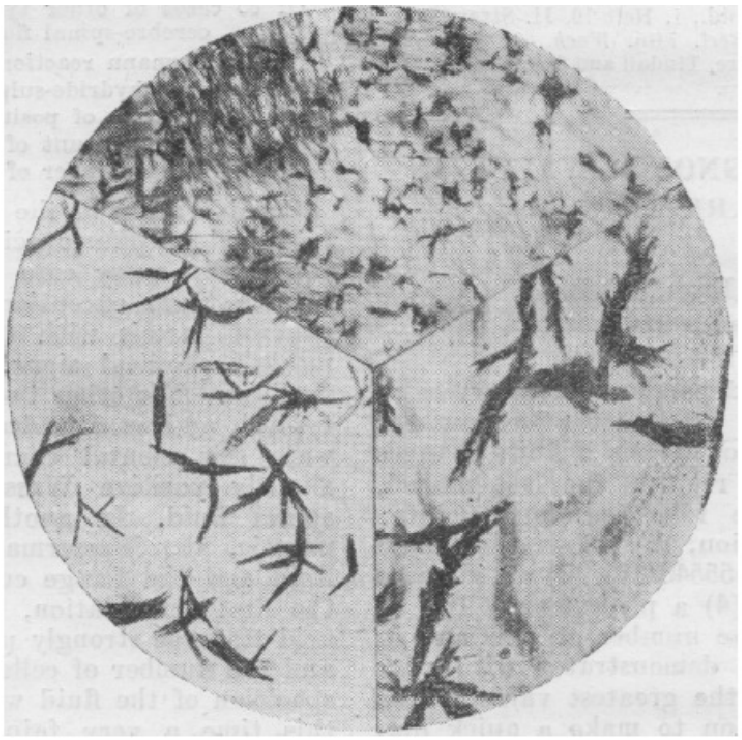


Figure 7: Haemochromogen Crystals from the Teichmann's Crystal Test[16]

However there still are some limitations to these tests which are mostly due to overheating of the sample or the acid not being pure. In order to overcome these issues, numerous modifications have been made to the test. The procedure of the test itself is quite simple and easy to follow. A small quantity of sample is placed on a microscope slide and initially a drop of a solution of 5% sodium iodide or potassium iodide in water is added. According to the modified Teichmann's Test protocol, it is also said that all chlorides gave a strong positive result. The sample is carefully dried on an asbestos mat over a Bunsen flame. A

cover glass was then placed over it and the lactic acid was allowed to run under the cover slide so as to cover the entire stain. The slide is then warmed till small bubbles start to appear underneath the cover slide [22].

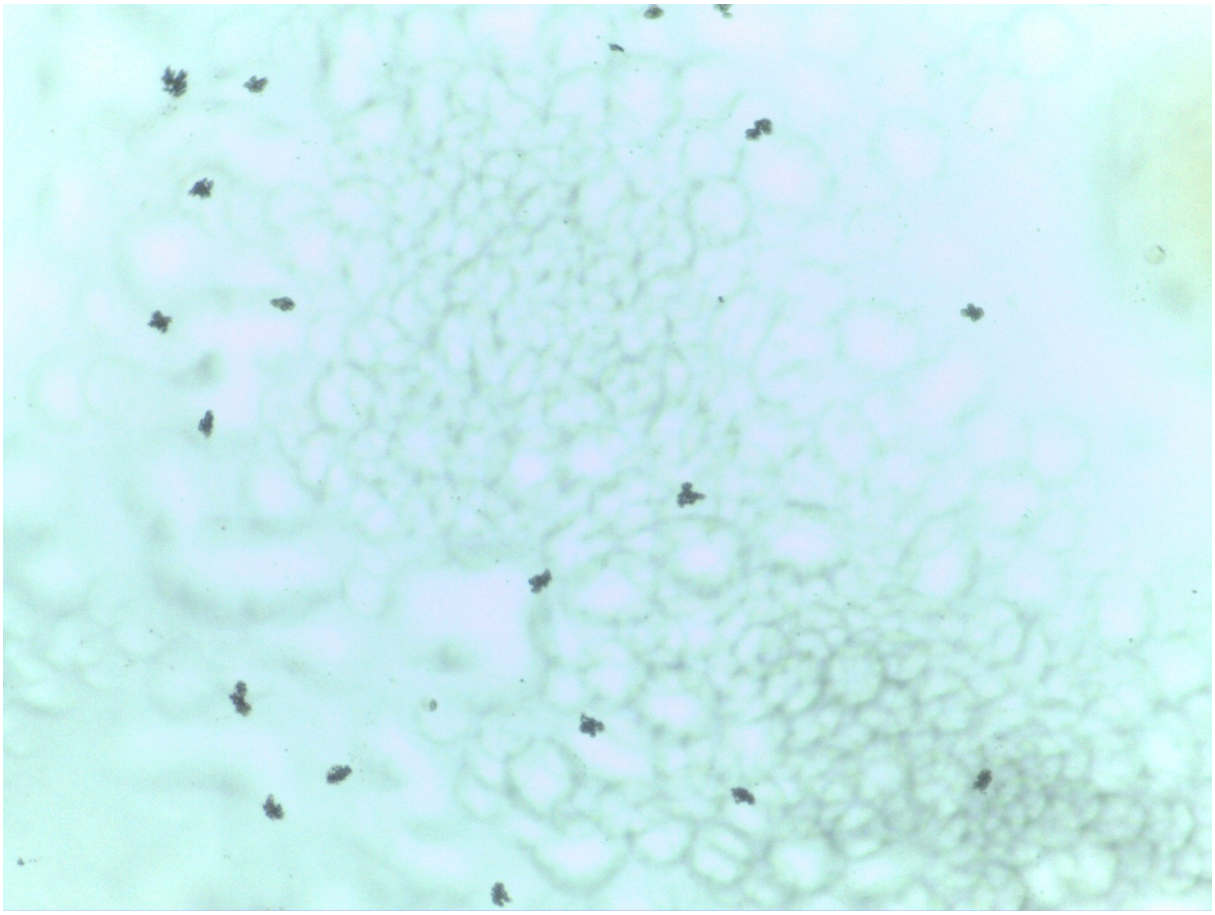


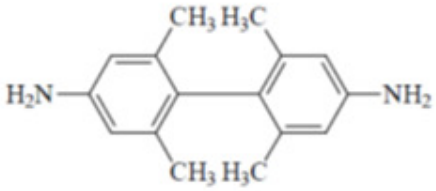
Figure 8: Teichmann's Crystals produced in a sample of 1hour old blood stain

- Catalytic Tests

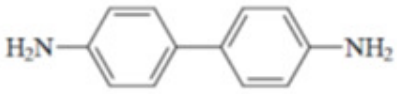
These tests depend on the fact that the haem group of haemoglobin possesses a peroxidase-like activity which catalyses the breakdown of hydrogen peroxide. The oxidising species formed in this reaction can then react with a variety of substrates to produce a visible colour change. Reagents that are commonly used for this type of testing include benzidine and various substituted benzidines, leucomalachite green, leucocrystal violet and phenolphthalein. The reaction with luminol (3-aminophthalhydrazide) to form a

luminescent rather than a coloured product is also a catalytic test. These are intended for the detection of blood in urine in clinical situations but are equally useful as a screening test for dried bloodstains[23].

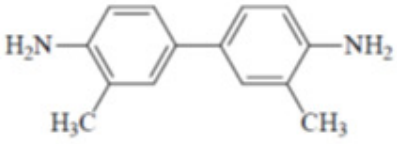
The following diagrams represent the different compounds that can and are being used as presumptive test reagents[23].



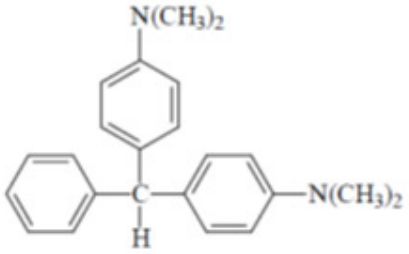
Tetramethyl benzidine



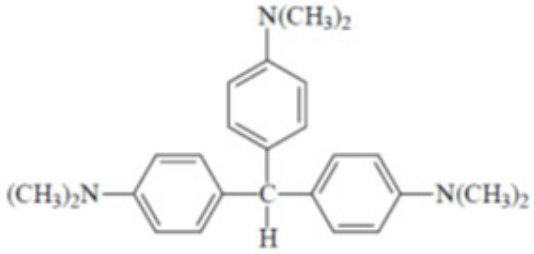
Benzidine



Orhto-tolidine



Leucomalachite green



Leucocrystal violet

The catalytic tests are extremely sensitive, but are attention to a number of interferences and are therefore not totally specific for blood. Substances which can interfere include enzymes such as catalase and peroxidases, oxidising chemicals and metals such as copper and iron. This is a very important factor that needs to be taken into account when this test is being performed in an external environment. Plant materials and many other objects that may contain interfering compounds can discredit the validity of results when

the test is performed outdoors. The general principle is that if the test is negative, blood is absent, but that if the test is positive, blood is probably, not definitely present. For this reason the tests are often described as "presumptive" tests.

In the Kastle-Meyer test the reduced phenolphthalein is kept in an alkaline solution. This is a colourless solution when kept under these conditions. Upon the oxidation of the solution with haemoglobin and in the presence of a peroxidase, the solution turns to a bright pink/purple colour[24].

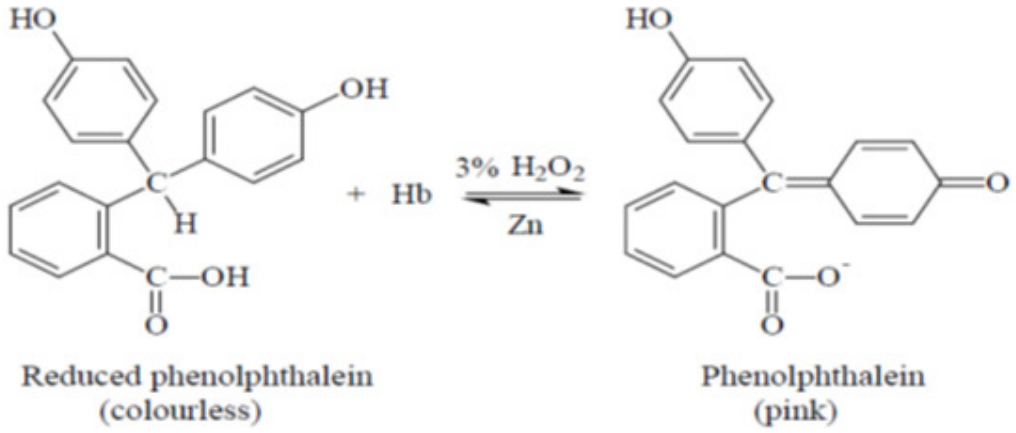


Figure 9: Oxidation of reduced phenolphthalein by haemoglobin and peroxidase[24]

In its original form, a small amount of the Kastle-Meyer reagent prepared is mixed withequal volumes of 95% ethanol and 10% hydrogen peroxide solution. The suspect stain is rubbed gently with a small piece of filter paper and a drop of the mixed reagent added to the paper. The development of a pink colour is indicative of the presence of haemoglobin, which has catalysed the breakdown of hydrogen peroxide to an oxidising species. However, used in this form, the test will give an apparently positive result with other oxidising materials[24].

Luminol is made up in an alkaline solution using sodium carbonate, and sodium perborate.Hydrogen peroxide can be used but provides us with a shorter-lived

luminescence than sodium perborate. The solution is applied as a spray and the presence of blood produces a bluish luminescence which stays luminescent for approximately 30-45 seconds. The luminescence can be restored by additional spraying but this needs to be done carefully as the stain will lose definition if too much liquid is added to it [25].

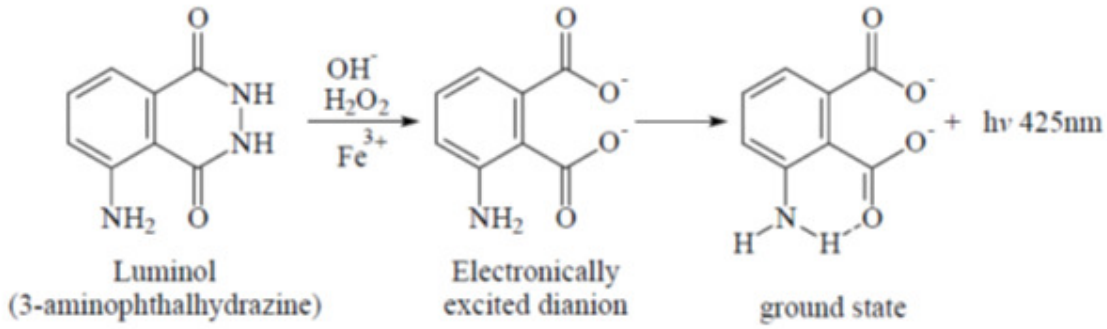


Figure 10: Reaction of Luminol[17]

Similar to other chemical tests, luminol is not blood specific and can give positive results in the presence of certain plant enzymes, oxidising agents and metals. However the result from blood can be differentiated from other false positives by the colour of the luminescence, how long it luminesces and the type of light that is initially given off during the reaction [25].

High Performance Liquid Chromatography is a chromatographic technique that is widely used for separation purposes in organic compounds. This technique is also used as a confirmatory test for the identification of blood by using the absorbance of haemoglobin. This particular method can also be used identify the species of origin from variations in the globin chain, to distinguish between foetal haemoglobin and adult haemoglobin. This is currently the most common instrumental technique used in the confirmatory analysis of blood [26].

1.1.4 Problems faced in Body Fluid Identification

Due to the advancement in modern day analytical techniques, performing certain test for the identification of body fluids has been made a lot easier as compared to the times when these techniques were not readily available. However, there are certain problems that still remain while trying to analyse samples either for identification purposes or any other sort of analytical processes. One of the main issues faced today is in maintaining the chemical and structural integrity of the sample. A lot of the techniques mentioned earlier require large amounts of chemical changes to occur in the samples and therefore compromise the integrity of the original compound. This is an extremely major issue as the sample available is not always large enough for it to be wasted on one particular analysis technique. Sometimes organic samples such as blood and semen are only present in trace amounts and thus testing such small quantities with methods like HPLC and Acid Phosphatase Test may not leave enough viable samples for further analysis like DNA profiling. Therefore the establishment of a non-invasive technique is highly important[30].

Another problem faced with body fluids is the accurate measurement of the age of the particular stain. The techniques that are currently being employed like HPLC (High Pressure Liquid Chromatography) are extremely invasive to the sample and leave little behind for other testing to be conducted[30].

1.1.5 Current techniques used in Body Fluid Identification and Forensic Investigations (Past Research)

Genetic Analysis

Advances in forensic genetics have led to the development of several new methods including messenger RNA (mRNA) and micro RNA (miRNA) expressions, as well as differential DNA methylation patterns. Especially, recent approaches based on tissue-

specific mRNA and miRNA expression have been proved to be useful because of their high tissue specificity to forensically relevant body fluids[31].

A recent study acknowledges identifying the formulation of a multiplex reverse transcription polymerase chain reaction (RT-PCR) method for the definitive identification of the body fluids that are commonly encountered in forensic casework analysis such as blood, saliva and semen [31]. Using selected genes, the research group was able to identify the expression of these genes is a tissue specific manner[31].

Specificity was established by demonstrating that the mRNA of the candidate gene was present in one type of body fluid stain but absent from all others. The body fluids tested have been mentioned above earlier[32]. This is another approach that has been used and is continuously being used in trying to test samples and to confirm the presence of these body fluids.An mRNA based approach, such as this multiplex process described in the paper allows the simplistic identification of the tissue components present in a body fluid stain [29]. One of the main issues regarding techniques related to genetic analysis of body fluids is the time that is required to prepare and run the sample. These techniques are an invaluable tool in forensic investigations but are extremely time consuming and at some stages, very expensive. Another drawback to this particular technique is that it is lab based and these tests cannot be performed on the crime scene. This would require packaging and evidence collection to be done and therefore risk contamination[32].

Raman Spectroscopy:

A very promising technique from the past has been shown to give some impressive results in terms of body fluid identification as well as in trying to establish a technique to accurately determine the age of a blood stain[73].

Raman Spectroscopy has been used in the past for the identification of blood. A recent study suggests the use of Raman Spectroscopy for not only the identification of blood, but also for other fluids like vaginal fluid, saliva, sweat and human semen [72]. The results suggested that all the spectra for the different body fluids provided specific peaks characteristic of the different types of body fluids.

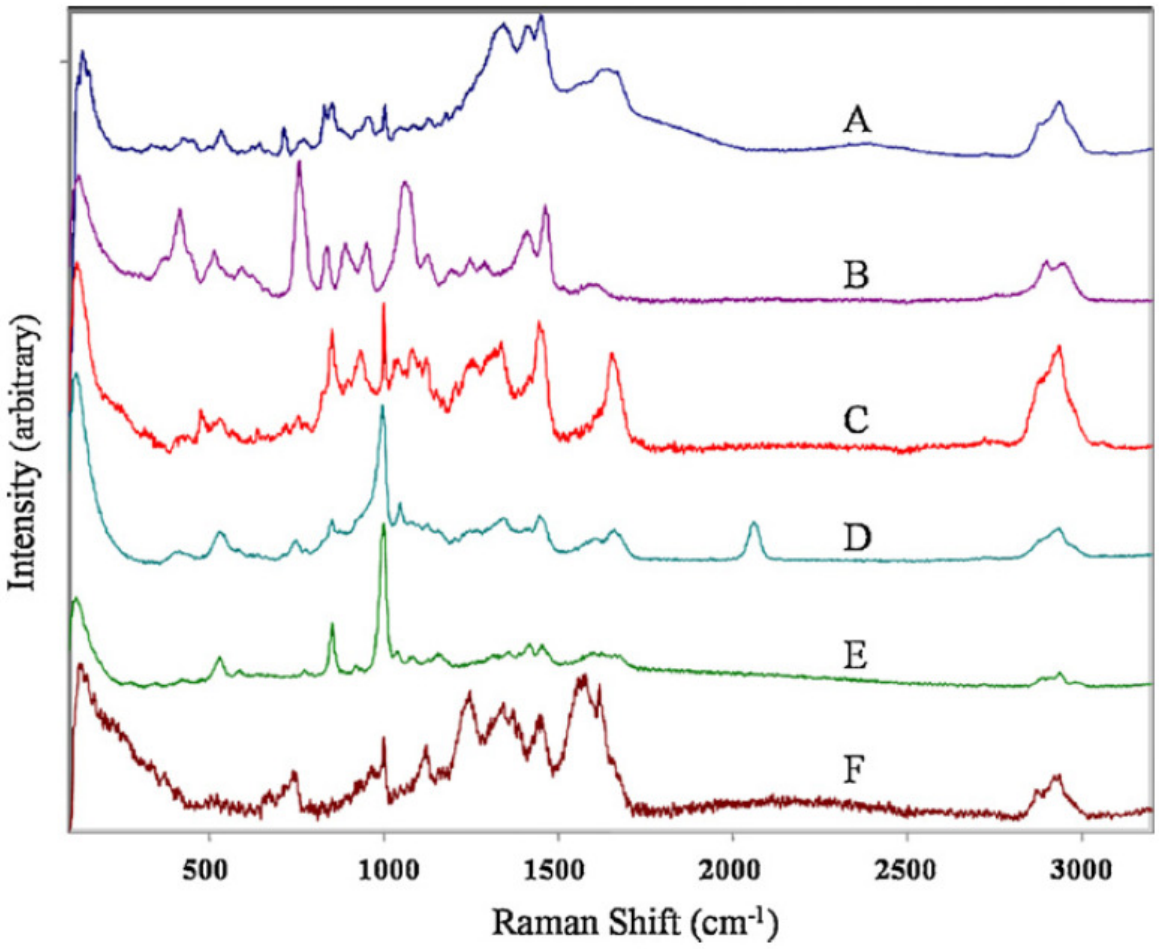


Figure 11: Spectra of body fluids (a) human semen, (b) canine semen, (c) vaginal fluid, (d) saliva, (e) sweat and (f) blood[34]

The spectrum above also shows a very integral part of forensic body fluid investigation which is species specificity. It shows considerable differences between samples of both human and canine semen samples. Although the spectra for both semen samples look quite similar, they exhibit some certain key peaks that can be used to distinguish between

different species. These include different relative intensities of peaks common to both spectra as well as certain bands present in one of these spectra only. For example, the very strong peak at 760 cm^{-1} in the canine semen spectrum is due to the presence of tryptophan in an undetermined protein which is also present in the human sample in a lower concentration. Another peak at 1001 cm-1 from a combination of glucose and fructose is very strong in the canine sample and barely evident in the human sample keeping in view all these results, it shows that this technique not only shows promise in body fluid identification, but can also be used to distinguish between species [72].

- **Distinguishing blood from other biological fluids[75]:**

There are different types of body fluids that could be found at a crime scene including saliva, sweat, vaginal fluid, semen and blood[34]. All of the mentioned fluids were scanned with the Raman spectrometer to compare each spectrum and highlight any differences that could differentiate between the different body fluids. In this case, all the body fluids showed different peaks. This shows that it is possible that body fluids could be distinguished using Raman spectroscopy[75].

Another study expanded on the previous project by increasing the number of different types of samples for comparison. A 785nm confocal Raman microspectrometer was used to scan the samples, which included saliva, canine semen, sweat, vaginal fluid and human semen. A single sample per body fluid type was mounted onto a microscope slide covered with aluminium foil and left to dry. The inclusion of non-human samples was to observe whether there were differences in composition of body fluids across different species. Focussing on the blood sample, there were certain components of the blood that were specific to this type of fluid when compared to the others, which included albumin, glucose, haemoglobin and fibrin. The main component in the blood, haemoglobin,produced peaks at 1367cm^{-1} (CH_3 symmetric stretch), 1575cm^{-1} (C-C stretch) and 1618cm^{-1} (C=N stretch) [75].

- **Distinguishing blood from other species:**

The importance of blood being identified at a crime scene is extremely important, however, if the blood is not human then the value of the evidence then decreases, if the investigation is considering only human foul play. Another project conducted looked into if different species have a different composition of components in blood, which could be used to distinguish them from human blood[74].

Human, canine and feline blood samples were taken for this experiment for comparison and were scanned at three separate wavelengths. However, in all three cases the composition of the blood components was too similar and so the three species could not be distinguished. A longer wavelength was tested (752.6nm) and this also showed that all three samples were very similar[74].

Further progress was made on the previous experiment (discussed above), where statistical reasoning was used to compare the composition of each of the three blood samples. The samples were run on a 785nm Near Infra-red (NIR) Raman spectrometer and as previously the three spectra looked the same. However, using statistics, the concentration of each of the components were compared with the positive control. It was found that human males had a higher concentration of ascorbic acid, glucose, niacin and insulin than the other two species[73]. In addition, haemoglobin which is the main component in blood was found to have a varying concentration between the three samples; human males presented a higher concentration than canines, which in turn has a higher concentration than [73].

- **Ageing of bloodstains:**

Bloodstain ageing is a vital part in forensic investigations, as this would indicate when an alleged crime took place. Previous work in this area have included other types of instruments however, none of these instruments have been introduced into forensic practise as most of them involve some preparation of the sample beforehand, which could

lead to contamination or irreversible changed to the sample. Some of these instruments include the use of visible spectroscopy to detect the colour change from red to brown, which is a chemical process involvingoxyhaemoglobin that contains a strong Soret band at 475nm and two weaker bands α at 576nm and β at 540nm. Other instruments included electron paramagnetic resonance, atomic force spectroscopy (AFM), measurements of RNA degradation and high performance liquid chromatography (HPLC)[76].

When blood dries the oxyhaemoglobin is oxidised, which turns it into methemoglobin and this then denatures into hemichrome overtime. This particular reaction causes the colour of the blood to change from red to brown. The NIR Raman was used in a similar way to visible spectroscopy to detect the components in blood.This was done in order to determine the drying stage at which blood was present [37]. Due to the face that these types of materials (cotton substrates) produce a high absorbance in the spectra, a wavelength was selected that removed this interference so it did not have much affect the sample. The results show that initially the spectra was dominated by water peaks at 1454 and 1940nm, after which the characteristic blood peaks for albumin, globulin and haemoglobin were seen[75]. This experiment was carried out over one month and included a number of different coloured materials as substrates to observe whether the colours affected the sample detection. Some promising results were obtained however, the older the bloodstain was, the higher the error margin seemed to beand this was said to increase after 15 days[76].

Comparison of the two shorter wavelengths was carried outto obtain the optimal spectra. Initially, the blood samples contained 1155 and 1511cm^{-1}, which were found to disappear when the blood samples were dry. Also, the decrease in oxyhaemoglobin peaks at 1369 and 1636cm^{-1} decreased overtime[77].

Histories of two samples were compared, the first sample was taken directly from a person and immediately analysed whereas the second sample was stored in a vacutainer containing EDTA, stored in a refrigerator. The first sample was immediately analysed and then scanned at hourly intervals. The second sample was analysed after one week in the storage conditions described above. The first sample exhibited two peaks at 1155 and 1511cm^{-1} that after one hour disappeared and when the second sample was analysed after the week the two peaks were still present. This shows that changing the temperature and storage conditions of the sample can affect the ageing process, which in this case delayed the ageing process of the second sample[77].

- **Long term bloodstain ageing:**

A cotton substrate was used for this experiment that was carried out to determine the long term effects of bloodstain ageing. Storage of the sample between analyses consisted of the samples being kept at 22.3±0.5℃ in a dark laboratory. The results of this experiment were that initially, oxyhaemoglobin peaks were present in the spectrum at 540 and 576nm, which later over time deteriorated and could not be identified in the year old sample.This was mainly because the spectra were not as distinctive due to the presence of hemichrome. For age determination of a bloodstain, three separate haemoglobin derivatives were used which included oxyhaemoglobin, methemoglobin and hemichrome.A fraction of these three components was monitored during the experiment to see if the amount of the components could in any way help in the ageing of the bloodstain. There was a comparison of the experimental spectra to literature spectra and overtime the R^2 value decreased significantly, showing less of correlation between the measured spectra and the multicomponent blood-fit[78].

Colorimetry

Colorimetry and more specifically, spectrophotometry have been used in numerous fields spread over all respects of science. It is an important tool for the quantitative analysis of

solutions and provided valuable information on the identity of chemicals present in an unknown sample. It has great significance in terms of forensic investigations ranging from drug analysis up to analysis of biological material and DNA.

- **UV-Visible Micro-spectrophotometry (MSP) of dyed textile fibres [36]**

UV Vis MSP uses a microscope to gather light from the sample and transmit it to a UV-visible spectrometer. The use of an instrumental technique such as MSP is significant because it is repeatable, non-destructive and requires little to know sample preparation time. This technique can distinguish between coloured fibres that may appear visually similar. However, it is important to note that MSP is not capable of identifying particular dyes or mixtures of dyes, but rather identifying the spectral characteristics of a sample for the purpose of comparison[36].

According to typical MSP protocols, several fibres need to be examined per item. Upon examination, man-made fibres are usually found to have a homogenous chemical composition as the dye is bonded to a relatively constant chemical environment. Cotton and other natural fibres are composed of many different chemical components which are not uniformly distributed throughout the fibre. In addition, fibres often fluoresce due to optical brightness or dyes applied to them. MSP is also limited in cases where dyes have similar structures or where fibres are either way lightly or very darkly dyed. Despite these limitations, MSP is an extremely important method is colour comparison of individual fibres. MSP has shown to discriminate between cotton fibres of similar colour quite well and the degree to which is differentiates is highest for blue followed by red cotton fibres. The use of MSP along with thin-layer chromatography is also a common practice in the investigation of coloured fibres. MSP is regarded to be a complimentary technique alongside thin-layer chromatography. Spectra obtained from MSP are often normalized and compared to each other visually[36].

1.2 Proteins

1.2.1 Introduction to Proteins and Amino Acids

Proteins are a family of biological macromolecules that provide a variety of three dimensional structures uniquely shaped for their many different functions. These include the following[37]:

- Structural Proteins: from the keratins of the outermost layer of the epidermis (skin) to the exterior coats of viruses
- Enzymes: chemical compounds produced naturally that catalyse the reactions involved in metabolism, and the replication and transcription of DNA
- Antibodies: that recognize and repel invading pathogens that enter the host body
- Regulatory Proteins: including those that control the transcription of genes
- Sensors: that detect and implement signals generated within our bodies and those that impact our bodies influenced by the outside environment
- Transporters and pumps: that control the movement of compounds both in and out of cells
- Transducers: motor proteins that convert chemical to mechanical energy, such as the contractile proteins of muscle contraction and the kinesins and dyneins of intracellular transport. ATP (adenosine tri phosphate) synthesis that converts chemiosmotic energy generated by respiration to high energy chemical bonds of ATP (adenosine tri phosphate) [37].

Proteins create the structures that they require for specific functions by variations on a common underlying chemical system. Proteins are linear polymers, all containing the same polypeptide backbone. Along this backbone a variable sequence of side chains that distinguish different proteins from each other. The great range of protein structures and functions depends on the variety in the properties of the side chains. The 20 naturally

occurring side chains vary in size and in other physiochemical properties. Some are polar or charged and they have the ability to participate in hydrogen bondingand electrostatic interaction with other residues and with a solvent. The charged atoms occur at or near the ends of the relatively long and flexible side chains. Other side chains are non-polar. These have an unfavourable interaction with water called the hydrophobic effect. Clustering of hydrophobic side chains in the interiors of proteins provides a thermodynamic drive force for the folding of proteins [37].

Amino acids are the raw material of proteins. Each amino acid has the structure of a central carbon atom to which is attached: a hydrogen atom, an amino group, a carboxylate group, and a side chain. Within each protein, the main chain forms peptide bonds between the COO^- group and one of the amino acid and the NH_3^+ group of another[38].

Natural proteins are formed from a basic collection of 20 standard amino acids.

Non Polar Amino Acids			
(G) Glycine	(A) Alanine	(P) Proline	(V) Valine
(I) Isoleucine	(L) Leucine	(F) Phenylalanine	(M) Methionine
Polar Amino Acids			
(S) Serine	(C) Cysteine	(T) Threonine	(N) Aspargine
(Q) Glutamine	(Y) Tyrosine	(W) Tryptophan	
Charged Amino Acids			
(D) Aspartic Acid	(E) Glutamic Acid	(K) Lysine	(R) Arginine
(H) Histidine			

Table 1: The Different Amino Acids

The combination of these 20 different amino acids results in the formation of natural proteins. These amino acids are held together in a crystalline lattice by charged interactions and these strong forces contribute to high melting and boiling points. Charged

groups are also responsible for electrical conductivity in aqueous solutions, their relatively high solubility in water and the large dipole moment associated with crystalline material[40].

At pH 7.0, the amino and carboxyl groups are charged but over a pH range from 1 to 14, these groups show a series of different equilibria which include the binding and the dissociation of a proton. This binding and dissociation represents the weak acid and weak base behaviour of an amino acid. This is extremely important as this property influences the eventual properties of the protein that it forms and also permits the identification methods for different amino acids. This particular property also dictates the reactivity of the amino acids and later of the protein. Amino acids form peptide bonds via a condensation reaction and the elimination of water in a process that normally occurs on the ribosomes found in cells. The formation of one peptide bond covalently links two amino acids forming a dipeptide. Polypeptides or proteins are built up by the repetitive formation of these peptide bonds[41].

1.2.2 Structure of Proteins

Primary Structure

The primary structure for proteins is mainly the linear order of amino acid residues which lie along the polypeptide chain. It is formed by the covalent linkage of individual amino acids via peptide bonds. Every protein is defined by a unique sequence of amino acids and all successive levels of organization rely on this primary level of structure. Some proteins are related to one another leading to certain degrees of similarity in primary sequences. When a change occurs in the primary sequence, it frequently involves two closely related residues[42].

Secondary Structure

Due to the flexibility of the polypeptide chain, any primary structure is compatible with a very large number of spatial conformations of the main chain and side chains. The main chain conformation defines the secondary structure. The two conformations that occur in proteins are regarded as α-helices and β-sheets. All other possible structures are variations of one these structural conformations[42].

A-Helices (α) are formed from a single consecutive set of residues in the amino acid sequence. They are a local structure of the polypeptide chain; that is they form a set of residues consecutive in the sequence. The hydrogen-bonding pattern links the C=O group of residues to the N-H group of residues[42].

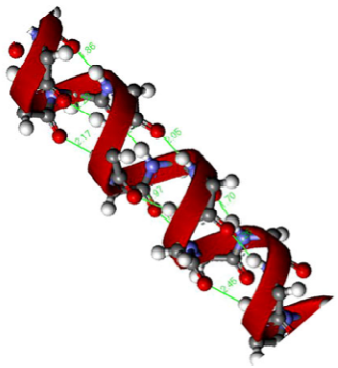


Figure 12: Alpha Helix[28]

B-Sheets (β) are formed by the lateral interactions of several independent sets of residues. They can bring together sections of the chain separated widely in the amino acid sequence. These strands are not completely flat, but each strand is rotated from its neighbours so that the sheet appears to be twisted. Each of the central strands has two neighbouring strands in the sheet and the two edge strands have only one neighbour in the sheet[28]. The strands of some sheets form a closed structure, with no edge strands and are known as β-barrels. The strands lie roughly on the surface of a cylinder. A common irregularity in β-sheets is the β-bulge, in which one or two residues in an edge strand of a

sheep loop out. The bulged residues do not participate in the regular hydrogen-bonding pattern of the sheet[43].

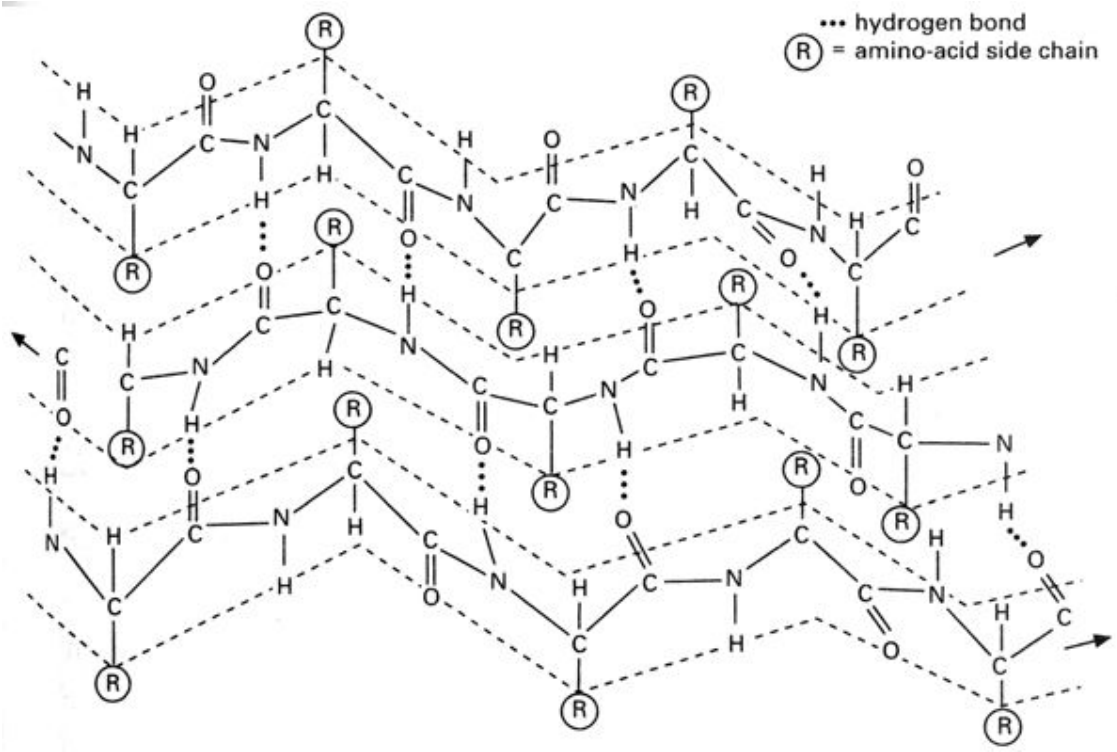


Figure 13: Beta Sheet for Proteins[28]

Tertiary and Quaternary Structures:

Different proteins combine helices and sheets in different ways to create different three-dimensional arrangements of the chain, along with different patterns of interactions between the helices and the sheets. This is known as the tertiary structure or folding pattern. It is from their folding patterns that the versatility or proteins and the polypeptide chains can be determined in the formation of structures adapted for different roles. Many proteins contain more than one subunit, or monomer. These may be multiple copies of the same polypeptide chain, or combinations of different polypeptide chains. The assembly of the subunits is called the quaternary structure[44].

Some common examples of proteins that have tertiary structures include the insulin protein and chlorophyll. Insulin is a polypeptide hormone produced naturally in the human body and has the ability to make our body's cells absorb glucose from the blood. The glucose is stored in the muscles and liver in the form of glycogen and it stops the body from using fat as a primary source of energy. If there is very little insulin being produced by the body, glucose is not taken up by most of the cells present in blood. When this happens, the body starts to use fat as the primary source of energy. Insulin is also responsible for controlling the signals to other body systems such as amino acid take up by body cells [45].

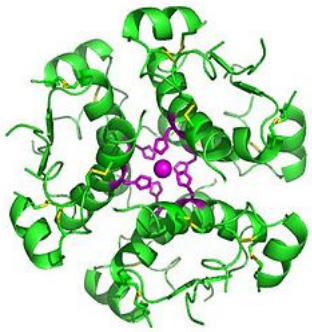


Figure 14: Structure of Insulin[30]

1.2.3 Protein Quantitation:

Accurate protein quantitation is essential to all experiments related to proteins in wide range of applications including molecular biology, cell biology, and forensic biology. Different methods have been developed to quantitate proteins in a given assay for the past, for the total protein content as well as for a single protein. Total protein quantitation methods comprise traditional methods such as the measurement of UV absorbance at 280 nm, Bicinchoninic Acid (BCA) and Bradford Assay, as well as alternative methods like Lowry or novel assays which have been readily developed. Individual protein quantitation methods include enzyme-linked immunosorbent (ELISA) assay, western blot analysis and more recently, mass spectrometry techniques.

Ultraviolet (UV) absorbance at 280 nm (range: 0.1-100 µg/ml)

Aromatic amino acids tyrosine and tryptophan give proteins the characteristic ultraviolet (UV) absorption spectrum at 280 nm[47], which is routinely used to estimate protein concentrations. Phenylalanine and disulphide bonds also contribute the absorption at that wavelength, although slightly. The method is simple and requires an extremely small sample volume since new spectrophotometers employ a sample retention system during the measurements. However the protein sample that is to be tested needs to be pure and must not contain any non-protein components [48].

Bicinchoninic Acid (BCA)

The Bicinchoninic Acid (BCA) assay was invented in 1985 and it employs the same principle as the Lowry assay which is the conversion of Cu^{+2} to Cu^{+1} under alkaline conditions. This reaction is influenced by four amino acid residues which are cysteine, cystine, tyrosine and tryptophan along with the peptide backbone. BCA is a specific chromogenic reagent for Cu^{+1} and in the second step of the reaction; two BCA molecules react with one Cu^{+1} ion. The amount of Cu^{+2} reduced is a function of protein concentration that can be determined spectrophotometrically by a colour change of the sample solution into purple. The absorbance is directly proportional to the amount of protein present in the solution and it can be estimated by comparison with a known protein standard[50].

The BCA assay is more tolerant to a range of ionic and non-ionic detergents such as Triton X-100 and denaturing agents such as urea[33]. These compounds tend to interfere with other colorimetric protein assays such as the Lowry assay. Some chemical molecules, such as reducing sugars can still interfere with the BCA assay. The effects of these interferences can be eliminated or reduced through several methods like removing the interfering substance through gel filtration or if the protein concentration is high enough, by diluting the sample[50].

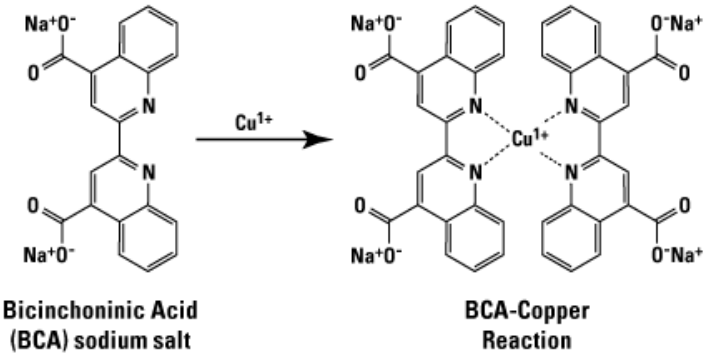


Figure 15: BCA Assay Reaction[50]

Bradford Assay

Bradford assay is one of the popular methods that arecurrently being used for determining protein concentrations. It relies on the formation of a complex between Coomassie brilliant blue G-250 dye and proteins in a solution. The dye binds to proteins and has an absorbance at a wavelength of approximately 590 nm. The protein concentration can be evaluated by determining the amount of dye in the blue ionic forms and thus measuring the absorbance of the solution at 595 nm using a spectrophotometer. The dye binds mostly to arginine, tryptophan, tyrosine, histidine and phenylalanine residues of the protein[51].

An advantage of using this method is that its compatibility with reducing agents that are used to stabilize proteins in a solution is farm more than that of those used in the Lowry assay and up to some extent the BCA assay as well. The limitation of this particular method is the incompatibility with low concentrations of detergents, which are quite frequently used to solubilize the membrane proteins. However, similar to BCA assay, these can be removed using gel filtration or precipitation of the proteins using calcium phosphate. Another advantage that this technique possesses is the possibility to measure high molecular weight proteins since the dye does not bind to the peptides with low molecular weights[52].

Lowry Protein Assay

Lowry assay is based on two fundamental chemical reactions. The first reaction is the reduction of copper ions under alkaline condition, which forms a complex with peptide bonds (Biuret reaction). The second reaction is the reduction of Folin-Ciocalteu reagent by copper-peptide bond complex which subsequently causes a colour change of the solution into blue with an absorption range of 650 to 750 nm. The amount of protein in the sample can be estimated using a standard curve of a selected standard protein such as Bovine Serum Albumin (BSA)[53].

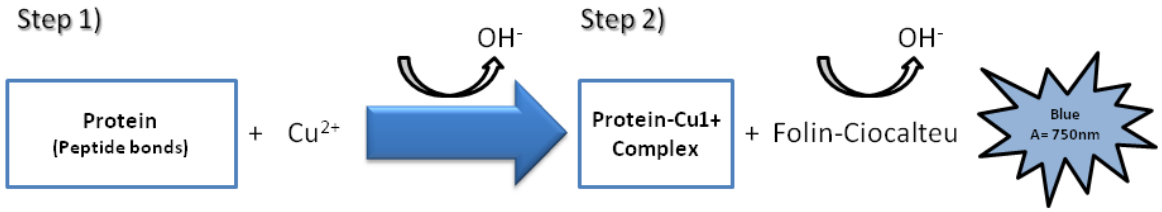


Figure 16: Lowry Assay[36]

The advantages of this assay are its sensitivity and most importantly, its accuracy. However, it requires more time than other assays and many compounds that are commonly used in buffers for protein preparation interfere with the assay and form precipitates. These interfering compounds include detergents, carbohydrates, glycerol, EDTA and Tris buffers. Nonetheless, the effects of these substances can be negated or to some extent minimised by diluting out the sample but only if the protein concentration is sufficiently high. It has also been shown by previous studies that the time to perform the assay can be reduced through raising the temperature or even by using a microwave oven[53].

1.2.4 Transition Metal Complexes and Colour Change Reactions

Transition metals are metallic elements that have incomplete d or f shells in their neutral states. They make up 56 from the total 103 elements on the periodic table. These

transition metals are classified into the d-block metals, which consists of 2d elements from Sc to Cu., 4d elements from La to Lu and actinoid elements from Ac to Lr. Although Sc and Y belong to the d-block, their properties are much more similar to lanthanoids[54].

Properties of d-block transition metals differ considerably between the first and the second series metals, although the difference in properties between the second and third series metals is not very different. Metallic radii of elements from Sc to Cu are significantly smaller than those of Y to Ag or those of La to Au[37]. Further metal compounds of the first series of transition metals are rarely 7-coordiante, whereas transition metals from the second and third series have the potential of being either 7 or 9 coordinate. Higher oxidation states in the second and third series transition metals are considerably more stable than those of the first series of the transition metals. Compounds in the first series of the transition metals in higher oxidation states are stronger oxidants and therefore are readily reduced. On the other hand, whereas M (II) and M (III) compounds are common among the first series of transition metals, these oxidation states are generally uncommon in compounds of the second and third series[54]. Metal carbonyl cluster compounds of first series of transition metals in low oxidation states exist but halide or sulphide cluster compounds are rare. In general, metal-metal complexes are much more easily formed in the 4d and 5d metals than in the 3d ones. Magnetic moments of the first series transition metal compounds can be explained in terms of spin-only values. Properties of the d-block elements are different not only in the upper and lower positions in the periodic table but also in the left and right groups[37]. The groups 3 to 5 metals are mostly referred to as early transition metals and they are generally oxophilic and halophilic. Smaller number of d electrons and the hardness of these elements explain their affinity toward hard oxygen and halogens. The d-block transition metals have s, p and d orbitals and those with n electrons in the d orbitals are termed ions with a d^8 configuration[54].

Compounds of metal ions coordinated by ligands are referred to as metal complexes. Most ligands are neutral or anionic substances but cationic ones, such as tropylium cation are also known. Neutral ligands such as ammonia or carbon monoxide are independently stable molecules in their free states whereas anionic ligands, such as Cl^- or $C_2H_5^-$ are stabilized only when they are coordinated to central metals. Those ligands with single ligating atoms are called monodentate ligands and those with more than one ligating atoms are referred to as polydentate ligands which are also known as chelate ligands. The number of atoms bonded to a central metal is the coordination number [55].

Molecular compounds which consist of d-block transition metals and ligands are referred to as complexes or coordination compounds. The coordination number is determined by the size of the central metal, the number of d-electrons or steric effects arising from ligands. Complexes with coordination numbers between 2 and 9 are known. In particular, 4 to 6 coordination numbers are the most stable electronically and geometrically [55].

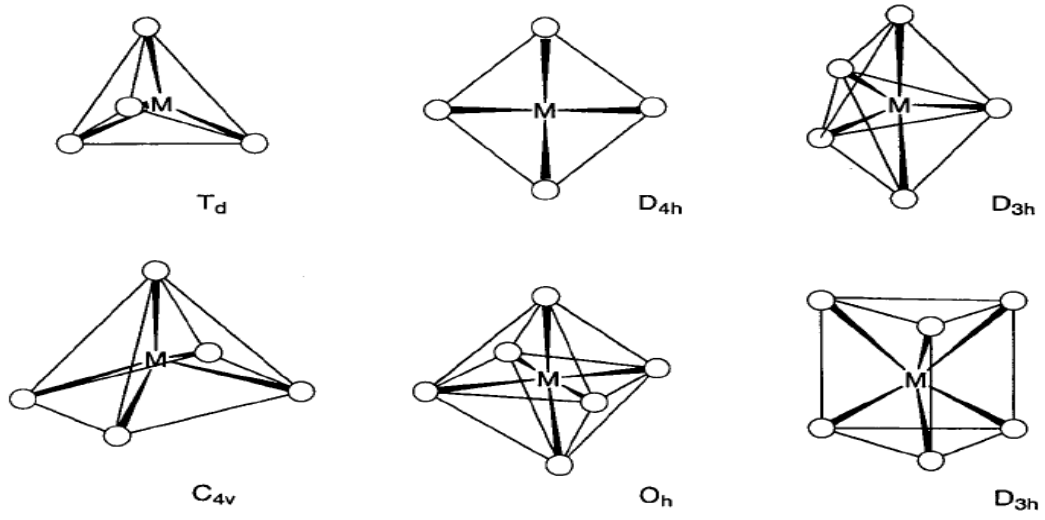


Figure 17: Structure of 4-6 coordination[55]

1.2.5 Application of Transition Metals

The particular property of transition metals enables them to be used in a wide variety of applications. As well as having electrical conductivity, the transition elements can be used in the production of electrical energy through their chemical reactivity. One of the most common examples of such an application is the dry cell battery. Any number of chemical reactions may be usedin this particular scenario and as a consequence, metals such as manganese, nickel, zinc, silver, cadmium or mercury may be found in dry cells [56].

The magnetic properties of transition metals are also of great commercial importance. A common example is that of magnetic recording used in floppy discs and hard discs along with similar processes in the magnetic recording of tapes. Small high-intensity permanent magnets are important in the construction of compact yet powerful motors such as those used in car windows. A quite different use of the magnetic properties of these metal ions is provided by the use of lanthanide ions. Eu^{3+}ions are used to separate signals obtained in NMR measurements and Gd^{3+} to enhance the contrast of images obtained using an MRI [58].

The unique property of transition metals can be used in a variety of catalytic processes. An everyday example of such a process is the heterogeneous catalyst used in car exhausts. This contains a platinum/rhodium alloy supported on a ceramic matrix and converts the mixture of oxygen, carbon monoxide, hydrocarbons and oxides of nitrogen in the exhaust gases to water, carbon dioxide and nitrogen. An important modern example of homogenous catalysis is provided in the Monsanto process in which the rhodium compound catalyses a reaction, resulting in the addition of carbon monoxide to methanol form ethanoic acid [59].

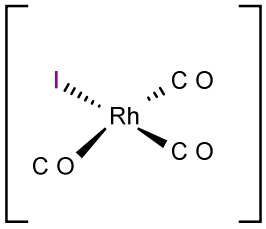


Figure 18: Rhodium Complex used in the Mosanto process[40]

Some f-block elements are also used in various organic processes. A number of the transition metals are essential trace elements for living organisms, hence the possibility of the use of transition metals have been exploited for medicinal purposes and treatment deficiency studies. For example, iron containing compounds can be used to treat anaemia and the use of dietary supplements containing cobalt in the form of vitamin B12. The reverse of this type of treatment involves the removal of excess metal ions from the body using compounds sometimes referred to as sequestering agents. Other important therapeutic uses of transition metal compounds include the treatment of cancer using platinum drugs such as carboplatin and rheumatoid arthritis using gold compounds such as auranofin [59].

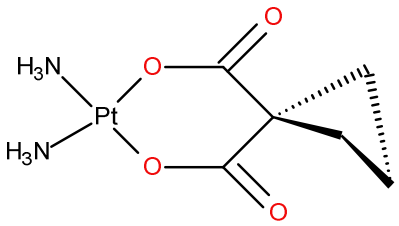


Figure 19: Carboplatin[40]

1.2.6 Copper Chemistry

Copper has a single s electron outside the filled 3d shell and it is for that particular reason that the +1 oxidation state is significant. The relative stabilities of Cu^{+1} and Cu^{+2} in aqueous media are strongly dependent on concentration and particularly the anions and ligands present.

$$2\ Cu^{I} \leftrightarrow Cu^{0} + Cu^{II}$$

This particular reaction is known as a disproportionation reaction. The equilibrium is pushed to the left by polarisable ligands or those capable of π bonding, such as CN^- or thioethers. With anions which are capable of covalent bonding or forming bridges, or with ligands which form particularly stable complexes with Cu^{II}, the equilibrium shifts to the right[59].

Copper (I) complexes with halides and other simple ligands have a wide variety of structures ranging from mononuclear to dinuclear chains to tetranuclear structures. As well as CuI, insoluble CuBr and CuCl can be made in aqueous solution. They are easily prepared by reducing a solution containing a mixture of Copper (II) Sulphate and sodium bromide or chloride with a stream of SO_2. CuF is known to be potentially soluble in water and cannot be prepared in the similar way the other halide compounds of copper are produced. It is unknown at room temperature but can be made by heating CuF_2 to about 1000°C[59].

$$CuF_2\ (s) = CuF(s) + \frac{1}{2}F_2(g)$$

On cooling this, it decomposes by disproportionation to the following products:

$$2\ CuF\ (s) = \ CuF_2(s) + Cu\ (s)$$

Copper (I) forms alkene and alkyne complexes in which the bonding from the hydrocarbon to the metal is predominantly σ donation from the π bonding orbitals to an acceptor orbital on the copper. Copper (I) ammine complexes react with monoacetylenes, to give oligonuclear important reagents in the synthesis of a variety of organic acetylenic compounds by reaction with aryls and other halides and be a key intermediate in the oxidative dimerization of monoacetylenes[59].

1.2.7 Colour Change Reactions

Transition metal complexes are involved in reaction that produce products that are coloured or may be involved in reactions that result is a change of colour in the final product. When light passes through a solution containing transition metal complexes, the colour that the solution becomes visible at is the light from the spectrum that is being transmitted. For example, most of the Cu (II) complexes appear to be blue in colour. This is because when light passes through the solution containing a Cu (II) complex, absorption occurs in the orang-red-yellow portion of the spectrum and green-blue-violet wavelengths are transmitted giving it the blue colour [58].

Ion	Colour
Copper (II)	Blue
Cobalt (II)	Red
Manganese (II)	Pale Pink
Iron (II)	Green
Iron (III)	Orange
Vanadium (II)	Purple
Vanadium (III)	Green
Vanadium (IV)	Blue
Vanadium (V)	Yellow

Table 2: Transition metal ions and their corresponding colours[58]

The absorption band corresponds to the energy that is required to excite and electron from the ground state to excited state [57].

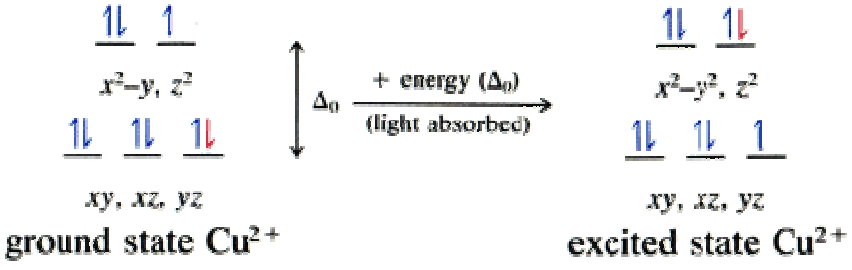


Figure 20: Excitation of Electrons[42]

1.2.8 Current Colour Test Techniques and Uses

- **Biuret Test**

Compounds which contain two carbonyl groups ($CONH_2$) joined either directly together or through a single atom of carbon and nitrogen. Proteins and peptides which contain these groups therefore give a positive biuret test whereas free amino acids do not[60].

Reagents used: 10% NaOH, 1.0% $CuSO_4$.

Quantitation of Milk serum Proteins[60]:

The biuret reaction was applied to quantitative colorimetric test for serum protein solutions. The method is simple and rapid, the solutions are free of turbidity and the response is nearly linear. The complete development of the Cu-protein complex took 30 minutes to develop after which the colour was reported to be stable for up to 24 hours. Lactose and calcium phosphate were found to interfere in direct application of the test for whey. Dialysis of the whey was performed as it was necessary to obtain normalized values. The colour response of casein was seen to be different than that for milk serum proteins therefore the study concluded that a mixture of proteins could not be analysed using the biuret test [60].

- **Xanthoproteic Test**

Certain substituted benzene compounds react with nitric acid to form yellow nitro or nitroso compounds which are more deeply coloured in alkalis, because of their behaviour as indicators. Tyrosine, phenylalanine and tryptophan contain substituted benzene rings and are responsible for the positive test given by most proteins[61].

Reagents used: Concentrated HNO_3, 20% NaOH[61].

- **Millons Test**

Phenols yield mercury derivatives when they are heated with mercuric salts. In the presence of nitric acid, mercury derivatives are nitrated to form a brick red coloured complex. The phenol found in proteins is tyrosine. Proteins which contain this amino acid will give a positive Millons test[62].

Reagents used: Mercury, Concentrated HNO3, Water, 0.5% Phenol solution.

Quantitation of Plasma Tyrosine levels[62]:

This study was carried out on premature babies and was aimed at understanding the way premature babies metabolize tyrosine in different ways from that of older and mature adults. The babies were fed different formulae under the guidance of the attending physician and the amount given was recorded at each feed. The protein intake was estimated on the basis of the manufacturer's guidelines. Blood was obtained by femoral punctures and collected into oxalated bottles. The cells and plasma were separated and the plasma was stored by freezing. Millons test was performed on the urine samples also collected and showed the presence of tyrosine. These results proved interesting and the test was used to compare the levels of tyrosine in the plasma which had been collected and stored earlier. It was seen that negative and trace reactions with Millons test were associated with plasma tyrosine levels below 10mg/100ml in all but 3 instances. With plasma, tyrosine levels above 10mg/100ml, the Millons reaction was nearly always positive. It was concluded from this study that a negative or trace Millons test on the urine nearly always indicated a plasma tyrosine level of less than 10mg/100ml but that no reliable information about the plasma tyrosine levels could be inferred from a positive Millons Test [62].

- **Hopkins Cole or Glyoxylic Acid Reaction**

When tryptophan reacts with glyoxylic acid (CHO-COOH), a purple colour product is formed. Proteins containing tryptophan give a positive test. Gelatin lacking tryptophan will not give a coloured ring[63].

Reagents used: Hopkins Cole Reagent, Oxalic Acid, Magnesium powder, Glacial Acetic Acid, Concentrated H_2SO_4.

Methods of Tryptophan Analysis[64]:

Tryptophan is a vital constituent of proteins. Its destruction can be associated to off flavour in irradiated foods and with yellowing of wool. Many methods have been tried for determining tryptophan specifically in the presence of other amino acids and constituents of biological products. Most amino acids can be determined accurately by ion-exchange or gas chromatographic analysis of protein hydrolysates. Tryptophan however is destroyed by an acid hydrolysis even under conditions which may be optimum for other amino acids. The Hopkins-Cole Reaction is one of the earliest methods that have been used to determine the quantity of tryptophan in proteins. The procedure demonstrates the use of glyoxylic acid as an essential reagent in the glacial acetic acid test for indoles. The tryptophan was precipitated as a mercury salt and then re-dissolved and treated with glyoxylic acid to take advantage of the indole ring, resulting in the development of a coloured compound. This compound was then analysed spectrophotometrically and a quantitative value was achieved for tryptophan[64].

- **Amino Acid Sulphur Test**

When proteins are heated in strongly basic solutions, sulphur in the protein molecule will react to form sulphides. The sulphide can then be detected by the formation of lead sulphide upon the addition of lead acetate. The amino acid cysteine and cysteine are responsible for a positive sulphur test in proteins. This test is also positive for albumin.

Reagent Used: 10% NaOH, 5.0% Lead Acetate.

2.0Materials and Methods

2.1Raman Spectroscopy

The instrument was initially calibrated by varying the power of the laser as well as the exposure time and average count. These needed to be calibrated in order to obtain the optimum conditions that could be used for blood analysis. The type of surface that was used for analysis was also tested. Numerous surfaces were used including a plain microscope slide (Fisher Scientific), a microscope slide covered in aluminium foil, a microscope slide scratched and roughened up by using a glass cutter and sandpaper.

The laser power was tested between 30mW to 300mW. Initially, powdered Paracetamol (4-Acetamidophenol) acquired from a local pharmacy was used. The pellet was physically ground to a powder using a pestle and mortar. The resulting powder was placed on a microscope slide directly under the Raman Spectroscope (1064nm Xantus-1). The Paracetamol sample was used to test other variables such as the exposure time and average count. A number of different spectra were produced that can be referred to in the appendix section. From these tests, the optimum conditions were found out to be, laser power 300mW, exposure time 1000ms and average count of 1.

After confirming the optimum conditions, the project was progressed to the use of blood samples. De-fibrinated equine blood (Fisher Scientific) was used as this could be acquired easily and in large quantities. Quantities of up to 5 ml were used. Initially, liquid blood was used to attain a standard reference spectrum which was run on the optimum condition discovered by the Paracetamol runs. 2 ml ofblood was placed in a glass vial and inserted in to the dark chamber of the spectroscope and the sample was run. In order to acquire a much more specific spectrum for haemoglobin, the horse blood was centrifuged at 12000 RPM for approximately 25 minutes, in order to separate the plasma from the red cells. The red cell concentrate was then removed and placed on a microscope slide and examined under the spectroscope (1064nm Xantus-1). In order to test the surface interference on our readings, all the surfaces mentioned earlier were tested and results presented later in the results section.

Human haemoglobin powder (Sigma Aldrich UK) was used in order to attain a spectrum for human haemoglobin. This was done as a reference so that when the human blood was run, there would be a reference to which the spectrum can be matched. The human haemoglobin was purchased as an anhydrous powder form and was dissolved in distilled water to form solutions of different concentrations ranging from 20% to 80%. These concentrations were run and the resulting spectra were recorded.

For human blood, samples were donated by volunteers abiding by the stipulations stated in the ethical approval document. A uni-stick prick method was adopted where by the finger was pricked and a few drops of blood were collected on the different surfaces mentioned earlier. The blood was tested initially to see if a spectrum matching the previously acquired spectrum of human haemoglobin could be attained. The study was further extended by ageing the blood stain. This was done by leaving the blood in normal room conditions (temperature of approximately 20^{o}C) for a fixed amount of time. The

sample was then run under the spectroscope too see any changes in peaks that might occur over time.

2.1.1Equipment:

The equipment used was provided by the department and consisted mostly of analytical instrumentation. The Raman Spectrometer was provided by Rigaku and is a 1064nm Xantus-1 Handheld Raman Analyser.

Figure 21: 1064 nm Rigaku Xantus Raman Handheld Analyser

The Raman Spectrometer of 785nm wavelength was also provided by the same company and is a Xantus-1 785 nm Handheld Raman Analyser.

Figure 22: 785 nm Rigaku Xantus Raman Handheld Analyser

Several attachments were also provided with the Raman Analysers that provided us with the ability to measure our samples in different forms as well as in different quantities.

Figure 23: Raman Analyser Tube Holder

The tube holder shown above encases a glass vial which in turn allows the analysis of liquid samples to be possible. Large quantities of powders can also be analysed. These attachments came as a standard for both the analysers that were used.

Both the Raman Analysers mentioned above can be used as individual, portable devices as well as being mounted on microscope stands for singular position analysis. The preferred method used by myself was attaching the analyser to a microscope stand. This provided the most stability while analysing the samples and negated some of the human error that may be cause during the process.

Figure 24: Raman Spectrometer on a fixed stand

The sample is placed on the lower end of the tray on which the Raman Spectrometer is then pressed on making sure that the area of our sample is completely covered by the laser emission hole. This minimises the risk of any external fluorescence from entering the analyser and interfering with our results.

As an individual device, the analyser provides us with the option of attaching a tube holder towards the laser emitter end of the instrument. This provides us with the option of analysing liquid samples as well as the option of analysing samples directly from the surface.

Instrument Background

Recent years have seen a growing use of Raman in the study of biological systems. With advances in laser sources, Rayleigh rejection filters, and detector technology, the weak Raman signal is easier to detect and analyse. Raman spectroscopy is currently being used for such applications as tissue diagnostics, blood analyte detection, and cellular examination, among others. The greatest benefit of using this technique is due to its sensitivity to very small molecular changes as well as its capability for non-invasive sensing. In addition, Raman offers generally narrow bandwidths, minimal sample preparation, and is easily integrated with microscopes to provide very small spatial resolution[65].

When light comes into contact with any surface or object, some of it gets absorbed while the rest is scattered. This scattering can be either inelastic (Rayleigh) or elastic (Raman). The inelastically scattered light undergoes no transfer of energy and therefore has the same wavelength as the incident light. The elastically scattered light does exchange energy with the atoms of the scattering material and thus the wavelength of this scattered light is shifted by some certain amount. This shift is known as the Raman shift after its

discoverer C. V. Raman. It is an excellent tool for probing the molecular structure of the material [66].

Theoretical Description

By considering the bound atoms in a molecule as point nuclei and the bonds between them as springs it can be shown that there are 3N-5 possible vibrational modes (3N-6 for linear molecules) where N is the number of atoms. The assumption is that the molecule's diameter is much less that the wavelength of the incident monochromatic light. The field then induces a dipole moment in the molecule and as such a polarization given by the following equation[67].

$$P(t) = \alpha E(t) \qquad (Eq\ 1)$$

The incident field can be written as a simple oscillating function with peak amplitude $E0$ and a frequency v_{inc}. The response of the atoms will be to displace from their equilibrium positions.

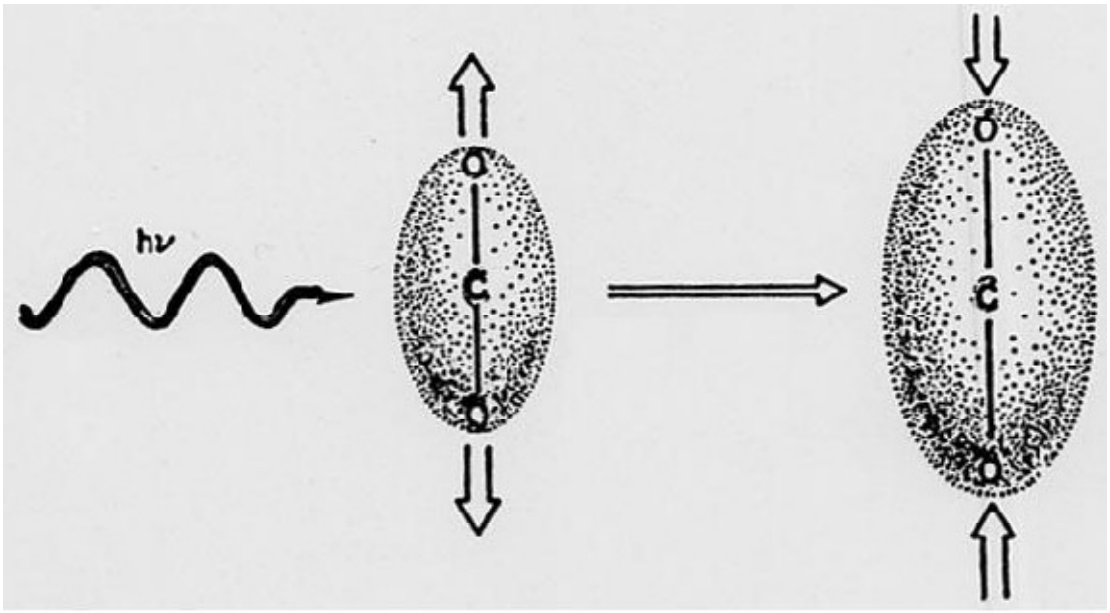


Figure 25: Light Induced Polarisation[66]

$$E(t) = E_o \cos(2\pi v_{inc} t) \qquad (Eq\ 2)$$

The atoms' displacement can be described by a normal coordinate q_j. The mode is assumed to oscillate although not necessarily with the same frequency[26].

$$q_j(t) = q_j^{(o)} \cos(2\pi\nu_j t) \qquad \text{(Eq 3)}$$

The induced dipole moment due to the vibrational modes is then given by:

$$\alpha = \alpha_o + \left(\frac{\delta\alpha}{\delta q_j}\right) q_j + \ldots \qquad \text{(Eq 4)}$$

Simply substituting these equations

$$P(t) = \left[\alpha_o + \left(\frac{\delta\alpha}{\delta q_j}\right) q_j\right] E_o \cos(2\pi\nu_{inc} t) \qquad \text{(Eq 5)}$$

$$P(t) = \alpha_o E_o \cos(2\pi\nu_{inc} t) + \left(\frac{\delta\alpha}{\delta q_j}\right) q_j^{(o)} \cos(2\pi\nu_j t) E_o \cos(2\pi\nu_{inc} t) \qquad \text{(Eq 6)}$$

Equations 1-6 show that the response of the molecule to the incident radiation has a term that responds with the same frequency as that radiation. This term corresponds to Rayleigh scattering. The next, much weaker term, is a frequency response that reacts with two components, one as the sum of the two frequencies and one as the difference. These correspond to the anti-Stokes Raman scattering and the Stokes Raman scattering respectively[67].

Although this derivation is classical and therefore incomplete, it does highlight most of the important features. First, both the Rayleigh and Raman scattering are linear with input intensity. Second, only vibrations that affect the polarizability exhibit Raman scattering. Finally, it shows that the Raman shift can be either positive or negative. All of these same features are derived from the quantum description as well many others[67].

Although the classical description of light scattering does reveal most of the important characteristics that are necessary to make use of the phenomena for spectroscopy, it is limited because it does not take into account the quantum nature of molecular systems[66].

The incident radiation is treated as a changein the Eigenstates of the molecule. This deviation creates virtual vibrational states (as opposed to electronic states). The incident photon excites the ground state. This state can decay back down to the original state with no net change in energy (Rayleigh scattering.) The state can also decay to a state just above or below the original resulting in an emitted photon that has either slightly more or less energy. This energy difference in the emitted photon is then due to the spread in energy level within the vibrational level and corresponds to the Raman shift[66].

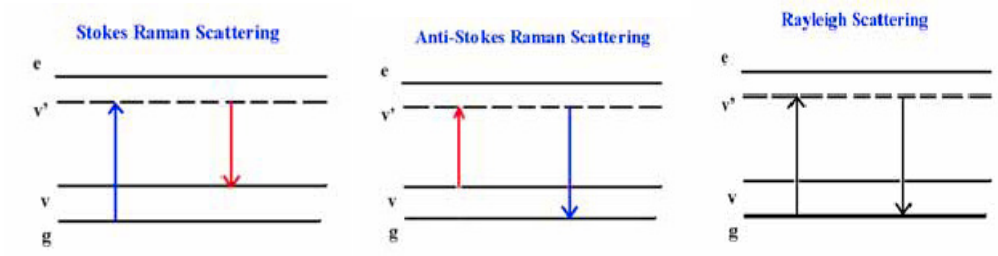


Figure 26: Stokes, Anti Stokes and Rayleigh scattering[66]

Micro-Raman Spectroscopy

Combining a Raman set up with a microscope allows for the spectroscopic sampling of very small volumes. Because the volumes are small, very little Raman signal is necessary for detection and therefore the power of the input source can be kept low. This is an ideal tool for biological systems where the targets are small and optical damage can occur at very low laser power levels. Keeping the sample undamaged is a requirement for repeated experiments but also to study dynamic variables in a biological system[68].

The laser and detector are on the same side of the sample at a 180 degree plane. A beam splitter is used to insert the laser into the collection axis. The backscattered light reflects from the sample and then passes through the beam splitter to the detector. The spatial resolution of the system is then limited by the laser and objective lens[68].

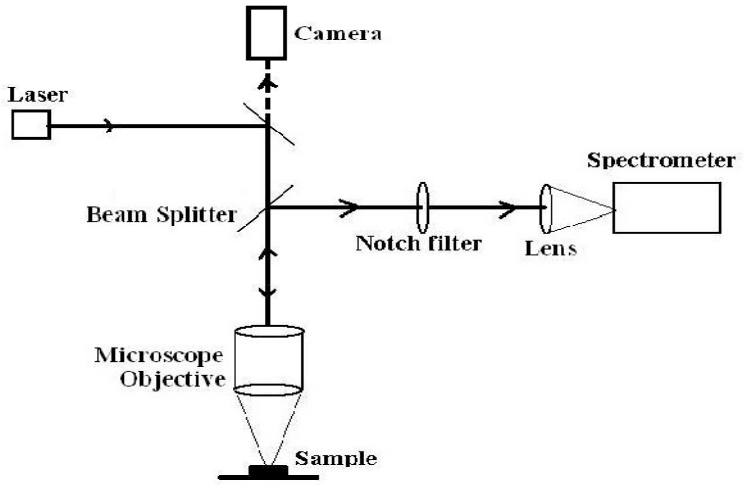


Figure 27: Micro Raman setup schematic[68]

Using a Raman microscope the sample can simply be placed on a three dimensionally translational stage and the portion of the samples that is of interest is selected optically through the viewing system of the microscope. With micro-Raman the vibrational spectra can be measured form micron-sized particles which makes it well suited as an analytical tool in chemistry and biotechnology. Raman micro-spectroscopy has proven to be an informative and non-destructive technique[69].

Surface Enhanced Raman Spectroscopy (SERS)

Surface Enhanced Raman Spectroscopy (SERS) is a Raman Spectroscopy technique that provides greatly enhanced Raman signal from Raman-active analyte molecules that have been adsorbed onto a certain specially prepared metal surface. The importance of SERS is that it is both surface-selective and highly sensitive whereas conventional Raman Spectroscopy is neither [79]. SERS is used primarily for analytes adsorbed onto surfaces (Gold, Silver and Copper) or alkali (Lithium, Sodium and Potassium) metal surfaces with the excitation wavelength near or in the visible region.

This particular surface selectivity and sensitivity extends Raman Spectroscopy utility to wide range interfacial systems previously in accessible to Raman Spectroscopy as it is not

surface sensitive which include in-situ and ambient analysis of electrochemical, catalytical, biological, and organic systems [79].

In recent years, the utilization of charged coupled detectors (CCD), confocal microscopes and notch filter in the Raman Spectrometer design have provided with an increased sensitivity together with much more detailed and accurate description for the scatter peaks. Together with SERS, this technique enables us to study the single molecule Raman Spectroscopy [79].

2.2 Small Angles X-Ray Spectroscopy

For SAXS, samples in liquid form were used to attain any information that could be gathered regarding their identity and to see any changes that could take place over time. Initially, Bovine Serum Albumin (Sigma Aldrich UK) in solution having a concentration of 1% by weight was used to be run as a standard. The solution was acquired from the biological sciences department from the University of Huddersfield. This BSA solution had already been pre-made to 1% as this was being used as a standard for some other projects. The solution was placed in a glass capillary tube (Bruker) and tested.

Equine blood (Fisher Scientific) was used in extremely small volumes (approximately 5µl) and was placed in the capillary for analysis. This sample was initially run to see if a scatter pattern could be attained and later was used in the age estimation experiments.

Human blood was acquired using the same method used in Raman Spectroscopy. A uni-stick was used to prick the skin and blood was collected directly from the source by means of the glass capillary (Bruker). This was then run on the machine and the same sample was saved for further use in the age determination section of the project. .

Instrumentation

A dedicated 2D SAXS system typically has a long primary beam path for modifying the beam, a third pinfole accurately aligned to remove unecessary scatter, and a long sample to detector chamber to enhance angular resolution. The primary beam path, secondary beam path and the sample chamber are all kept under vaccuum conditions to remove any scatter that can come forward due to the presence of air. The 2D detector is the most important and essentail component in the whole system. The detector needs to have a large active area, good spatial resolution and very low noise level[84].

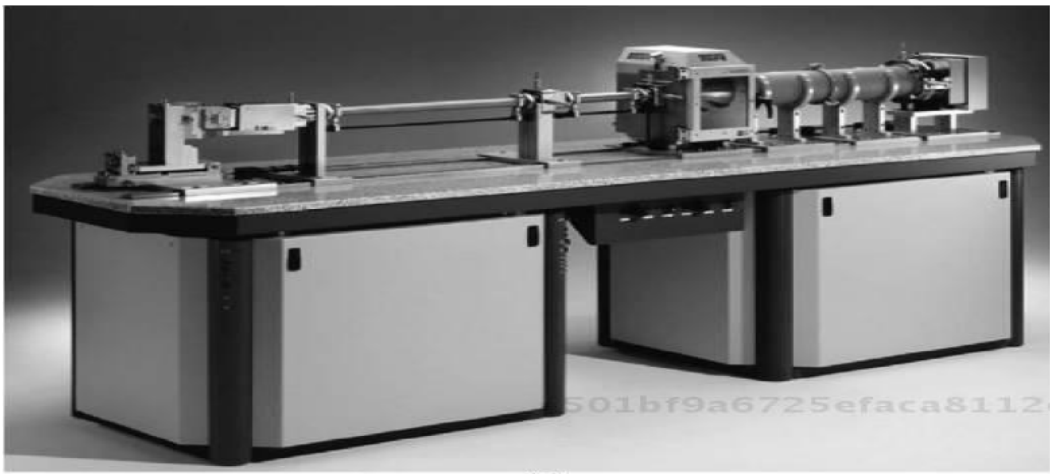


Figure 28: 2D SAXS system – Side view

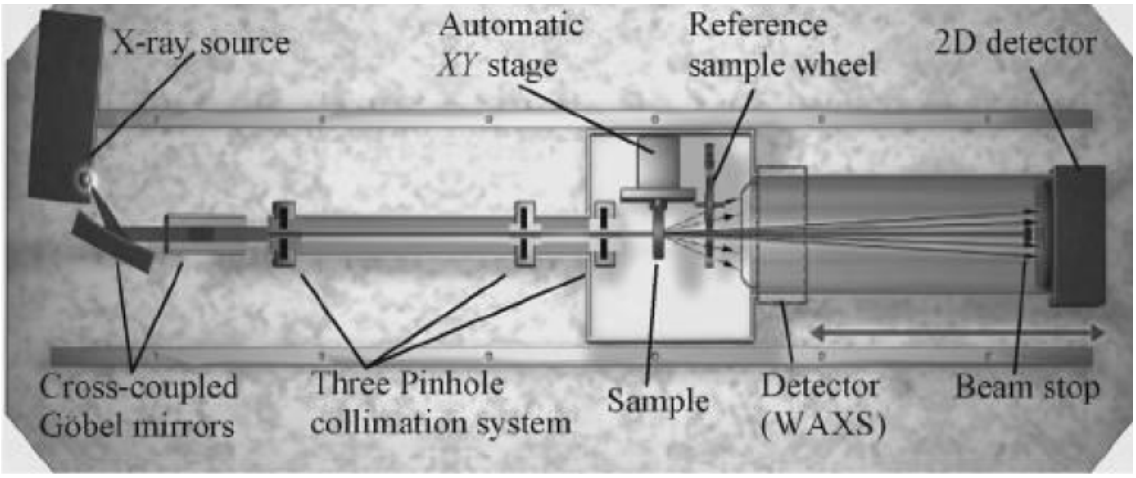


Figure 29: 2D SAXS system – Top view showing the complete beam path

The figure above show a representation of a 2D SAXS system, similar to the one used by us in the current project provided by Bruker. The types of X-Ray source can either be a fine focus sealed tube or a rotating anode generator. Two parabolic shaped mirrors and the cross coupled in the K-B scheme monochromatize the X-Rays to Kα radiation and condition the divergent X-Rays from the source into a two dimensional parallel beam. Due to the low Bragg angles of the mirrors and the cross coupled modification, the polarization of the primary beam is negligible. The large space between the first and the second pinholes results in a parallel beam with very low divergence. The third pinhole also known as the anti-scatter pinhole consists of an aperture much larger than the first and second pinholes. When this is properly aligned, no direct beam reaches the third pinhole therefore no scatter is generated from the direct beam by the third pinhole. The basic and main function of the third pinhole is to block the scatter that might have been created from the second pinhole[85].

The sample is mounted on the XY stage which has the capability of allowing several samples to be loaded in the chamber at the same time. The XY stage can oscillate the sample while collecting data to improve the sampling statistics. The stage also allows the sample to be scanned through the X-Ray beam during data collection[85].

Instrument Background

Small Angle X-Ray Scattering (SAXS) and Small Angle Neutron Scattering (SANS) is a widely used diffraction technique used to study the structural characteristics of matter. This method of elastic scattering is used in various branches of science and is commonly used to investigate the spatial structure of matter based on wave diffraction phenomenon. A common example of this technique is the determination of the atomic structures of crystals i.e. the arrangement of atoms in a unit cell[85].

In SAXS, the wavelength used is on the order of several Angstroms. The most important feature of this technique is its potential for analysing the inner structure of disordered systems and therefore provides a detailed sketch on the structural information of systems that have a random arrangement[85].

Theory

X-Rays are electromagnetic waves similar to visible light but having a much shorter wavelength. The waves propagate, because an alternating electric field causes and alternating magnetic field and vice versa. The electric field, magnetic field and the direction of propagation are always at right angles to one another.

There are two main interactions that take place between X-Rays and matter which are namely absorption and scattering. When an X-Ray hits a material, some of it will be absorbed by the material as it passes through it and is transformed into other forms of energy such as heat, radiation, fluorescence etc. The other fraction will be scattered into other direction or propagations[86].

Irradiation of an atom with X-Rays can result in the expulsion of an electron from the atom. In doing so, the energy from the X-Ray is used up and the photon is said to have been absorbed. This in turn, leaves the atom in an unstable form with a vacant shell where an electron previously resided hence leaving the atom to restore to its original configuration. This is achieved by the atom by re-arranging its other remaining electrons in such a way as to fill the hole that had been caused by the vacating electron initially. As a result of this, the atom emits fluorescent radiation. The absorption of an X-Ray photon is most efficient at the absorption edges. This is the region from where the electrons have the highest probability to be expelled. Absorption occurs at all wavelengths with various efficiencies. Depending on the atomic species of the material and the wavelength, these absorption efficiencies can be referred to as mass-absorption coefficients. In order to obtain a high

quality SAXS data, the absorption needs to be kept to a minimum. The optimum sample thickness d_{opt} depends on the linear absorption coefficient[86],

$$d_{opt} = \frac{1}{\mu}$$

Scattering is another form of interaction that occurs between and X-Ray and matter. It can occur with or without the loss of energy. This means that the scattered radiation can have a different wavelength than the incident wavelength. This sort of scattering is referred to as Compton Scattering. When both the scattered radiation wavelength and the incident wavelength are the same, the scattering is referred to as Rayleigh and Thomson scattering or elastic scattering[86].

Compton scattering takes place when a photon hits an electron and is subsequently bounced away. The photon loses a fraction of its energy which is taken over by the electron. The scattered radiation results in having a different wavelength and has no particular phase relationship to the incident radiation[86].

Rayleigh and Thomson scattering occurs when photons collide with strongly bonded electrons without any energy transfer. The electrons start oscillating at the same frequency as the incoming radiation. Due to this oscillation, the electrons emit radiation that has the same frequency as the incoming radiation. Because the emitted wave of neighbour atoms oscillate in sync to each other, they result in producing coherent waves which have the ability to interfere at the detector. These interference patterns provide information about the structure of the particle[86].

This technique observes the clear scattering from a sample as a function of the electron distribution in the sample. The resulting scattering pattern or the scattering intensity distribution pattern contains information about the particle size, size distribution, particle

shapes, orientation and other structural information. Small Angle X Ray Scattering can also provide information such as lamellar repeat distance, radius of gyration, large scale functions, and the arrangement of a column structure, its diameter and the distance between the columns[86].

Current Research

Numerous researches has been carried out using SAXS in the fields of biological sciences and chemical sciences all ranging from protein structure determination to the analysis of bones and in chemistry, the structural changes in bonds and crystal structures in reactions.

- **Proteins in solution[87]**

The main purpose of structural studies for molecular biology include identifying structural changes and changes of biological macromolecules along with correlating these changes to their biological functions. Over the past few years, there have been discoveries of a large number of biological molecules using techniques such as X-Ray crystallography and nuclear magnetic resonance. However, these high resolution methods have their own limitations and therefore can be applied only when rather specific conditions are met. For example, a structure determined by X-Ray crystallography requires high quality protein crystals which are extremely complex and costly to produce and their preparation alone is one of the major disadvantages. The recent advancements in the development of X-ray scattering instruments allow simultaneous measurements of small and wide angle X- Ray scattering from proteins in solution. Usually SAXS is used to determine the tertiary and quarternary structures by investigating the overall size and shape. Due to the limited information in the relatively small q region, proves to be an issue to the capability to restore 3-D structures. Experimental set ups with simultaneous and continuous q range measurements directly probe distance correlations on length scales that are small

compared to the overall protein dimensions and may contain rich information of fine-structure details of proteins in solution. Scattering data in the higher q region is sensitive to protein conformation, and also enables scattering patterns to be compared quantitatively with calculated patterns from detailed structure models. SAXS data can be used as additional information input for the evaluation of NMR data as well[878991].

- **Cancer Cells[92]**

Breast cancer is a very frequent form of cancer and the leading cause of cancer death in women. The lifetime risk of a woman developing breast cancer is as high as 10-13%[78] in the urban world. Currently diagnosis is based on a process known as triple assessment. This is a combination of physical examination, mammography using X-Ray or ultrasound, and fine needle aspiration cytology or core biopsy. The histo-pathological assessment relies on changes to cellular morphology and tissue structure. Although triple assessment is effective in detecting most malignant lesions, its sub-optimal specificity means that in some cases open biopsy surgery is required to exclude malignancy. There is therefore a considerable interest in developing diagnostic tools that are specific for the presence of malignant breast tissue. Each tissue in the breast has its own small angle scattering pattern which is directly related to its structure[92 93]. In particular, SAXS is an essential tool for studying the supramolecular arrangement for the collagen fibrils which is the main component of the connective tissues. The formation of collagen accompanies the development of breast tumours, either benign or malignant. The supramolecular arrangement of collagen fibrils degrade upon cancer invasion and it can be revealed by SAXS. Scattering characterisation of the tissues and their pathologies is therefore possible. A SAXS pattern not only carries information about the comparison of the sample, but also about the pathology of the tissues. A SAXS pattern not only carries information about the composition of the sample, but also the pathology of the tissue, hence it can

help identify the presence of the cancer cells and the amount of area that they have spread to[84].

Advantages and Disadvantages

Small Angle X-Ray Scattering presents us with a unique set of both advantages and disadvantages when compared to other similar techniques currently available. One of the biggest advantages with this technique is that small angle scattering system set up does not require the sample to be in a crystal medium. This means that the sample can be presented in a liquid medium and would still be suitable to analyse. Since small angle scattering provides information without the need of requiring repeated structure, there is no need to obtain a near perfect crystal structure. This makes the method quite useful for substances that are in liquid from such as blood and other fluids. This also reduces the risk of any contamination that might take place when samples are crystalized and reduces the risk of invasiveness of different reagents on the delicate aspects of biological samples such as DNA and other nuclear material. Another very important advantage that small angle scattering possesses is its ability solutions. This is something that cannot be achieved is a vacuum using other techniques such as SEM (Scanning Electron Microscopy)[96]. This is due to the fact that SAXS responds to the change in electron density and this in turn has no effect by the phase in which the sample is presented. While TEM (Transmission Electron Microscopy) can also be useful in this particular regard, SAXS is not as prone to damaging the sample through contact with an electron beam as provided by TEM. Plus with the introduction of synchrotron, SAXS has become even more useful for analysing RNA strands in this type of medium. Another advantage that SAXS commands is that it allows the analysis of much larger particles than many of its counterpart techniques such as crystallography and TEM. SAXS has the ability to measure particle sizes ranging from 5-25 nm with weakly repeating structures of up to 150 nm. The

implementation of synchrotron, as stated earlier, allows for even larger sizes to be measured through controlling the wavelength of the outgoing X-Ray via filtering devices[97].

Despite all these advantages, there are some disadvantages that have presented themselves while using this technique. Firstly, there is a loss of information over techniques like TEM. This is due to the fact that SAXS provides a more generalized view regarding the structure. Techniques like TEM provide information about individual grains by actually looking at them optically whereas SAXS provides information about the average particles. Another disadvantage to SAXS when comparing it to Small Angle Neutron Scattering (SANS) is the ability to control the scatter length density with a lot more ease. However this particular issue can be rectified by introducing systems that have filtering capabilities such as synchrotron[97].

2.3 Colorimetric Analysis/Spectrophotometry

Samples were collected from me along with volunteers that met the stipulations stated in the ethical approval agreement. Blood samples of sizes between 5-10 µl were collected by using a uni-stick pricking method and placed on the desired surface. These samples were tested both fresh and were tested over numerous time intervals to see if any changes could be detected. In order to investigate the effects of surfaces, different materials were used to test the blood sample. Initially, white filter paper (Fisher Scientific) was used as a standard surface. Variations were made and instead of a white filter paper, other surfaces such as cotton cloth, nylon cloth and silk cloth were also used. To test whether a change in colour to the surface had an effect on our readings, a piece of blue denim cloth was acquired and the blood sample was placed on it. All these material were provided by the in-house staff at the University of Huddersfield and nothing was purchased for this section.All samples run were collected in their raw data form and background noise was subtracted before analysis was done.

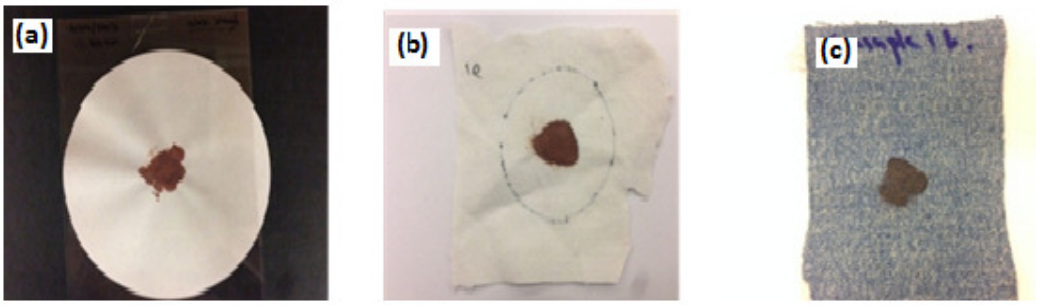


Figure 30:Blood Sample used for colorimetric analysis (a) white filter paper, (b) white cotton cloth and (c) blue denim

For this particular experiment, a Konica Minolta C-3700D spectrophotometer was used.

Figure 31: Konica Minolta C-3700D Spectrophotometer

This particular model has the following features:

- Illumination/viewing geometry – meets ISO and DIN standards for diffuse illumination/8^{o} viewing angle geometry and also conforms to CIE and ASTM standards for diffuse illumination/0^{o} viewing angle geometry.

- Pulsed Xenon lamp Light Source – provides high stability, long life and excellent repeatability on dark and high Chroma colours.
- 6-inch integrating sphere – has a powdered barium sulphate coating with superior optical characteristics.
- Double beam feedback system – monitors the light emitted by the xenon lamp and automatically compensates for changes in brightness or spectral characteristics.
- Silicon photodiode array sensor – quickly converts the light separated by the grating to electric currents.
- Changeable measurement areas – select measurement areas of 3x5mm.
- Variable UV – the amount of UV included in the illumination can be controlled in 1000 steps for measurements of fluorescent materials.
- Transmittance measurements – the spectral transmittance of liquids or of specimens in sheet or plate form can be measured using for diffuse illumination/0^{o} viewing angle geometry.

2.3.1 Instrument Background

The measure or matching of colours is frequently used as a quantitative method for the determination of materials which themselves are coloured or can be made to form coloured complexes by certain chemical reactions. Colorimetric analysis is concerned with the determination of the concentration of a substance based on relative absorption of light with respect to a known concentration of a substance. Most of the substances analysed in water are normally colourless and undetectable to the human eye. In order to test their presence and any other quantitative information, a spectrophotometer or colorimeter is used. This particular instrument is used to detect the presence of any colour in a seemingly colourless solution as well as having the capability to determine the subtle colour changes that can occur in already coloured systems[56].

A colorimeter or spectrophotometer can be used to photo-electrically measure the amount of coloured light absorbed by a coloured sample in reference to a colourless blank sample. The choice of having the correct wavelength for testing is extremely important for this technique to be effective. It is important to note that the wavelength that gives the most sensitivity for a test factor is the complimentary colour of the test sample[56].

As mentioned previously, this particular technique can also be used to measure subtle colour changes that can occur in samples over a certain period of time. The lightening or darkening of the already existing colour can provide useful information about certain chemical interactions that might be taking place in the sample being analysed. These changes can then be corresponded to the formation of new chemical compounds or the breakdown of the original chemical composition into several new products. This technique can be paired up with other chemical techniques that can provide further proof to these changes at a much more chemical level[57].

Theory

In colorimetric analysis, one of the most important aspects that need to be taken into account is the absorbance and the transmission of light [57].

$$Transmittance\ (T) = \frac{Light\ Transmitted\ (I)}{Incident\ Light\ (I0)}$$

$$Absorbance = \log\frac{1}{T} = abc$$

In the equation presented above for absorbance,(a) is a constant representing the ability of a given molecule to absorb a particular wavelength of light. (b) Is the path length which stipulates that the longer the path length is, the less light gets through. (c) Is the concentration which suggests that the more of the molecules present in a solution, more of the light is absorbed[97].

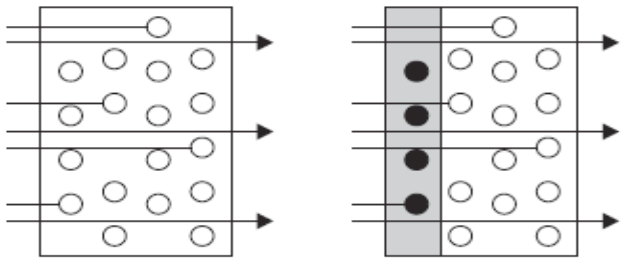


Figure 32: Sample containing fewer molecules will allow less light to be absorbed and more to pass through and vice versa[83].

In order to get a straight line calibration curve, a graph of absorbance versus the concentration is plotted and the equation of colorimetry is used for the calibration curve which is better known as the Beer's Law. The Beer Lambert's law describes the linear relationship between absorbance and concentration of an absorbing species[97].

$$A = \varepsilon c l$$

The above equation is a representation of Beer's Law. The quantity ε is the molar absorptivity and is also referred to as the extinction coefficient. This value changes with the wavelength of light that is used for measurements. The absorption spectrum is therefore sometimes represented as ε vs. λ rather than A vs. λ[98].

Some factors affect colorimetric measurements both in a good way and in some certain cases, may influence or hinder results. To get the greatest sensitivity and selectivity, the choice of wavelength as well as trying to get as close as possible to the absorbance peak are extremely important. Sample blanks and zero samples are therefore important to run as a means of referencing the final outcome. It helps to correct for stray light, sample colour or turbidity. Turbidity is the interaction of light and suspended particles in water. It is a qualitative measurement because the reflection the reflection and scattering of light from particles depends on the nature of the particular particles themselves. The size and shape

of these particles affects the direction as well as the intensity of scattered light as well as its colour. The scattering intensity and direction of scatter also changes with the wavelength of the incident light. Therefore, the greater the intensity of scattered light, the higher the turbidity. There is still a degree of difficulty when it comes to the measure of particle size from the information provided by this technique[97].

Spectrophotometry

An absorbance spectrophotometer is an instrument that measures the fraction of the incident light transmitted through a solution. Simply put, they are used to measure the amount of light that passes through a material, and by comparison to the initial intensity of light they indirectly measure the amount of light absorbed by a sample. Some compounds absorb light which is beyond the visible light spectrum and therefore can seem to be colourless. Because different compounds absorb different wavelengths of light, a spectrophotometer can be used to distinguish between different compounds in a particular sample. Spectrophotometers can also be used to estimate cell numbers along with identifying the different chemical compounds that may be present in a sample material[98].

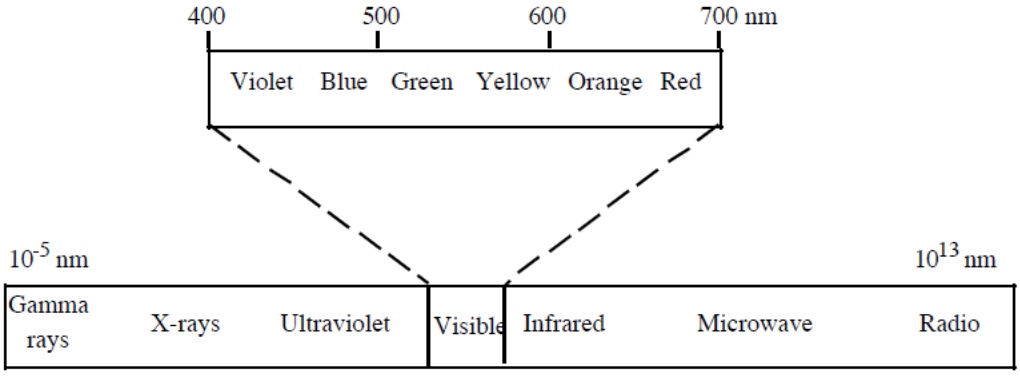


Figure 33: The Electromagnetic Spectrum[98]

The light from the light source of a spectrophotometer does not consist of a single wavelength rather consists of a continuous part of the electromagnetic spectrum. The light is separated into specific portions of the spectrum through the use of prisms or by using diffraction grating. High quality spectrophotometers that are frequently used for research purposes have a slit width of <2nm. Absorbance is detected by a photocell and measured into yield the absorbance value of optical density for the sample[98].

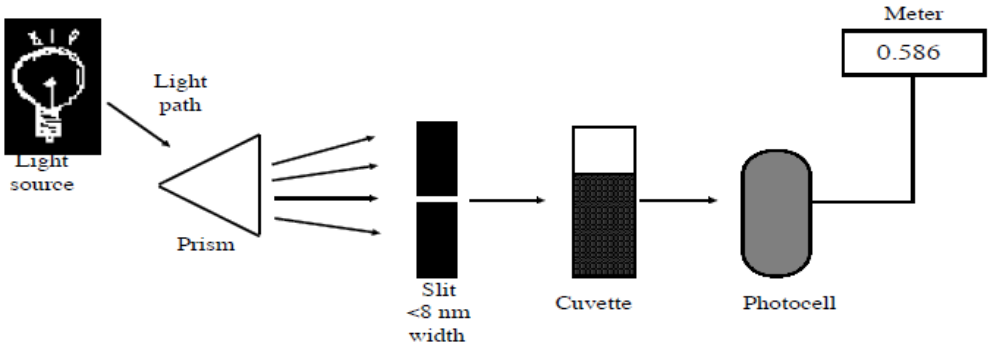


Figure 34: Schematic of a spectrophotometer[98]

2.4 Protein Identification

The Bicinchoninic Acid (Sigma Aldrich UK) used for the identification of proteins was. In order to make it viable for protein identification and quantitation experiments, 1% by weight of sodium Tetraborate was added to the stock solution mentioned earlier, provided by Sigma Aldrich. A solution of 4% Copper Sulphate (Sigma Aldrich UK) by weight was prepared by dissolving in distilled water. The colour of the Bicinchoninic acid solution presented itself as colourless whereas the aqueous copper sulphate formed a light blue coloured solution.

Samples were collected from myself and in accordance to the stipulations suggested under the ethical approval guidelines. Samples were collected by using a plastic swab (Sarstedt UK). Cotton swabs (Sarstedt UK) having a wooden stick were initially used but were seen to affect the results as the protein from the cotton and wood interfered with colour readings and therefor a switch was made to a plastic viscose swab (Sarstedt UK). In some cases, the tips of the swabs were cut using a disposable sample and transferred to 10ml glass sample vials. This was done in order to better see the colour and made it easier to visually judge any colour change that might occur over time.

The procedure for the test started off by swabbing a surface or suspected area. The swab was first moistened by distilled water and then applied to the area in question. The reagents are then added directly to the swab container or if needed, the end of the swab was cut and placed in a glass while, into which the reagents are added. To the tube or glass vial, 1960 µl of BCA Solution was added followed by adding 40 µl of the Copper Sulphate solution. The tube or vial was then swivelled in order to mix the sample and the reagents thoroughly. The samples were left to stand for 45-60 minutes. The initial colour of the solution in the sample was light green and if there was any protein material present in the sample, after reacting with the reagents, it would start turning purple indicating a positive result. If there was no protein materials present in the sample, after the allotted time, the solution remained light green. In some cases it was noted that the solution turned colour to purple even in controlled negative samples. But this was only observed after at least 2 to 3 hours. Once the colour was seen to have stabilized, the samples were then analysed using the spectrophotometer (Nanodrop 2000) and the information gathered was recorded.

The Nanodrop 2000 is a micro volume spectrophotometer used for measuring DNA, RNA and protein concentration from a solution. This instrument has the capability of measuring

samples as small as 0.5 µl and reports the results in the forms of sample concentration, purity ratios and full spectral data. The instrument itself is quite simple to use and does not require any plates or consumables, hence making it extremely cost effective. It is also useful for proteins with low wavelength absorbance, such as peptides at 205 nm.

3.0 Results

3.1 Raman Spectroscopy

3.1.1 Paracetamol

Initially, in order to calibrate and check the workings of the device, Paracetamol was analysed which already had a library spectra present for comparison. Different parameters were tested to see which one gave the best results. The parameters that were tested included laser power, average count, distance of the sample from the laser output and the surface on which the sample was analysed. The type of surfaces that was used to analyse our samples can be seen in the table below:

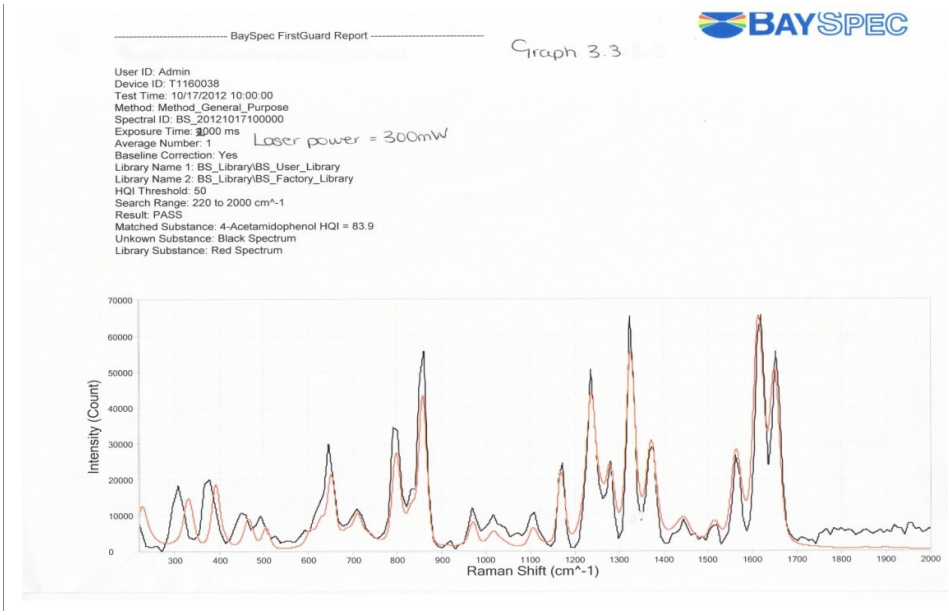
Substrate Number	Substrate	Description
1	Microscope slide /Aluminium foil/Sample	Microscope slide covered with aluminium foil with the sample mounted on top of the foil
2	Microscope slide/Aluminium foil/ Sample/Microscope slide	Microscope slide covered with aluminium foil with the sample mounted onto this and then covered with another microscope slide
3	Microscope slide/Sample	Sample mounted on top of a microscope slide
4	Microscope slide/Sample/Microscope slide	Microscope slide with sample mounted onto it and then covered with another microscope

Table 3: Surfaces tested for Analysis (Calibration Run)

In order to get an accurate measure and the differences that each parameter presented, all of these were kept consistent, changing one at a time for each run. The runs were then compared to the library already provided in order to determine how close of a match each run gave us. The surface that provided the best match was therefore used the primary surface of analysis for our blood samples which were run later on.

All the spectra that were acquired from the Paracetamol runs presented us with clear and defined peaks which could be easily distinguished from the background noise and provided us with a large intensity. This meant that the device which was being used clearly showed the ability of running and analysing Paracetamol on almost all the surfaces mentioned earlier. However substrate one (from table 2) provided us with the best match of 81.8% and therefore this particular method was regarded as the best to be used for other types of samples.

Spectrum 1 shows us the sample run and the library match both on the same plane and can be seen below.



Spectrum 1: Paracetamol Run against Library Spectra having a 81.8% match

3.1.2 Equine Blood

Blood analysis on Raman was carried out in order to formulate a method for the detection and age estimation of a blood stain. This however proved to be a very difficult task due to the nature of the samples and the principles of laser wavelength. As mentioned earlier, numerous researches have been conducted in the past for the identification of blood. The major problem faced by us was the difference of wavelength of the device that we were currently using to the ones that had already been used. We were using 1064 nm wavelength as compared to the 532-785 nm wavelengths used in the past. In order to establish the functionality of our wavelength, numerous tests were run in order to find the optimum parameters that could be used for our blood samples.

Initially, de-fibrinated equine blood was used as a substitute to human blood. This is a lot easier to acquire and can be made available in large quantities for repeated use. The different parameters used are described in the table below.

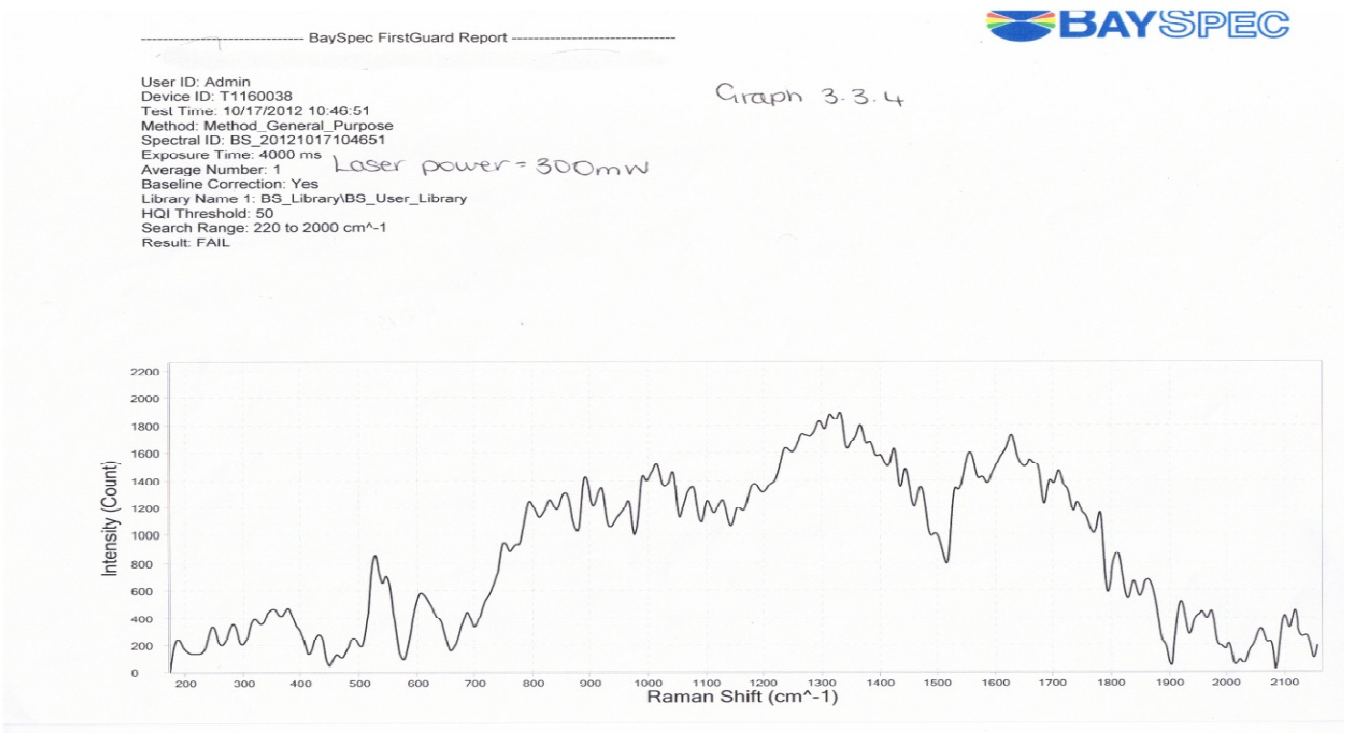
Parameter	**Laser Power (mW)**	**Exposure Time (ms)**	**Average Count**
Calibration Sample Number			
Background	30	4000	1
1	30	4000	1
2	150	4000	1
3	300	4000	1

Table 4: Parameters used for testing the suitability of fresh equine blood

The lowest power of the Raman was used for the background spectrum and the first sample, as the laser was not passing through a medium to scan the sample the laser may not need to be at as high a power as the increase in power may have damaged the blood sample. Also, the exposure time was maintained at 4000ms as this was the optimum

parameter for Paracetamol and suitable for preliminary work. The other two parameters were used as they covered a range of low to high laser power to observe whether the higher laser powers could damage the sample. The maximum power of the Raman was avoided as the laser was coming into direct contact with the sample. If the laser was set to full power it would have degraded the blood sample.

The spectra provided us with a much better view of how the samples reacted to the change in parameters. For the first two samples, there was no particular shape that could be observed in the spectra and therefore a third sample was run in order to identify what the problem was. This particular run provided us with a spectrum that looked to be that of blood. But because there was no spectrum available in the library to confirm this hypothesis, the result from this run was deemed inconclusive and this was added to the library so we may compare further runs that we did on equine blood. More experiments were hence carried forth to see if they matched the shape as well as the peaks that were



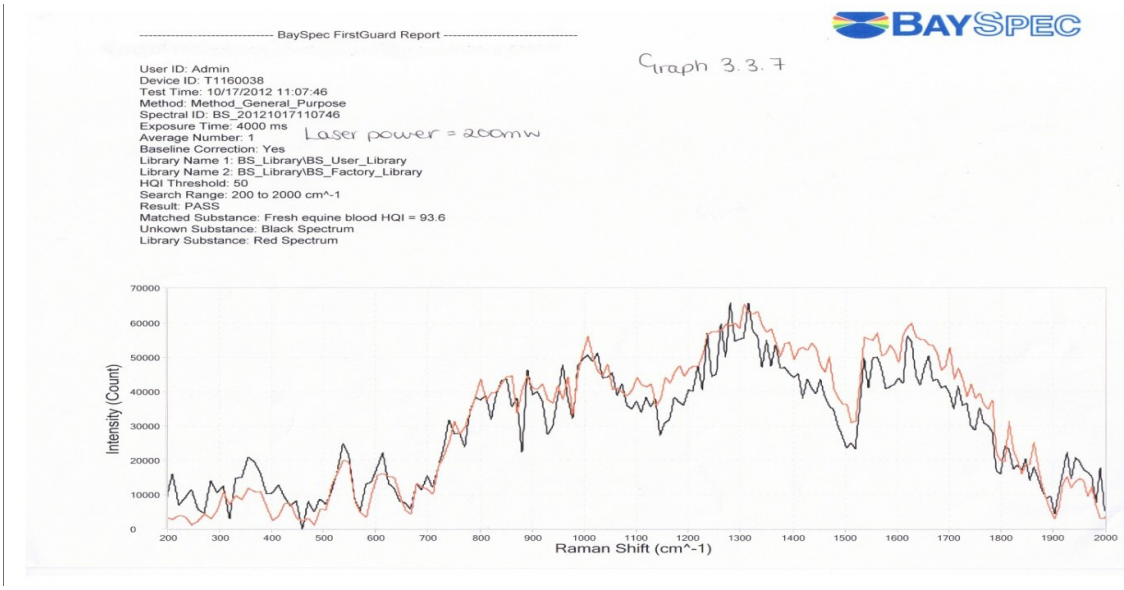
Spectrum 2: Spectrum of Equine blood (Presumption)

The experiment with equine blood was repeated in order to attain the same spectra and to confirm that the spectrum that was initially acquired was in fact that of equine blood. Two further samples were run in order to see what similarities and differences could be observed in them against our initial run. The first scan did not provide a substantial match to the initial spectrum. The second scan also failed to produce a significantly high match and therefore new samples were prepared to be run. This time however, some parameters were changed in order to determine if they had any sort of effect on our results. The laser power was lowered from its initial 300mW as we presumed that the high power could actually be denaturing our blood samples. The laser power was run at 200mW, 150mW and 100mW. This provided us with some interesting results and these are illustrated in the table below.

Calibration Sample Number	**Laser Power (mW)**	**Library Match (%)**
1	200	93.59
2	150	57.7
3	100	No Response

Table 5: Library match of fresh equine blood at different laser powers in the comparison experiment

The results not only replicated on of our results to the initial spectrum but also provided information that 200mW was the optimum laser power to be used for our blood samples. The lowest laser power of 100mW did not give us a spectrum and therefore we were confident that the laser power that was ideal for our sample type was 200mW as the spectrum acquired at 150mW provided us with a match of only 57.7%.

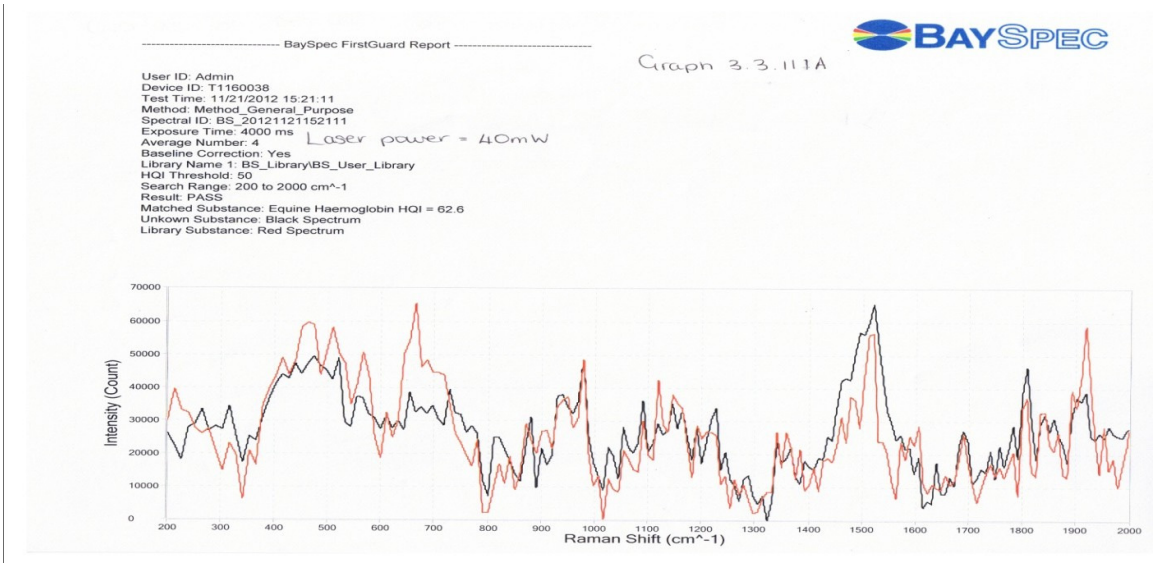


Spectrum 3: Comparison of a repeat experiment with Equine blood showing a 93% match

The spectrum above shows the comparison of the result obtained using the optimum laser power (200mW) to our initial run. Visually, it can be seen that the two spectra are quite similar and the by analysing the Raman Shift and peak values, it can be determined that there is a 93% match between both the spectra. According to these results which was taken as an average of numerous other runs conducted on the same sample at the same conditions, it was up to an extent, established that the instrument that we were currently using could be used for the identification of equine blood. Another issue that came up during these experiments was the presence of organelles and compounds in the equine blood. Due to the blood being de-fibrinated, it was established that it only contained red blood cells along with water. There were no other cells such as white blood cells and platelets and it was also assumed that there were no proteins present in our samples. Because of this, the blood that was being analysed did not have the ability to clot as there were no clotting factors and platelets present. Therefore to use this blood in our age estimation experiment would not be suitable as the formation and removal of certain compounds during the process of coagulation would not take place. According to the principle of scattering, the new compounds that are formed over time in blood would

provide us with new peaks and therefore in a system where the process does not take place, it would be difficult to use in age estimation. In order to overcome this particular problem, it was decided that blood would be acquired from volunteers to be run for this particular part of the project.

In order to confirm the presence of red blood cells and to use an identifying marker for the presence of blood, equine blood that was currently being used was centrifuged and the red blood cells were collected separately. This concentrate of cells was then analysed in the Raman Spectrometer in order to see any similarities or differences that could be present while comparing it to our whole equine blood sample. It was also believed that the spectrum produced from the centrifuged concentrate would also give a much better shape and would confirm the identifying peaks that we required for equine blood identification.



Spectrum 4: Centrifuged equine blood compared with non-centrifuged blood.

The spectrum above shows comparison of both centrifuged and non-centrifuged equine blood. The black line indicates the centrifuged and the red line shows non-centrifuged blood. It can be clearly seen that the centrifuged blood provided us with much sharper and much cleaner peaks where as there is still some distortion that can be observed in whole

non-centrifuged blood. These peaks were noted down and were kept as identifying peaks for blood (red blood cells).

3.1.3 Human Haemoglobin:

In order to see if the device that we currently were working on was suitable for human blood, another alternative was first tested. Human Haemoglobin was ordered out from Sigma Aldrich and was run in order to see if we could get some specific peaks for human haemoglobin. These were also run to provide us with a library spectrum which we could later compare to our blood samples and see if they provide us with any sort of similarities or differences. The haemoglobin powder ordered was anhydrous and had any extremely light and crystalline texture.

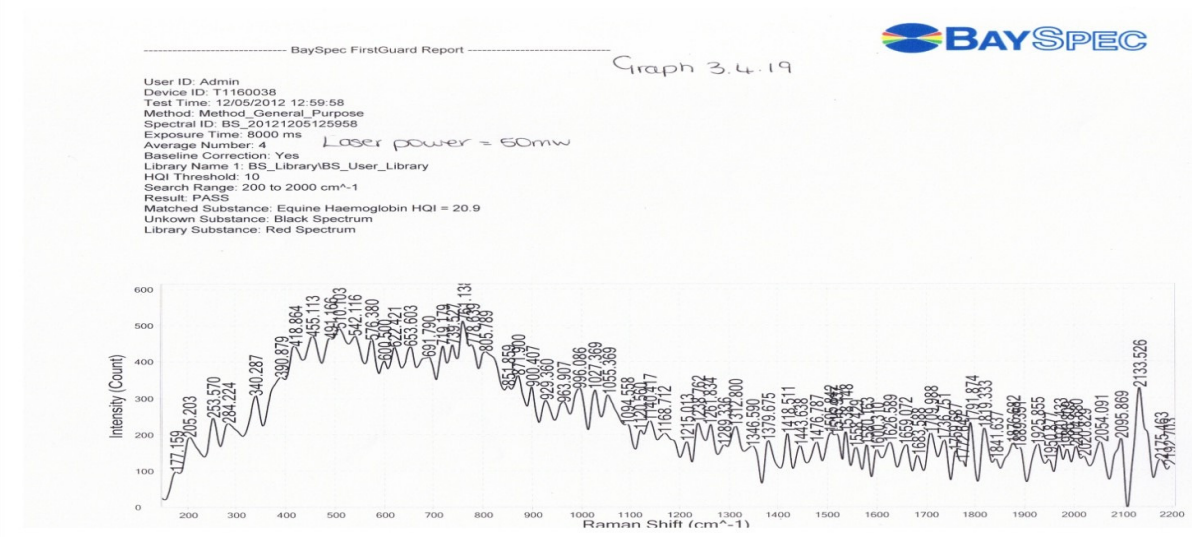
An initial sample of haemoglobin was run by dissolving it in deionised water at different concentrations and placing it on a sample slide. This transfer was done using a micro spatula and a record of all the concentrations was kept in order to determine the limit of detection with regards to the concentration of haemoglobin. The following parameters were used in order to see which would be the optimum conditions for the haemoglobin to be run on.

Calibration Sample Number	Laser Power (mW)	Exposure Time (ms)	Average Count
1	2000	30	4
2		40	4
3		50	4
4		100	4
5		150	4
6		200	4
7		250	4
8		300	4
9	4000	30	4
10		40	4
11		50	4
12		100	4
13		150	4
14		200	4
15		250	4
16		300	4
17	8000	30	4
18		40	4
19		50	4
20		100	4
21		150	4
22		200	4
23		250	4
24		300	4

Table 6: Conditions and Parameters for Human Hemoglobin

The results for these runs provided us with a suitable set of conditions in which the optimum results for human haemoglobin were achieved. Some of the runs that were done

failed to provide any sort of discernable shape or peak strength therefore those conditions were termed as being not viable. The spectrum below shows the peaks for human haemoglobin when it was run on its optimum condition of 300mW laser power, average count of 4 and an exposure time of 30ms.



Spectrum 5: Human Haemoglobin at optimum conditions

As it can be seen, a number of different peaks appear for this particular compound describing the numerous different structures and bonding that are present in it. According to an extensive literature search, numerous different peaks were determined that represent human haemoglobin and can be used to identify the compound. These peaks were compiled and kept as a reference in order to determine the identity of haemoglobin from unidentified and presumed blood samples.

Signature blood peaks for Hemoglobin at 1064nm (experimental)	Library peaks for Hemoglobin	Peaks Common to Porphyrin
756 - 760	751-757	745-750
1001-1005	962-967	1308-1312
1127-1131	1001-1005	1390-1395
1366-1368	1127-1131	1586-1590
1448-1453	1335-1338	
1555-1558	1366-1368	
1575-1576	1448-1453	
1621-1624	1555-1558	
1669-1673	1575-1576	
	1621-1624	
	1669-1673	

Table 7: Peaks obtained compared with library peaks

According to table 6, apart from all the other peaks that are present in our spectrum, certain peaks that are congruent with the structure of haemoglobin and which can be used as an identification tool are also present in our results. These peaks represent the different type of bonds that are present in the structure of haemoglobin. All of the peaks mentioned as 'Library Peaks' were seen to be present in almost all of the samples that were run using the optimum conditions as described earlier.

The samples that were prepared at different concentrations were then run on a separate device which had a wavelength of 785 nm. Again, according to the literature that we had of earlier work that had been done regarding this, it was said that haemoglobin and more importantly whole human blood provided much sharper and more intense peaks at lower wavelengths. Therefore these samples were run using the 785 nm Raman Spectrometer to see what sort of results could possibly be obtained.

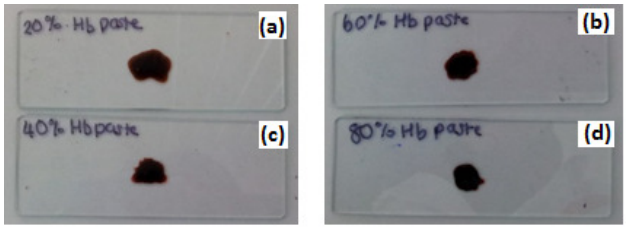


Figure 35: Different concentrations of Human Haemoglobin on glass slides (a) 20% conc, (b) 60% conc, (c) 40% conc and (d) 80% conc

Four different concentrations of 20%, 40%, 60% and 80% respectively were prepared and put on a glass slide for analysis on the 785 nm Raman Spectrometer. Along with this, another theory which regarded to the type of surface was also tested. It was noticed that scattering took place a lot more effectively on much rougher surfaces than the ones we already had in the form of a glass slide. In order to mimic these conditions, a few microscope slides were intentionally made rough in order to see how much more scattering could be achieved in comparison with a normal plain microscope slides. The slides in question were roughened up by using a common glass cutter which enabled us to make a crisscross pattern on the surface of the glass slide. This provided a clear and concentrated rough are on the slide on which the human haemoglobin, again of different concentrations was placed.

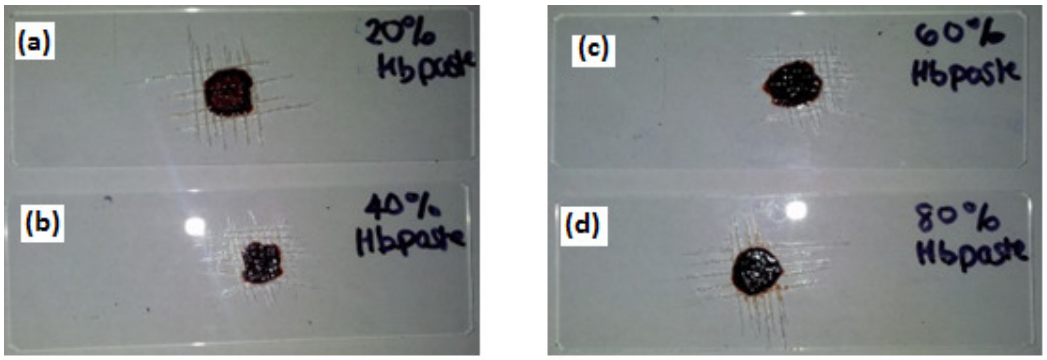
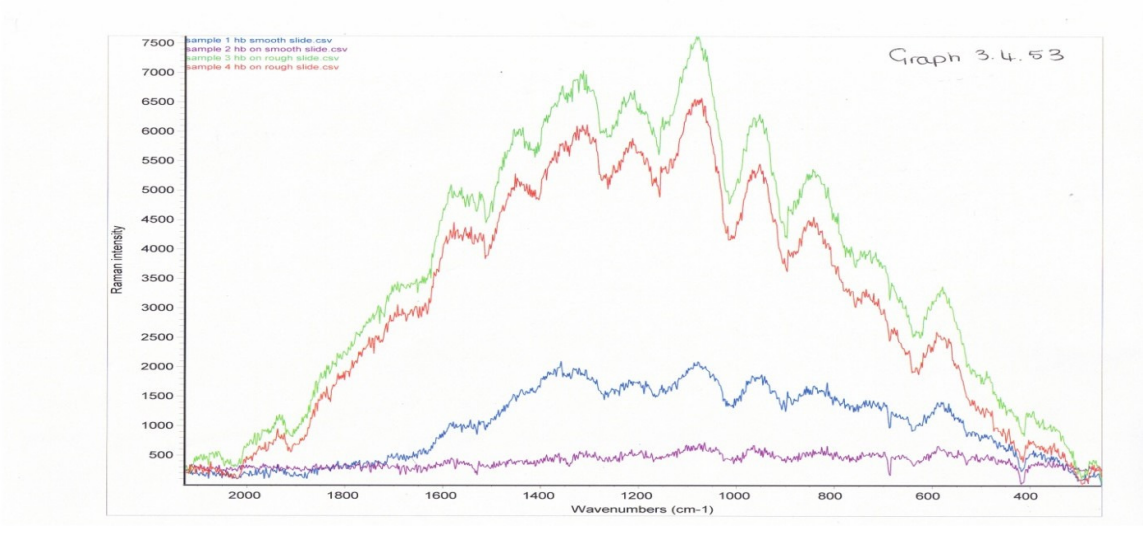


Figure 36: Different concentrations of Human Haemoglobin on rough slides (a) 20% conc, (b) 40% conc, (c) 60% conc and (d) 80% conc

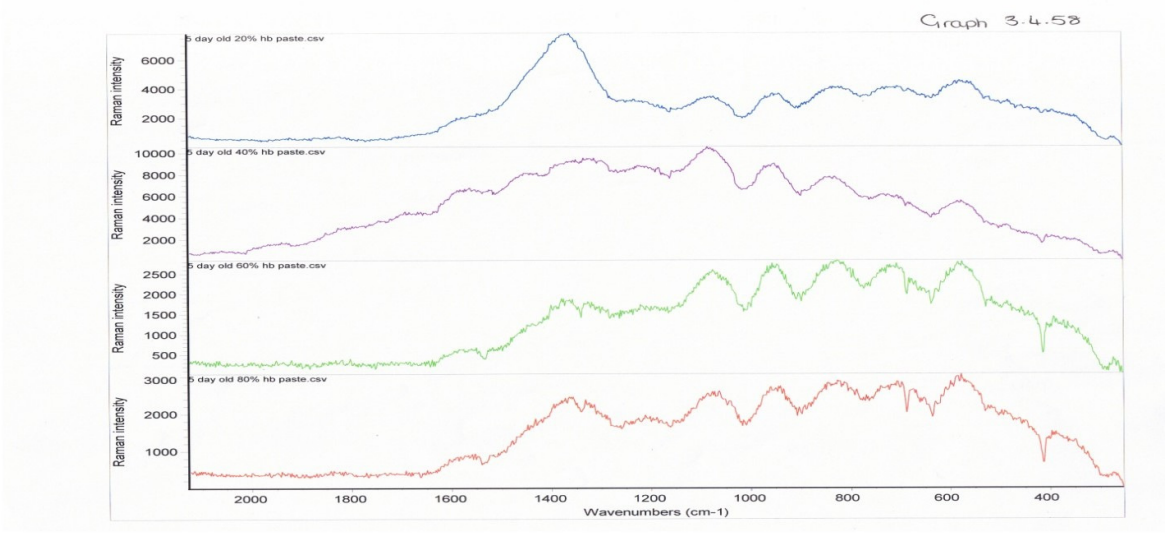
As seen on the figure above, the haemoglobin solution seemed to have seeped into the cracks of the slide and dried out providing a coating of the target sample on a comparatively rough surface. These samples were then run to see which type of surface would provide us with maximum scattering and the best spectra.



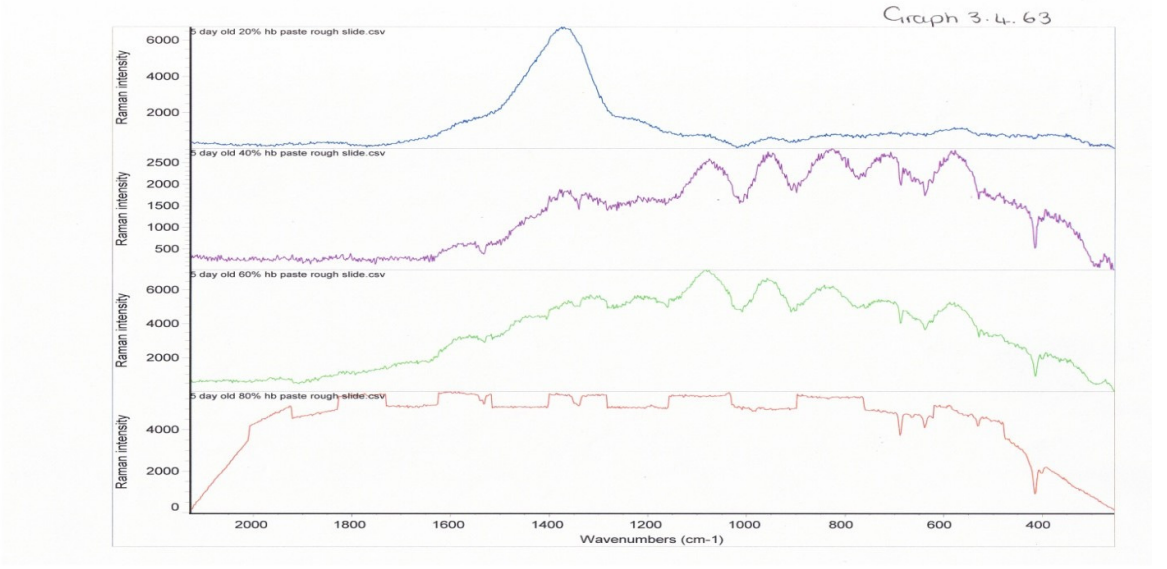
Spectrum 6: Comparison of smooth slide and rough slide spectra. The blue and purple lines represent Hb on smooth slides whereas the green and red lines represent Hb on roughened slides.

From this spectra, it can be clearly seen that the two samples of haemoglobin that were placed on a rough slide (green, red) provided a much sharper and more intense reading when compared to the two samples that were run on a normal smooth slide (blue, purple). This is because there was more scattering on the rough surfaces as compared to the much smoother surface of a normal slide. This was therefore deemed as the ideal surface to be used in terms of human blood.

The different concentrations of human haemoglobin were also tested to see what sort of concentration would provide us with the clearest results.



Spectrum 7:Comparison of spectra for the smooth slide at different haemoglobin concentrations.The blue and purple lines represent Hb on smooth slides whereas the green and red lines represent Hb on roughened slides.



Spectrum 8: Comparison of spectra for the rough slide at different haemoglobin concentrations.(blue line 80% conc, purple line 60% conc, green line 40% conc and red line 20% conc)

The smooth slide had similar spectra for 40%, 60% and 80% however; there was no distinct shape for the spectrum at 20% so this could show that the blood could not be detected at any lower concentration than 40% haemoglobin in a solution. The rough slide spectra had distinct spectra with possible blood peaks at 40% and 60%. In this case, the 20% had only one broad peak with no distinct shape or defined peaks and the 80%

sample had a raised baseline and so the blood may have degraded. The most suitable substrate was the rough slide and the optimum concentration of haemoglobin would be between 40% and 60%.

3.1.4 Human Blood:

To further extend our research, human blood was tested to see if we could represent this on our 785 nm Raman Spectrometer. Before the initial runs were done, it was suggested as well observed that we attained a maximum amount of scattering over surfaces that were rougher than the microscope slides we had been using. As described earlier, the slides that had been roughened up using a glass cutter provided us with a much intense signals for our peaks. Keeping the same principle in mind, another surface was used to see if it gave even better scattering in terms of using human blood. Commercial grade sandpaper was used as our analysing surface and a drop of whole human blood was placed on it. This was then left to dry which took approximately 15-20 minutes. The dried sample was them run through the 785 nm Raman Spectrometer.

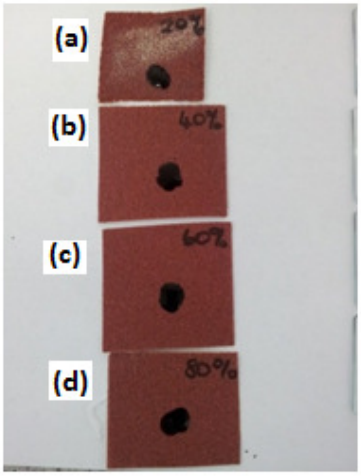


Figure 37: Dried Blood on Sandpaper (a) 20% conc, (b) 40% conc, (c) 60% conc and (d) 80% conc

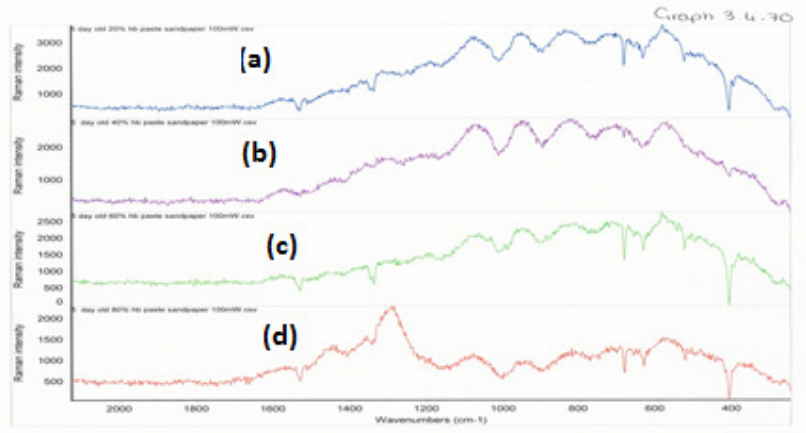
Different concentrations of blood were used and the concentration of the initial whole blood was considered 100%. The blood was then serially diluted with saline and water to provide us with the different concentrations as seen in figure 39.

The parameters set for this experiment are as follows:

Parameter	Value
Laser Power (mW)	300
Exposure Time (ms)	1000
Average Count	1

Table 8: Parameters for Human Blood

These parameters were formulated after testing all the other possible parameters in previous runs as mentioned earlier and these values were found to provide the best results.



Spectrum 9: Different concentrations of Human Blood on Sandpaper(a) 20% conc, (b) 40% conc, (c) 60% conc and (d) 80% conc

The resulting spectrum showed a very distinct shape and almost all concentrations that were used. The peaks that were mentioned in table 6 seemed to be present and we accounted all the indicative peaks for human haemoglobin as well. According to this particular run, it was established that the optimum concentration for human blood to be used on the 785 nm Raman Spectrometer was between 40% and 60%.

3.2 Small Angle X-Ray Spectroscopy

Initial runs of human blood, equine blood and BSA were run to see if there was any structural representation shown by the SAXS. The samples considered were freshly extracted as far as human blood is concerned and equine blood used was taken from the sealed pack. Both types of blood samples were tested in liquid form and a 1% solution of

BSA was tested along with it. Scatter patterns were obtained for all 3 samples as shown below.

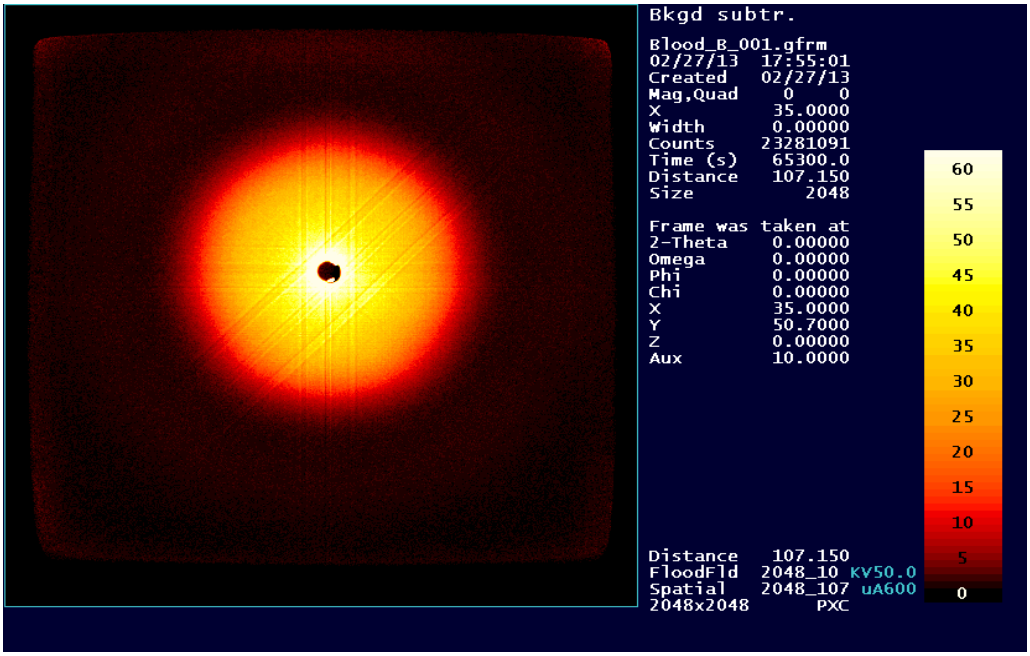


Figure 38: Scatter Pattern for Human Blood

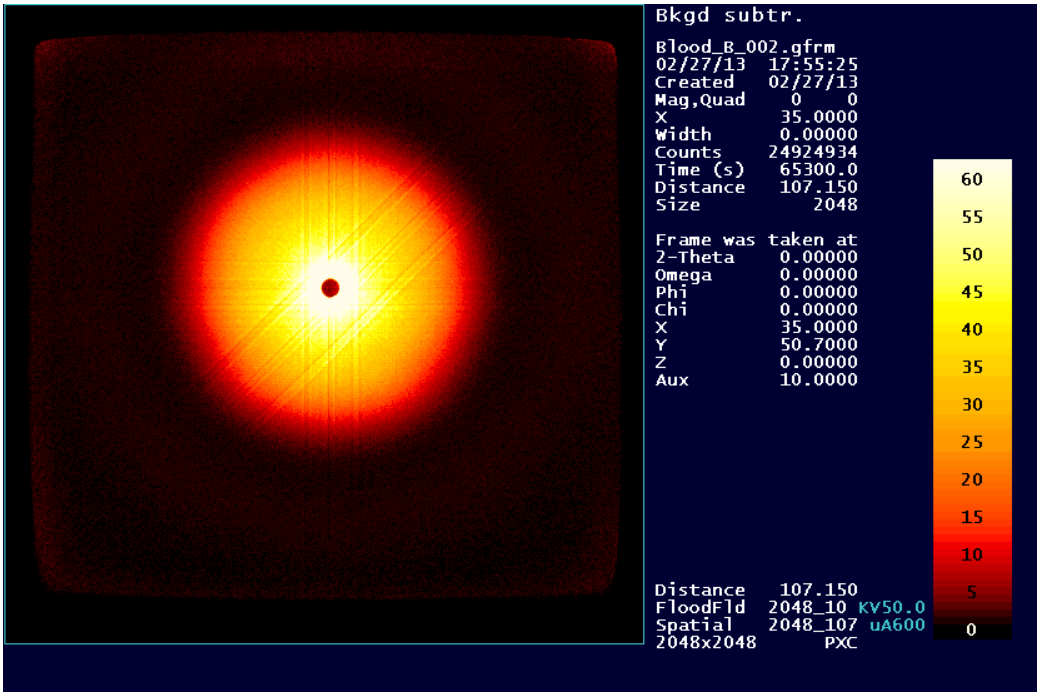


Figure 39: Scatter Pattern for Equine Blood

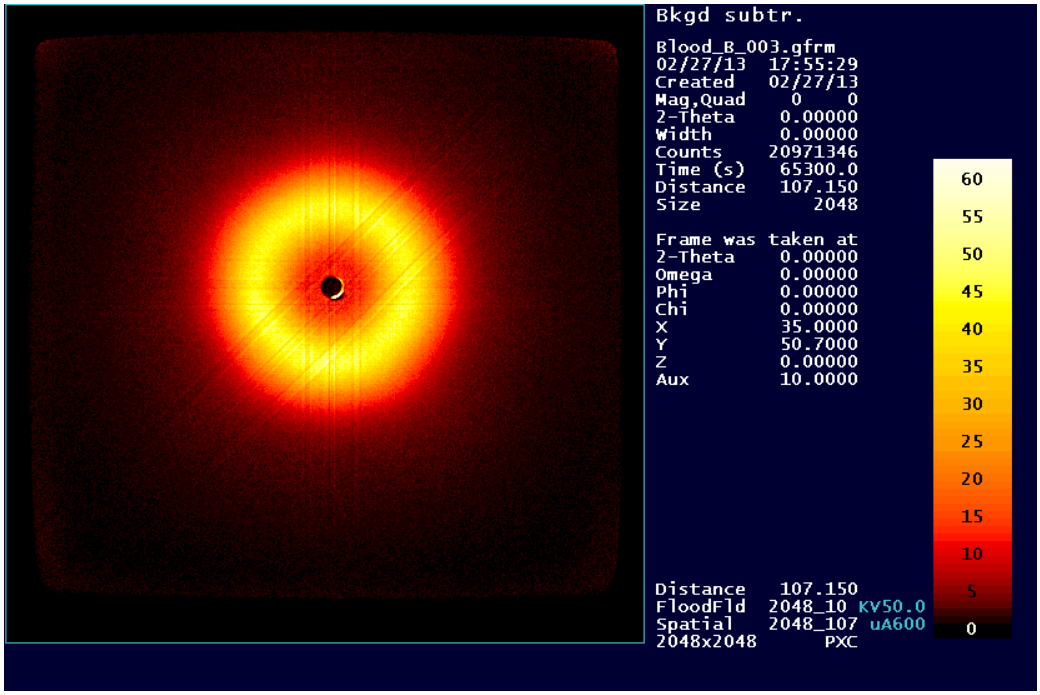


Figure 40: Scatter Pattern for BSA

As can be seen from the 3 scatter patterns displayed above, the two patterns for human blood and equine blood look more or less the same. This is due to the similar structure of haemoglobin present in both the types of blood. The results regarding these scatter patterns can be plotted as a graph as shown below.

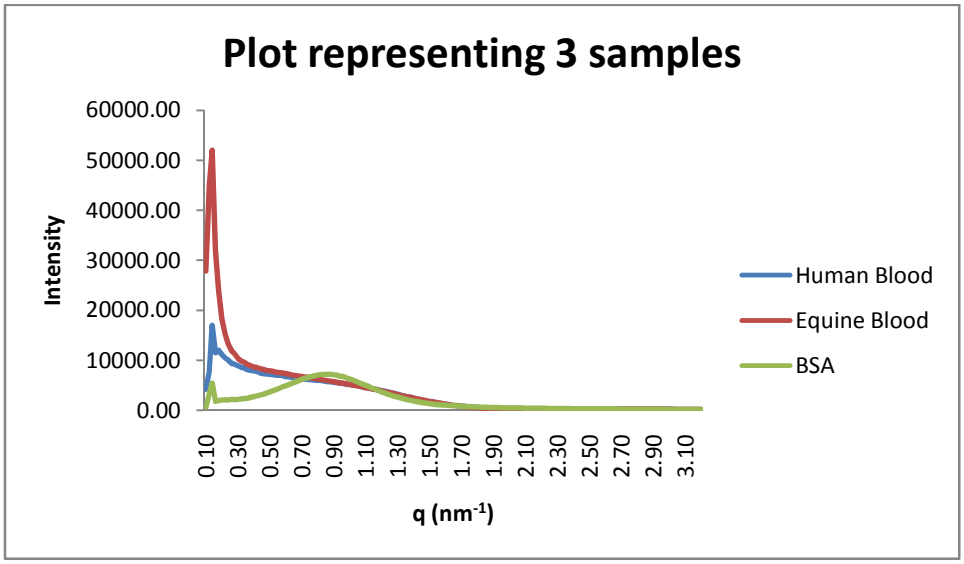


Figure 41: Graph representing the intensity values of Human and Equine blood as well as BSA solution. The difference in intensity values is due to the difference in structures causing different scattering patterns.

The values for all these points can be seen in the appendix section of this report. These values here represent the intensity change over time which on this representation is being observed by the value q (angle of scattering). The values plotted here can be logged to give a much more accurate representation in terms of finding out both the radius of gyration as well as gaining any structural information regarding the samples.

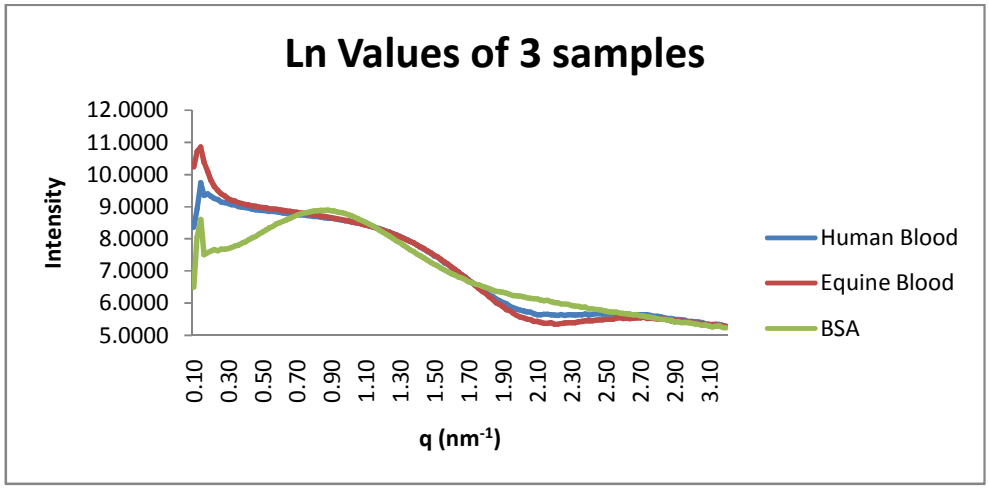


Figure 42: Graph representing the logged (ln) values of all 3 fluids

This graph can then be used to determine the radius of gyration which determines the dimensions of the samples which can be used in determining the type and shape of our samples.

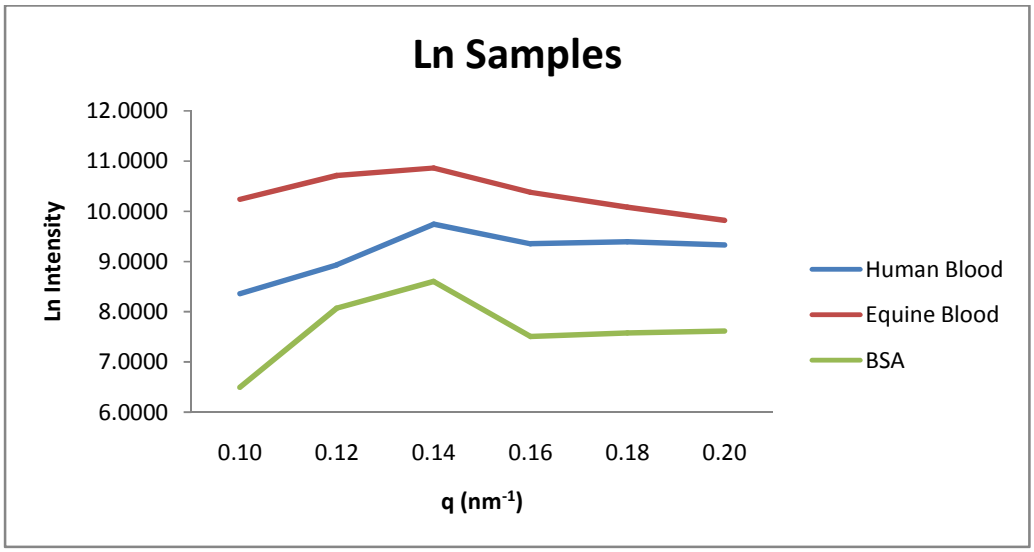


Figure 43: Graph of logged intensity values to investigate the shape of molecules

According to the features of PDDF, as provided earlier, it can be seen that both equine blood and human blood graphs follow under the same shape as that of a globular protein. This correlates to the fact that haemoglobin is in fact a globular protein and can hence be confirmed by this. The structure of BSA can be correlated to that of a cylindrical shape.

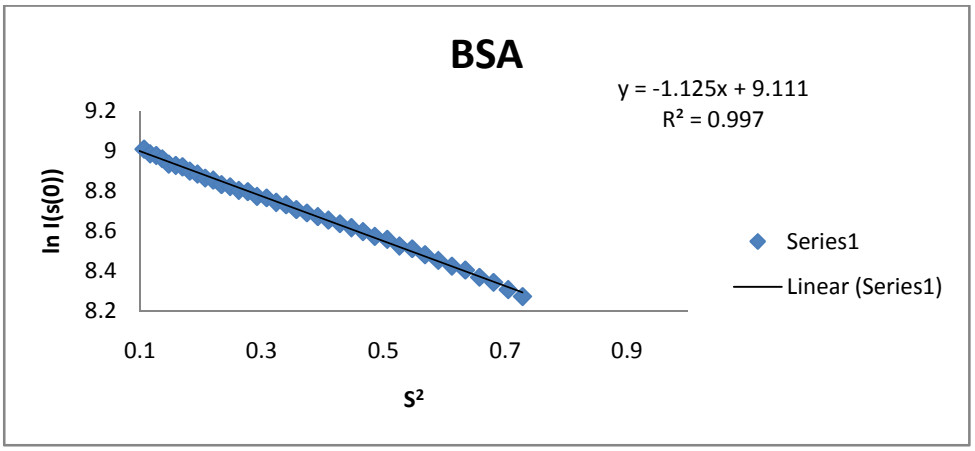


Figure 44: Graph representing Rg Values for BSA

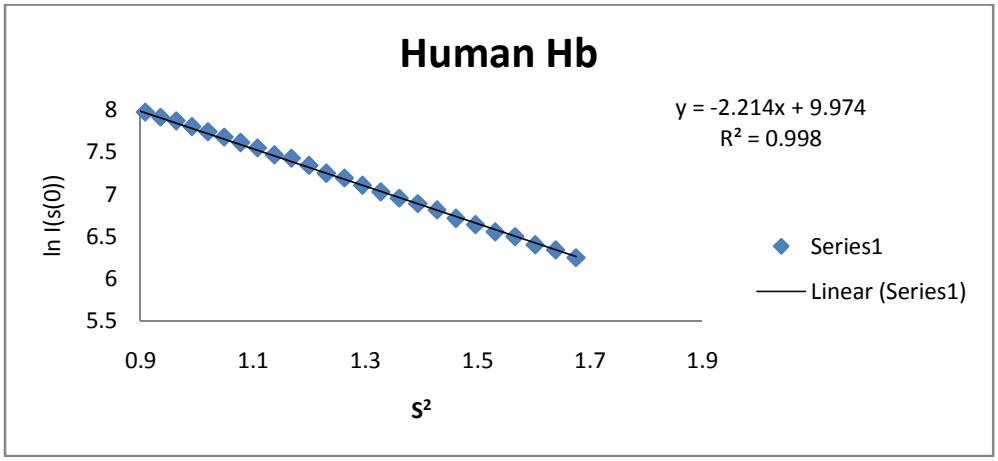


Figure 45: Graph representing Rg Values for Human Haemoglobin

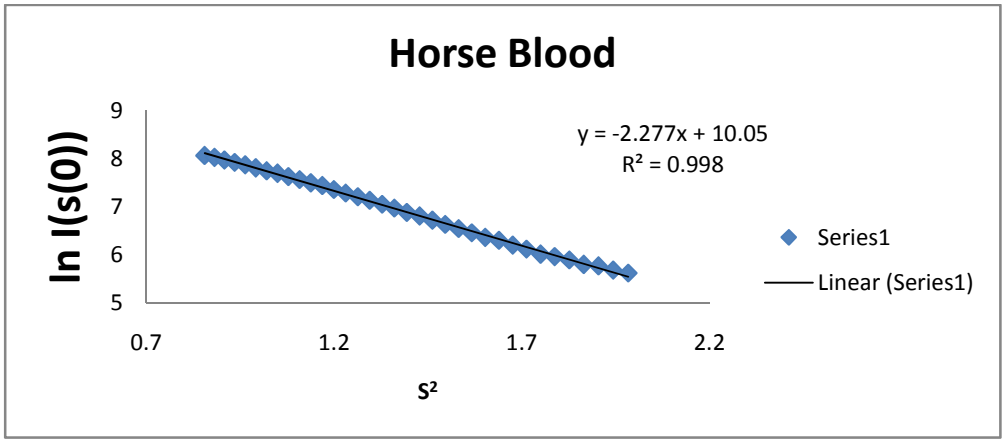


Figure 46: Graph representing Rg Values for Equine Blood

As mentioned earlier, the value for the radius of gyration (Rg) can be used to describe the dimensions and other structural information of our samples. The Rg values can be considered to be specific for specific structures and can be used as an identifying tool for certain types of proteins. The Rg values for the 3 types of samples can be seen below.

Sample Name	Gradient	Rg
In intensity Horse Blood	-2.2776	0.416025
In intensity Human Hb	-2.2145	0.410222
In intensity BSA	-1.1255	0.292451

Table 9: Rg Values for 3 different types of stains

Apart from general structural identification studies, aging was also performed on blood samples.

The SAXS patterns presented in the appendix were taken over a period of 6 hours. Pattern A shows human blood freshly extracted and placed on a piece of cotton cloth. Readings were taken at an hour interval. The final patterns produced are presented after background subtraction of both the possible scatter background along with subtracting the background template scattering, that of cotton. It can be seen from these patterns that there is a considerable drop in the count number of scatter over time. In the final two hours, there is

a significant increase in the count number. This can be associated with the formation of a new compound or an excessive increase in the structure size and therefore the increase in scatter. Theoretically, this can be associated with the formation of Hemichrome which has a larger structure than the earlier compounds in the dissociation of haemoglobin. This could explain the increase in scatter pattern over approximately 4-5 hours. This scatter pattern increases for 1 more hour and then reaches a stabilizing constant which lies within the average error range.

3.3 Colorimetric Analysis

3.3.1 White Filter Paper

Initially 2 runs were conducted in order to get a base reading for blood and to see if we could come up with any sort of numeric value that can be used to explain bloodstains being aged. Both the samples were collected from the same donor and labelled as Sample 1a and Sample 1b respectively. Runs were conducted over a period of 30 minutes and the results tabulated as shown below.

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
Sample 1a - 0 mins	47.19	19.02	6.65	------	------	------
Sample 1a - 30 mins	47.61	19.07	6.88	0.42	0.04	0.23
Sample 1a - 60 mins	48.04	19.22	7.19	0.84	0.2	0.55
Sample 1a - 90 mins	48.09	19.4	7.39	0.9	0.37	0.74
Sample 1a - 120 mins	48.2	19.55	7.53	1	0.52	0.88
Sample 1a - 150 mins	48.57	19.68	7.81	1.38	0.66	1.16
Sample 1a - 180 mins	48.62	19.63	7.87	1.43	0.61	1.22
Sample 1a - 210 mins	50.84	20.53	9.53	3.65	1.51	2.88
Sample 1a - 240 mins	50.92	20.56	9.6	3.72	1.53	2.95
Sample 1a - 270 mins	51.54	20.75	9.96	4.35	1.73	3.31
Sample 1a - 300 mins	51.33	20.71	9.82	4.14	1.68	3.17
Sample 1a - 330 mins	51.61	20.81	9.96	4.42	1.79	3.31
Sample 1a - 360 mins	53.03	21.34	10.84	5.84	2.31	4.19

Table 10: Sample 1a Filter paper 30 minute intervals

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
Sample 1b - 0 mins	50.27	19.82	9.09	------	------	------
Sample 1b - 30 mins	49.71	20.23	9.53	-0.56	0.41	0.44
Sample 1b - 60 mins	50.12	20.28	9.82	-0.15	0.46	0.73
Sample 1b - 90 mins	50.13	20.1	9.74	-0.14	0.28	0.65
Sample 1b - 120 mins	51.51	20.59	10.96	1.24	0.77	1.87
Sample 1b - 150 mins	51.87	20.63	11.12	1.6	0.81	2.03
Sample 1b - 180 mins	52.27	21.01	11.35	2	1.19	2.26
Sample 1b - 210 mins	50.82	20.71	10.58	0.55	0.89	1.49
Sample 1b - 240 mins	51.39	20.85	10.87	1.12	1.03	1.78
Sample 1b - 270 mins	52.28	20.98	11.13	2.01	1.16	2.04
Sample 1b - 300 mins	54.25	21.26	12.02	3.98	1.44	2.93
Sample 1b - 330 mins	55.85	18.56	11.32	5.58	-2.7	2.23
Sample 1b - 360 mins	55.88	15.25	10.25	5.61	-6.01	1.16

Table 11: Sample 1b Filter Paper 30 minute intervals

The graph provided by the machine was plotted across 3 axis which were Reflectance (%) and Delta-Reflectance (%) against the Wavelength.

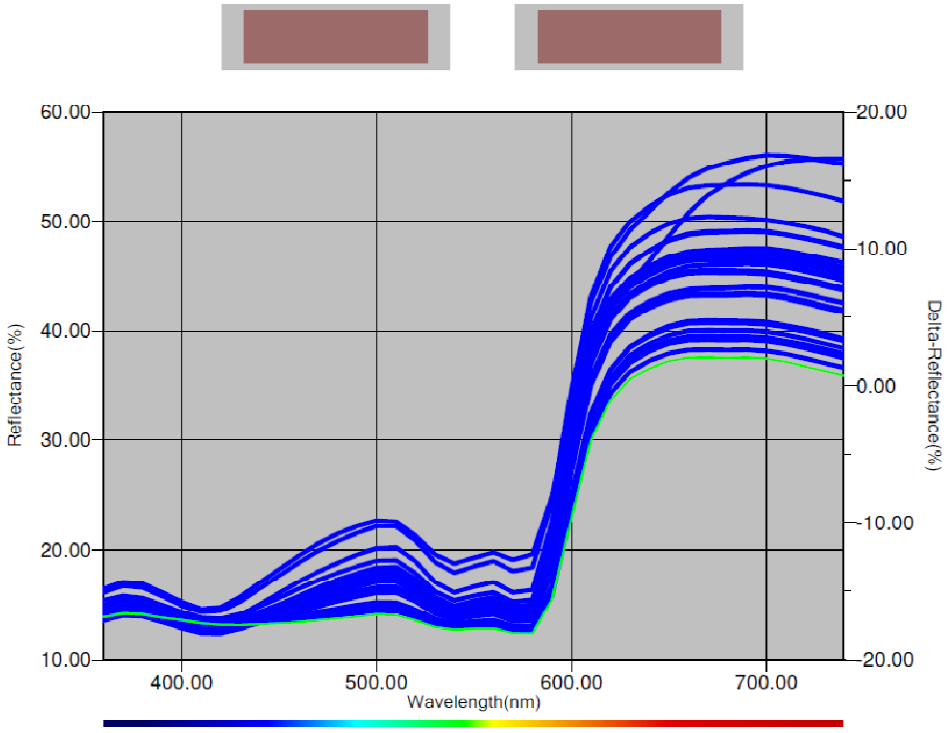


Figure 47: Graph representing Sample 1a and 1b run at 30 minute intervals.

The graph shown above shows the change in reflectance over a range of colour represented by the wavelength. There seemed to be a trend towards the higher wavelengths (red spectrum) which was expected from blood.

According to the numeric values obtained, a secondary graph of all the values obtained and their change over time can be presented. The values of both sets were averaged and are presented as follows:

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
0 mins	48.73	19.42	7.87			
30 mins	48.66	19.65	8.205	-0.07	0.225	0.335
60 mins	49.08	19.75	8.505	0.345	0.33	0.64
90 mins	49.11	19.75	8.565	0.38	0.325	0.695
120 mins	49.855	20.07	9.245	1.12	0.645	1.375
150 mins	50.22	20.155	9.465	1.49	0.735	1.595
180 mins	50.445	20.32	9.61	1.715	0.9	1.74
210 mins	50.83	20.62	10.055	2.1	1.2	2.185
240 mins	51.155	20.705	10.235	2.42	1.28	2.365
270 mins	51.91	20.865	10.545	3.18	1.445	2.675
300 mins	52.79	20.985	10.92	4.06	1.56	3.05
330 mins	53.73	19.685	10.64	5	-0.455	2.77
360 mins	54.455	18.295	10.545	5.725	-1.85	2.675

Table 12: Average values of 3 runs of blood on filter paper

According to the graph above, there does not seem to be much change in colour of the blood sample over time. Some slight change can be seen but nothing too exclusive that can tell us with a certainty as to something major changing in the system. In order to gain some more stable numeric values, a much more mathematical approach was taken to the values.

A secondary experiment was carried out to further our research into investigating the changes that took place in blood over time. 5 samples were run all from the same source on a white filter paper. The samples were again run on a 30 minute interval and the

measurements recorded. The values were then averaged and the data set represented as follows.

Average	L	L sd	a	a sd	b	b sd	a/b	a/b sd	a * b
0	40.732	3.535	33.366	0.707	20.842	1.313	1.601	0.034	695.414172
30	45.412	1.048	27.284	1.217	18.422	0.505	1.481	0.066	502.625848
60	44.850	1.219	23.156	0.372	16.622	0.539	1.393	0.022	384.899032
90	44.728	1.289	21.820	0.265	16.082	0.578	1.357	0.016	350.90924
120	44.596	1.242	21.264	0.284	15.808	0.545	1.345	0.018	336.141312
150	44.530	1.116	20.816	0.365	15.620	0.524	1.333	0.023	325.14592
180	44.446	1.368	20.378	0.297	15.454	0.582	1.319	0.019	314.921612
210	44.576	1.386	19.952	0.315	15.306	0.598	1.304	0.021	305.385312
240	44.374	1.291	19.698	0.323	15.146	0.574	1.301	0.021	298.345908
270	44.408	1.196	19.328	0.334	15.030	0.559	1.286	0.022	290.49984
300	44.264	1.232	19.192	0.381	14.936	0.551	1.285	0.026	286.651712
330	44.392	1.119	18.908	0.349	14.900	0.481	1.269	0.023	281.7292
360	44.544	1.224	18.576	0.497	14.808	0.492	1.254	0.034	275.073408
390	44.426	1.246	18.318	0.390	14.644	0.535	1.251	0.027	268.248792
420	44.446	1.357	18.142	0.440	14.608	0.599	1.242	0.030	265.018336
450	44.414	1.295	18.030	0.486	14.544	0.558	1.240	0.033	262.22832
480	44.424	1.323	17.828	0.417	14.498	0.561	1.230	0.029	258.470344

Table 13: Average values for 5 runs of Blood on filter paper

The graph provided from the software showed that this particular batch showed a much darker colour of red and still provided with the same trend as the previous results.

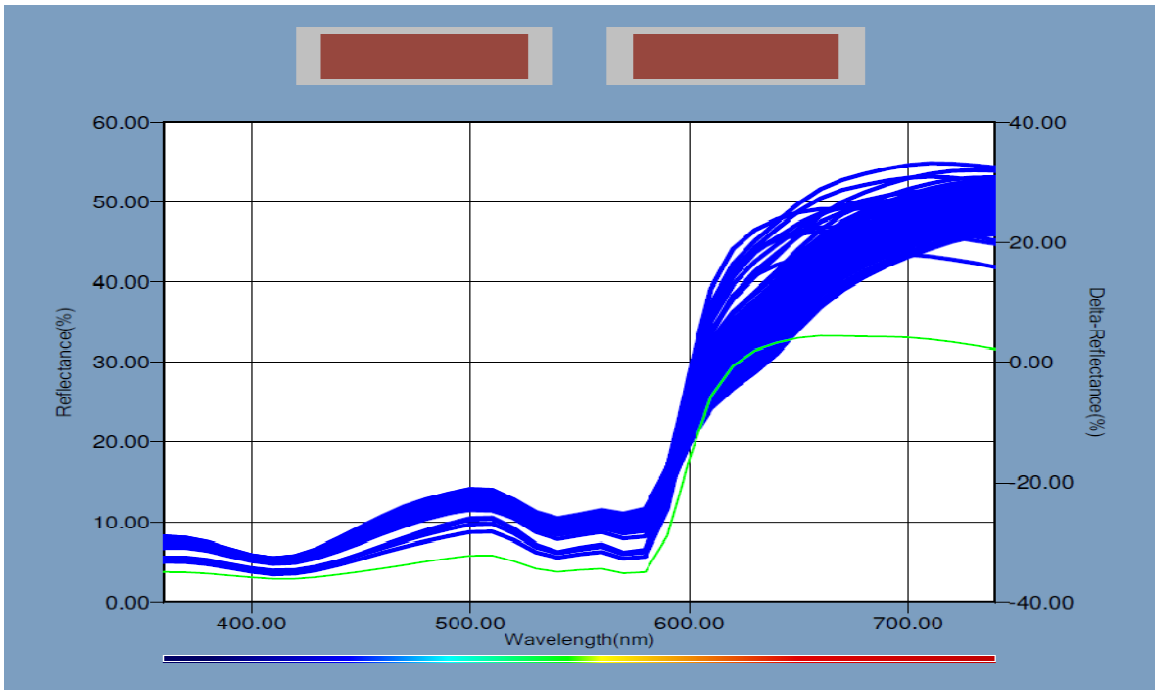


Figure 48: 5 runs of Blood on filter paper

Further mathematical values were obtained which included dividing and multiplying the chromatic values obtained to get a set of new values. Secondary values that used the difference between the chromatic values and then multiplying and dividing them to produce new numerical values was also done.

Time / mins	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)	da/db	da * db
0	40.732	33.366	20.842	0	0	0		
30	45.412	27.284	18.422	4.68	-6.082	-2.42	2.5132	14.718
60	44.850	23.156	16.622	4.118	-10.21	-4.22	2.4194	43.086
90	44.728	21.820	16.082	3.996	-11.546	-4.76	2.4256	54.959
120	44.596	21.264	15.808	3.864	-12.102	-5.034	2.4041	60.921
150	44.530	20.816	15.620	3.798	-12.55	-5.222	2.4033	65.536
180	44.446	20.378	15.454	3.714	-12.988	-5.388	2.4105	69.979
210	44.576	19.952	15.306	3.844	-13.414	-5.536	2.4230	74.260
240	44.374	19.698	15.146	3.642	-13.668	-5.696	2.3996	77.853
270	44.408	19.328	15.030	3.676	-14.038	-5.812	2.4153	81.589
300	44.264	19.192	14.936	3.532	-14.174	-5.906	2.3999	83.712
330	44.392	18.908	14.900	3.66	-14.458	-5.942	2.4332	85.909
360	44.544	18.576	14.808	3.812	-14.79	-6.034	2.4511	89.243
390	44.426	18.318	14.644	3.694	-15.048	-6.198	2.4279	93.268
420	44.446	18.142	14.608	3.714	-15.224	-6.234	2.4421	94.906
450	44.414	18.030	14.544	3.682	-15.336	-6.298	2.4351	96.586
480	44.424	17.828	14.498	3.692	-15.538	-6.344	2.4492	98.573

Table 14: Difference in chromatic values and new constants

A graph was plotted for the different new values that were calculated and are presented in the appendices for reference. The most reliable values that were observed to have a stable pattern used to get a numerical value of blood stain age estimation was from da*db.

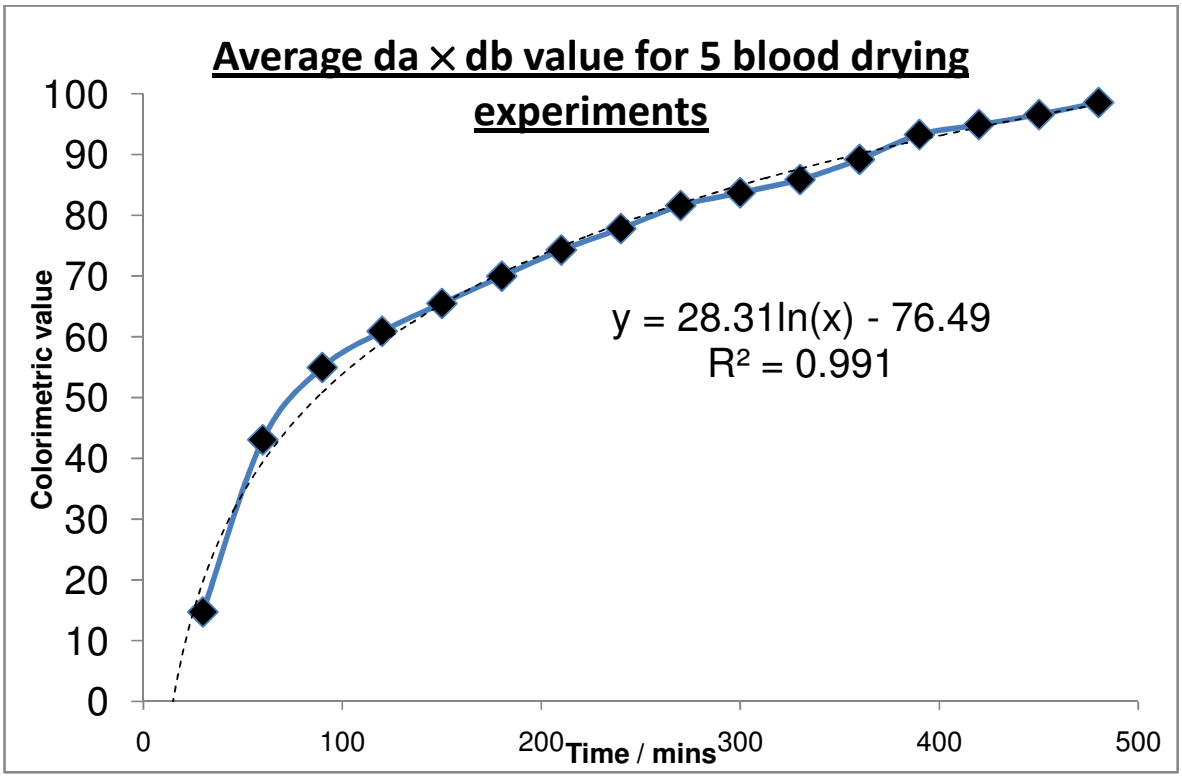


Figure 49: Average da x db value for 5 blood sample drying experiments

The graph produced showed a logarithmic increase in the average colorimetric values over time. This was seen as a basis to do more runs and try and compare the validity and reliability of these results. Therefore, 5 more samples were used but this time they were run at 10 minute intervals to see if there was any significant change over a period of short time.

Data Name (mins)	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)	a/b	da*db	da/db
0	45.960	33.563	22.810	------	------	------	1.471		
10	47.007	30.397	20.930	1.047	-3.167	-1.880	1.452	5.953	1.684
20	46.537	25.280	18.413	0.577	-8.283	-4.397	1.373	36.419	1.884
30	46.477	24.090	17.993	0.517	-9.473	-4.817	1.339	45.630	1.967
40	46.343	23.207	17.590	0.383	-10.357	-5.220	1.319	54.062	1.984
50	46.173	22.780	17.330	0.213	-10.783	-5.480	1.314	59.093	1.968
60	46.163	22.307	17.137	0.203	-11.257	-5.673	1.302	63.863	1.984
70	46.110	22.193	17.137	0.150	-11.370	-5.673	1.295	64.506	2.004
80	45.710	21.863	16.783	-0.250	-11.700	-6.027	1.303	70.512	1.941
90	45.680	21.470	16.600	-0.280	-12.093	-6.210	1.293	75.100	1.947

Table 15: 5 blood sample on filter paper run over 10 minute intervals

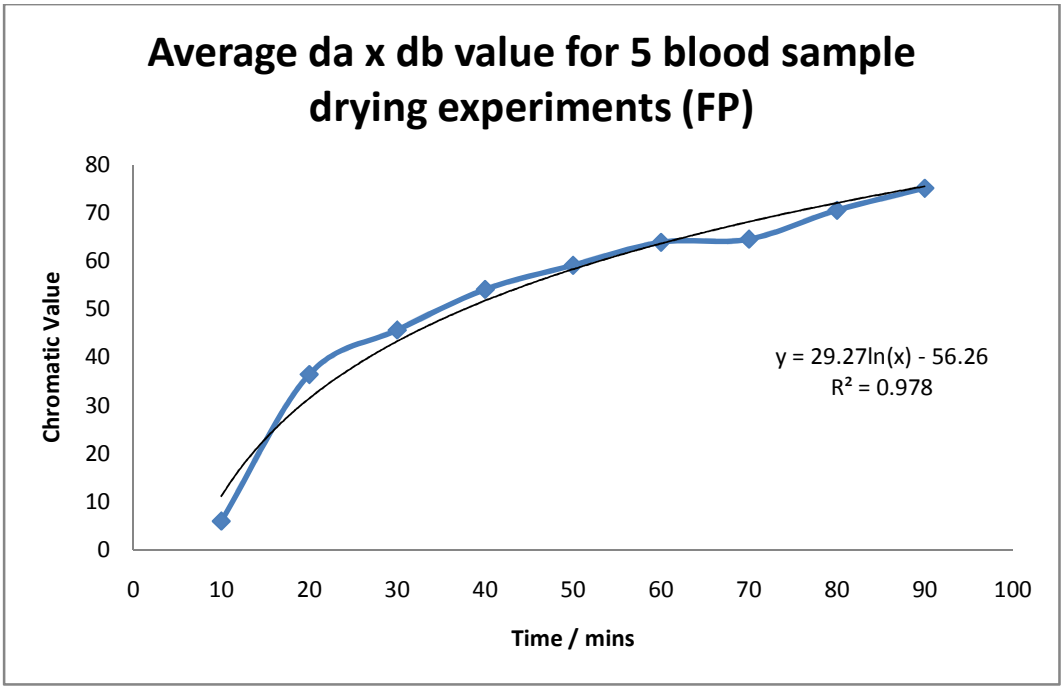


Figure 50: Average da x db value for 5 blood sample drying experiments on filter paper over 10 minute intervals

As can be seen from the both the graphs presented they produce a much similar pattern therefore the values da*db are taken aboard as being reliable to estimate the age of a bloodstain numerically.

3.3.2 White Cotton Cloth

Another issue that needed to be addressed was the type of surface our bloodstain was being produced on. In order to cover all the bases, a secondary surface than that to filter paper was used. A small piece of white cotton cloth was used on which blood was placed and left to dry. These samples were then tested using the same conditions. A total of 5 samples were run all from the same source and these were run at 30 minute intervals. The average values for all the runs are tabulated below.

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)	a/b	da*db	da/db
0	35.132	37.680	20.170	------	------	------	1.868		
30	45.040	25.912	17.556	9.908	-11.768	-2.614	1.476	30.762	4.502
60	44.794	22.336	16.222	9.662	-15.344	-3.948	1.377	60.578	3.887
90	44.556	21.244	15.708	9.424	-16.436	-4.462	1.352	73.337	3.684
120	44.460	20.508	15.480	9.328	-17.172	-4.690	1.325	80.537	3.661
150	44.428	20.244	15.344	9.296	-17.436	-4.826	1.319	84.146	3.613
180	44.756	19.700	15.196	9.624	-17.980	-4.974	1.296	89.433	3.615
210	43.224	18.346	14.796	8.092	-19.334	-5.374	1.240	103.901	3.598
240	44.004	18.826	14.600	8.872	-18.854	-5.570	1.289	105.017	3.385
270	44.528	18.636	14.772	9.396	-19.044	-5.398	1.262	102.800	3.528
300	44.308	18.724	14.812	9.176	-18.956	-5.358	1.264	101.566	3.538
330	44.460	18.294	14.620	9.328	-19.386	-5.550	1.251	107.592	3.493
360	44.192	18.214	14.568	9.060	-19.466	-5.602	1.250	109.049	3.475
390	44.012	18.032	14.502	8.880	-19.648	-5.668	1.243	111.365	3.466
420	44.442	17.636	14.430	9.310	-20.044	-5.740	1.222	115.053	3.492
450	44.560	17.330	14.318	9.428	-20.350	-5.852	1.210	119.088	3.477

Table 16: Averaged values of 5 sample of blood on white cotton cloth

A graph was produced representing the change in reflection and wavelength again showing the same trend as in the filter paper experiments.

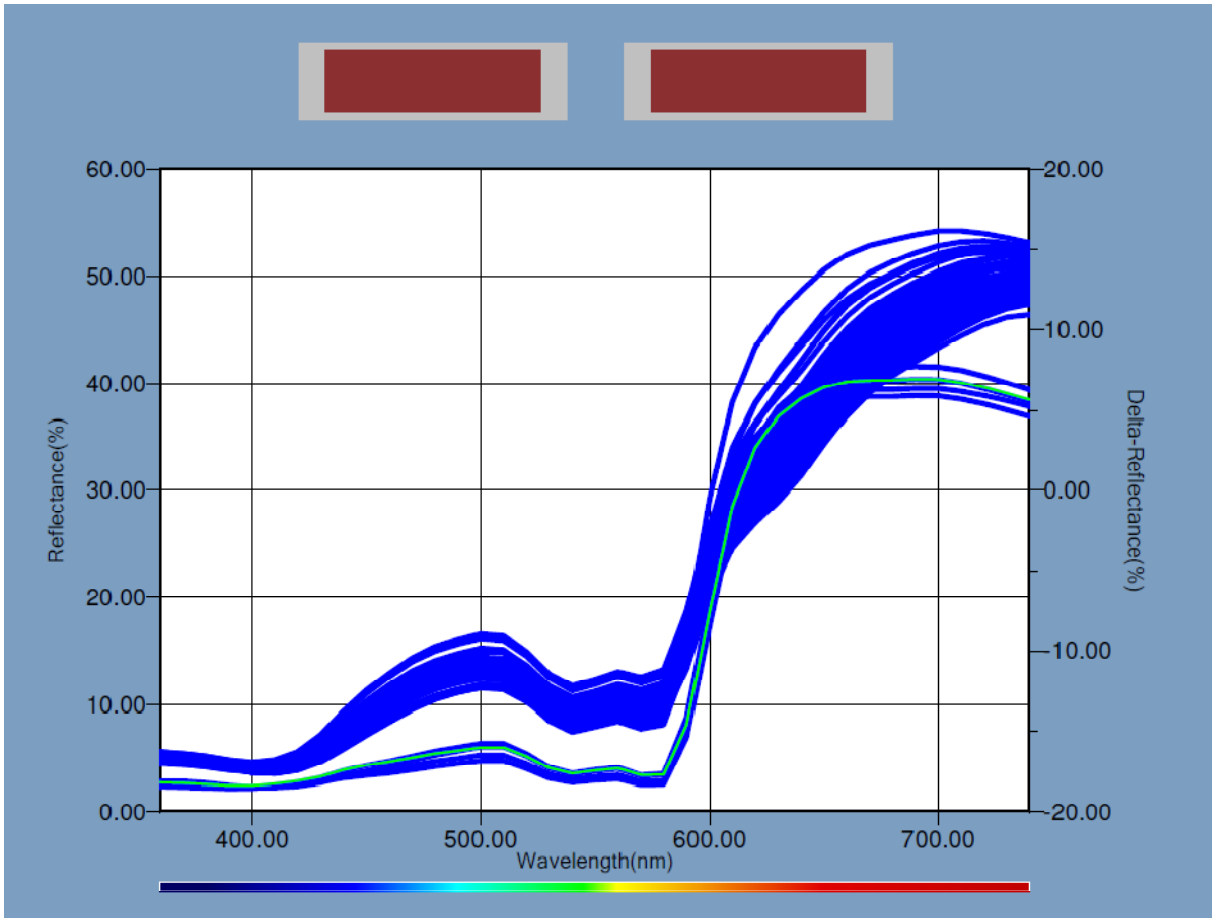


Figure 51: Averaged values of 5 sample of blood on white cotton cloth

Using the values acquired and the ones calculated, a graph was produced for da*db against time.

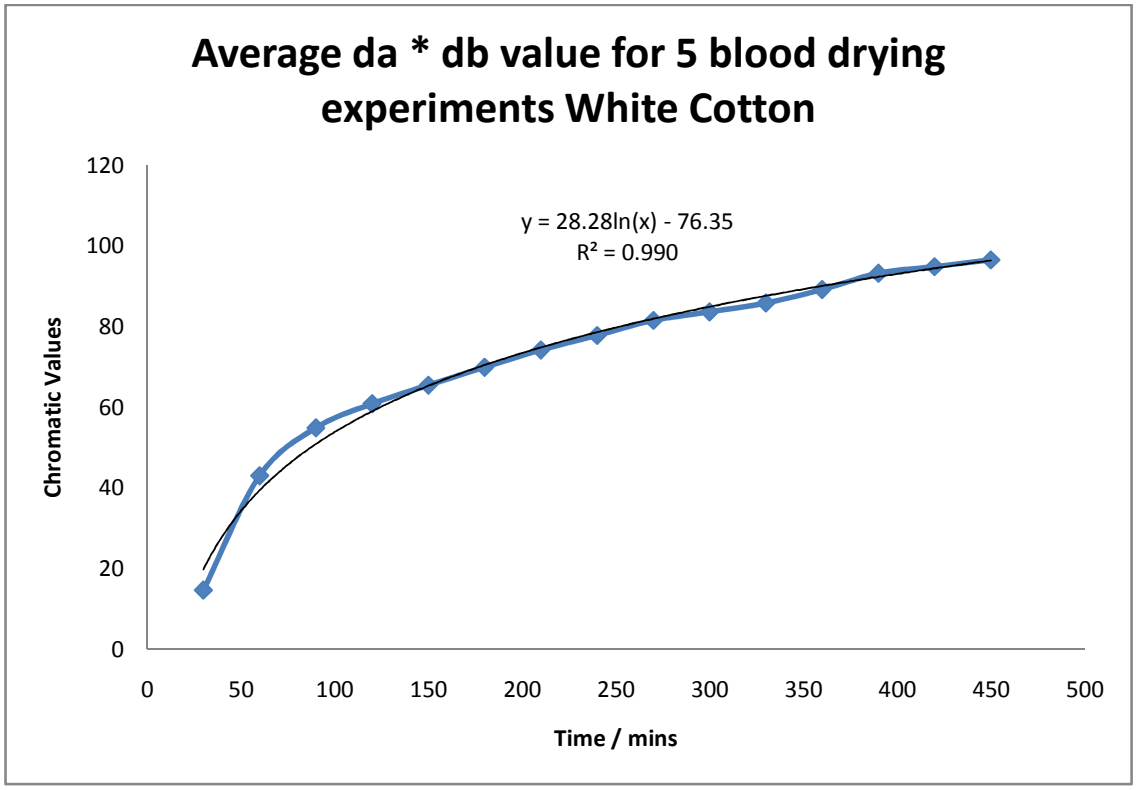


Figure 52: Average da * db value for 5 blood drying experiments White Cotton

The values represented in the graph correspond to the same trend as seen earlier for samples that were run on blank white filter paper. This provides us with a conclusion that the type of surface does not play that much of an important role in disrupting reading by using a spectrophotometer. In order to confirm the similarity of our readings, a graph was produced to represent both sets of data i.e. blank whit filter paper and white cotton cloth. This particular graph was presented using the values da/db for both sets of data.

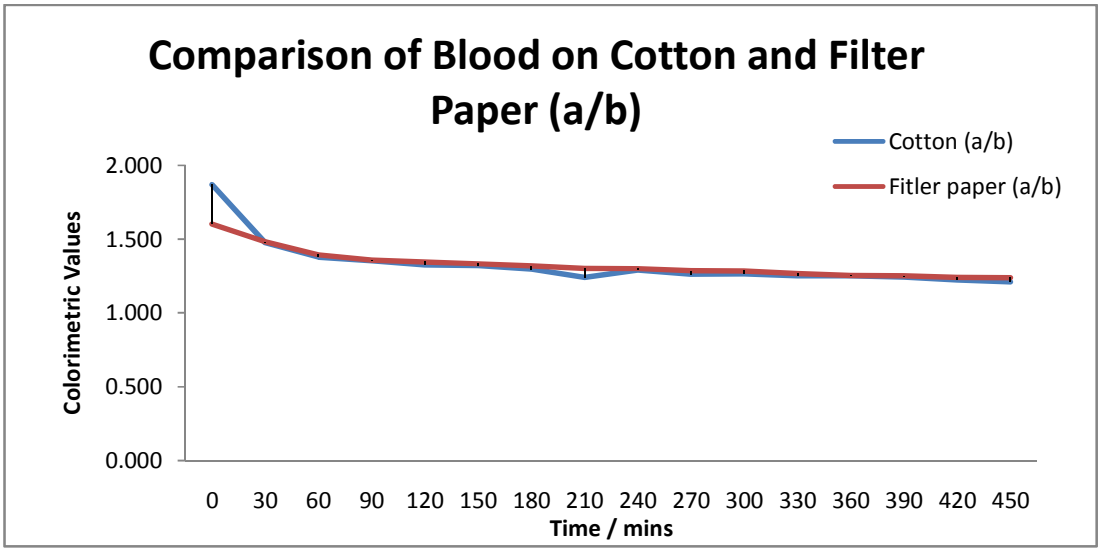


Figure 53: Comparison of Blood on Cotton and Filter Paper (a/b)

As it can be seen, there is not much difference between the values for both surfaces.

3.3.3 Blue Denim

Another consideration that was taken into account was the fact that the technique being used relied on colour so an investigation needed to be done as to see what effect did coloured backgrounds have on the reading for our stains. In principle, background subtraction should eliminate most of the background colour influence on our sample and the readings should only be that of blood. This in turn should give us a similar pattern in the values that have been previously used.

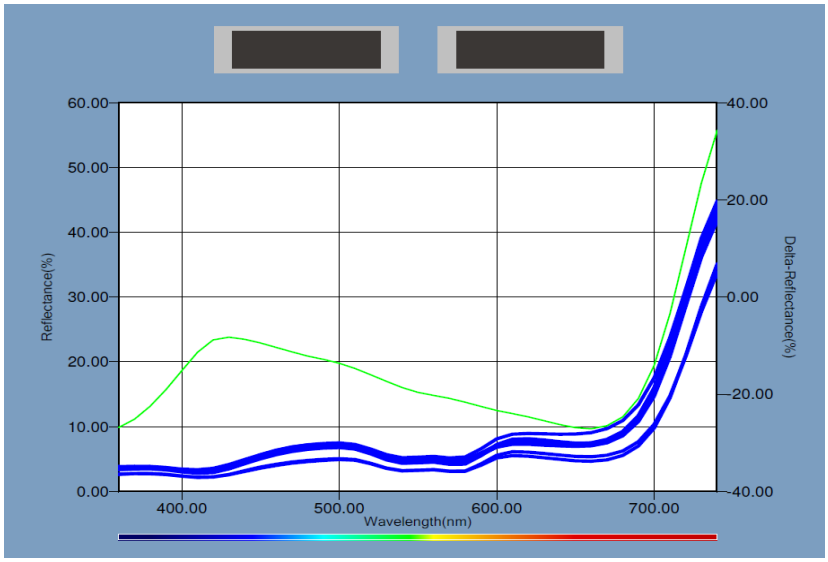


Figure 54: Blood on blue denim

The generic colour for these particular samples was a bluish red shade and presented with a different pattern of reflection and delta reflection when compared with previous surfaces. This was expected as there would have been background influence on the colour of the blood that was placed on the blue denim surface.

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)	da*db	da/db
0 mins	23.935	2.230	2.530	4.330	0.380	0.105	0.040	3.619
30 mins	28.265	2.610	2.635	4.890	-0.675	-0.185	0.125	3.649
60 mins	28.825	1.555	2.345	4.930	-1.275	-0.410	0.523	3.110
90 mins	28.865	0.955	2.120	5.155	-1.180	-0.390	0.460	3.026
120 mins	29.090	1.050	2.140	6.015	-0.355	0.445	-0.158	-0.798
150 mins	29.950	1.875	2.975	5.315	-1.310	-0.835	1.094	1.569
180 mins	29.250	0.920	1.695	-23.935	-2.230	-2.530	5.642	0.881

Table 17: 3 sample of blood on denim over 30 minute intervals

The results were taken from blood samples on 3 denim pieces. These were measured at 30 minute intervals and provide us with the following graph.

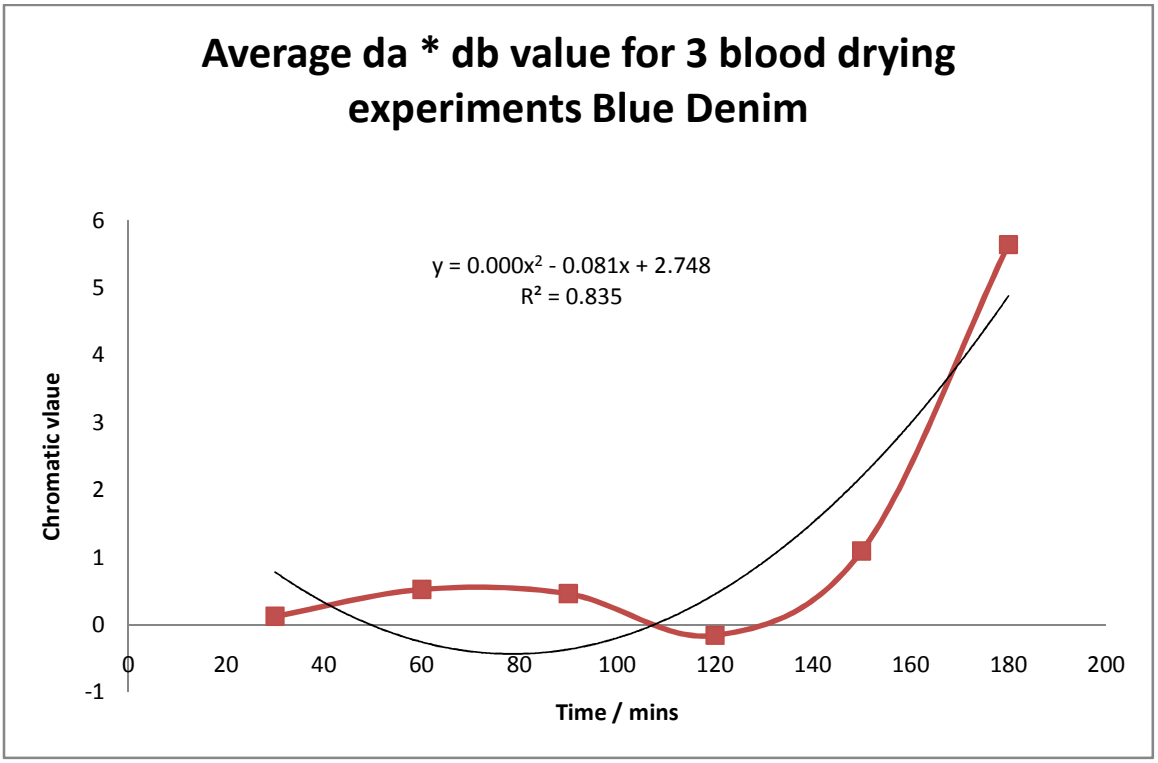


Figure 55: Average da * db value for 3 blood drying experiments Blue Denim

The results show a completely different pattern to what had been obtained previously on non coloured surfaces. As expected and mentioned earlier, the blue back ground of the denim surface plays a vital role in influencing the results of obtained for this experiment. The blue colour seems to influence the reading in such a way where the colour of the blood stain on the surface appears to have a much more bluish colour rather than the usual dark red colour seen previously on our other samples. It also needs to be taken into account that 2 different background subtractions were carried out for these sample. One subtraction was taken from the blue coloured denim surface and the other from the blood as soon as it was dried. This was done in order to get the difference in chromatic values from the initial blood stain at a theoretical time 0 against chromatic values taken at different time intervals. The double background subtraction also leads to gaining chromatic values that are quite low to when compared with values obtained from white backgrounds.

3.4 Protein Identification

The samples, as mentioned earlier were collected using plastic viscose swaps and were treated with BCA as well as Copper Sulphate in aqueous solutions (Refer to section on BCA). Initially, in order to test he validity and the fundamental working of this technique, a known protein was tested. The protein used was Bovine Serum Albumin (BSA) and a 1% concentrated solution was made up in water. Approximately 1.5 ml of this solution was added to 2 glass vials which were then treated with our reagents. The colour of both the solutions turned light green and approximately 70 minutes later, one of the vials which contained the protein solution turned purple whereas the other vial that contained distilled water along with the reagents remained light green. The colour change was noted and the time it took for the colour to change was also recorded.

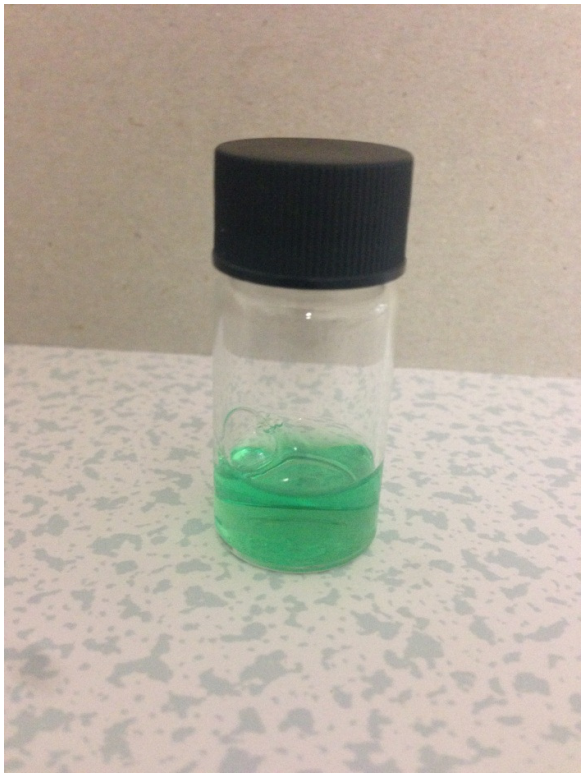


Figure 56: Initial colour of bovine serum albumin

Figure 57: Final colour of bovine serum albumin solution

A total of 10 different samples were treated with BCA in order to investigate as to how long it would take on average for the sample colour to change. From all these runs, it was estimated that it took between 60 to 90 minutes for the colour to be fully developed without introducing the samples to any heat source. If the samples were heated using a hot-plate, the time of development decreased to 30 minutes at 60^{o}C and to 60 minutes at 35^{o}C.

The samples were then analysed using the Nanodrop 2000 spectrophotometer. The results provided absorbance in the region of 562 nm as stipulated earlier in the BCA section.

#	Sample ID	User Name	1(nm)	1(Abs)
1	Solution with Protein +ve	Admin	562	1.085
2	Solution without Protein -ve	Admin	562	0.034

Table 18: Average Absorbance value for control test

A graph was produced by the machine that showed the specific peaks in question at 562 nm wavelengths.

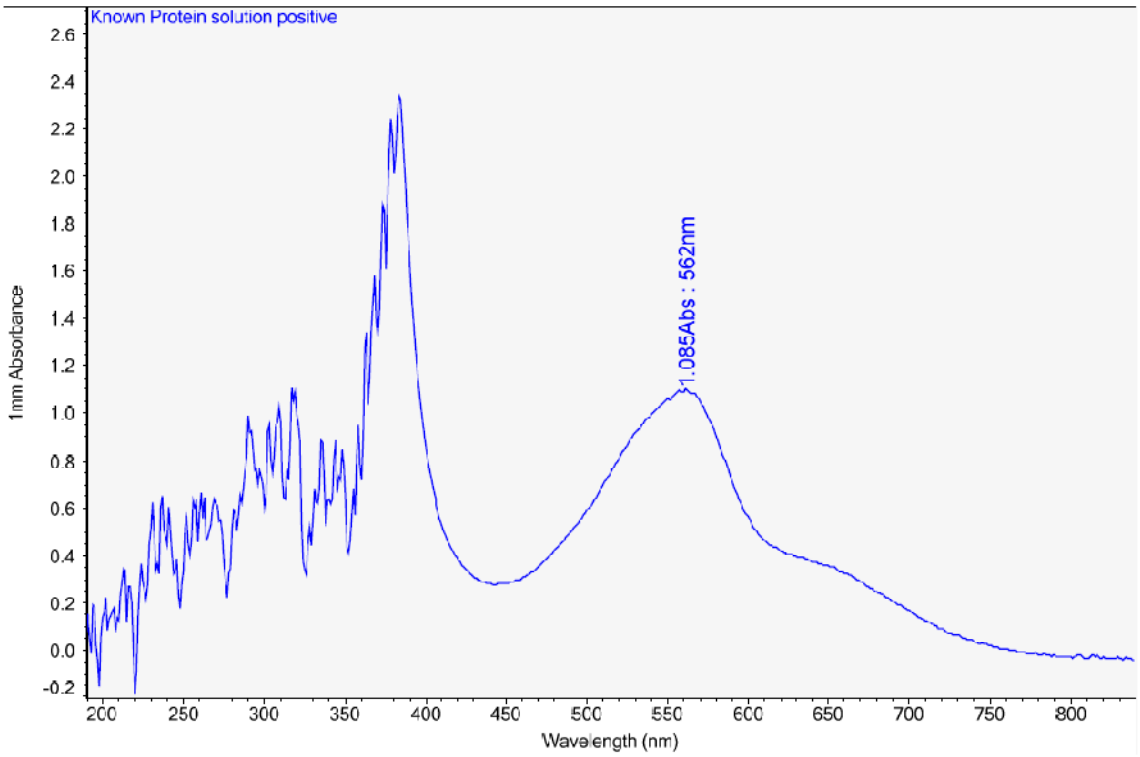


Figure 58: Absorbance at 562 nm for Positive Protein Reaction

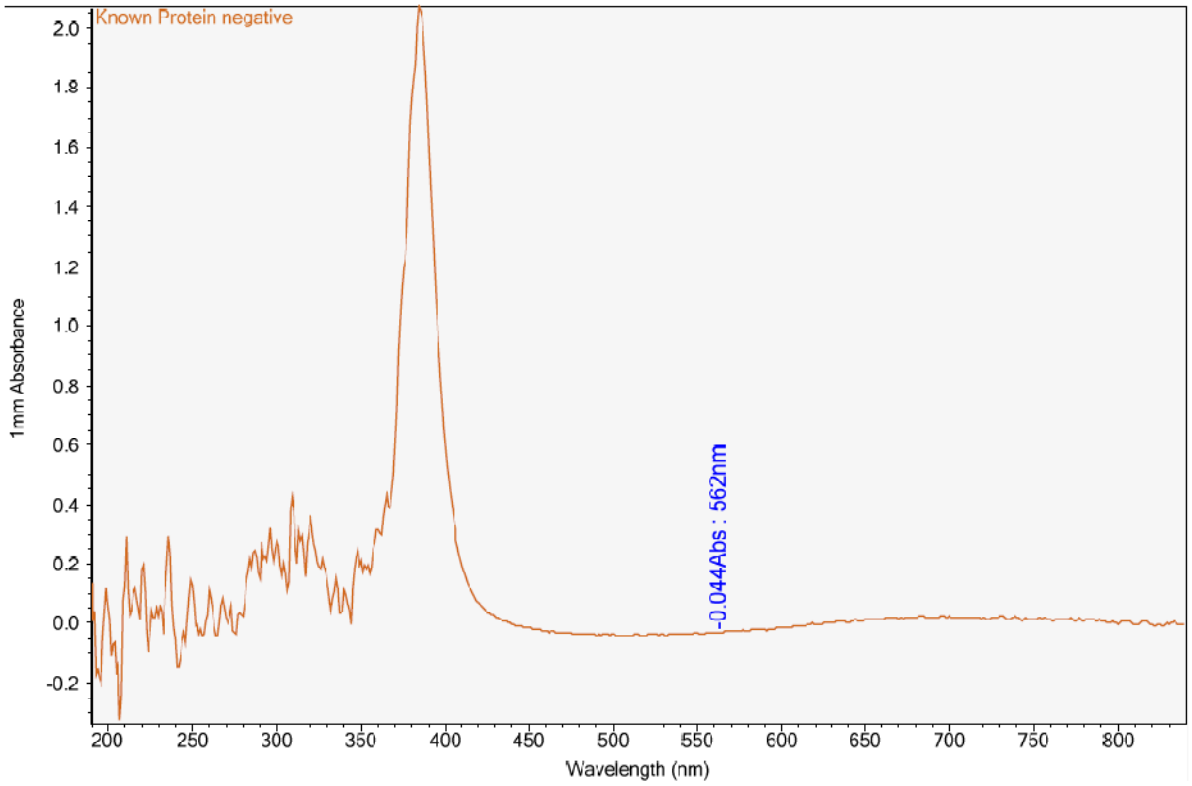


Figure 59: Absorbance of 564 nm for Negative Protein Reaction

The next step was to start work with skin cells. The process used was similar to that used in the initial study but instead of using glass vials, plastic viscose swabs were used. Skin scrapings were taken using these swabs that were moistened with distilled water before the scrape. The swap was used on the fingers and the skin region of around the nails. This was done in order to attain the most skin cells that could be gathered from shedding. The samples were treated the same way and after approximately 80 minutes, the colour of the solution in the swab containers was seen to change to purple from light green. A negative sample was also run which was that the swab was scraped against the hand that had a glove on.

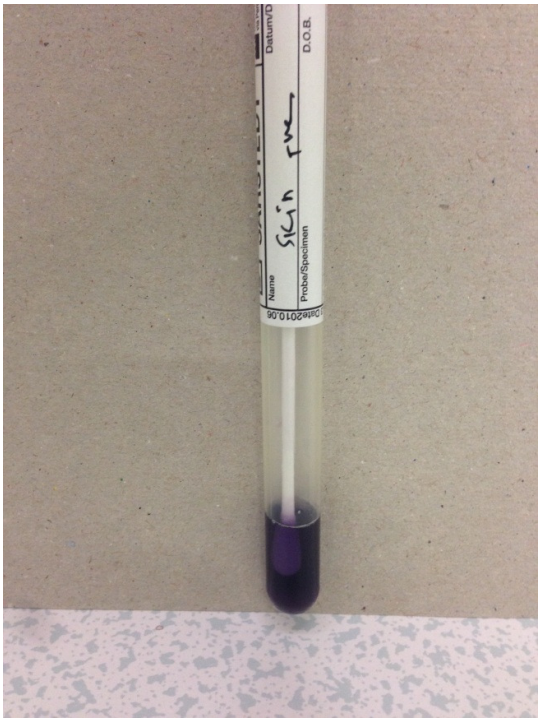


Figure 60: Positive test for skin cells

The samples were run on the spectrophotometer to attain the value of absorbance. The value of absorbance was estimated to be a lot lower than that obtained for BSA as the concentration of the solution used for that part was estimated to be a lot higher than the recovered skin cells.

#	Sample ID	User Name	1(nm)	1(Abs)
1	Skin Cells Positive	Admin	562	0.066
2	Skin Cells Negative	Admin	562	0.032

Table 19: Average Absorbance values for skin cells

Seeing that this particular process worked, the project was proceeded to identify skin cells from surrounding surfaces. An area on the bench top of our lab was designated for this experiment. One section was used to mimic conditions of a normal surface where numerous had touched it and the other area was cleaned of firstly using a detergent wash and then to be safe, by using a Microsol solution. 2 swabs were taken from each surface

and treated with the BCA and copper sulphate reagents and left to develop. After approximately 70 minutes, a colour change was seen on both the swabs.

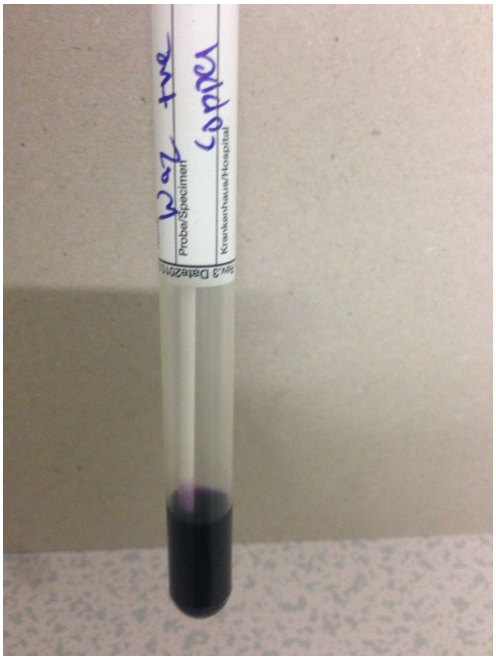


Figure 61: Positive test result from surface

Figure 62: Negative test result from surface

The swab that was taken from the free surface where contact was made without using gloves turned completely purple as expected. The negative however also showed a purple layer that had formed closer to the swab and the same light green colouration at the bottom as seen for the previous negative results. The positive colour change in our negative test could have come from contamination. The fact that the surface was not a covered or isolated area could mean the presence of dust particles or just proteins from the surrounding to have interfered. However this was not a surprise as a non-isolated system would have a lot of interference from the surroundings. There could also be some protein or biological material that could have been left behind and not properly cleaned initially. The experiment was repeated with 4 more samples and the same colouration was observed in all the sample tubes. The samples were run on the spectrophotometer to attain absorbance data.

#	Sample ID	User Name	1(nm)	1(Abs)
1	Surface Positive	Admin	562	1.132
2	Surface Negative	Admin	562	0.032

Table 20: Averaged Surface Absorbance values

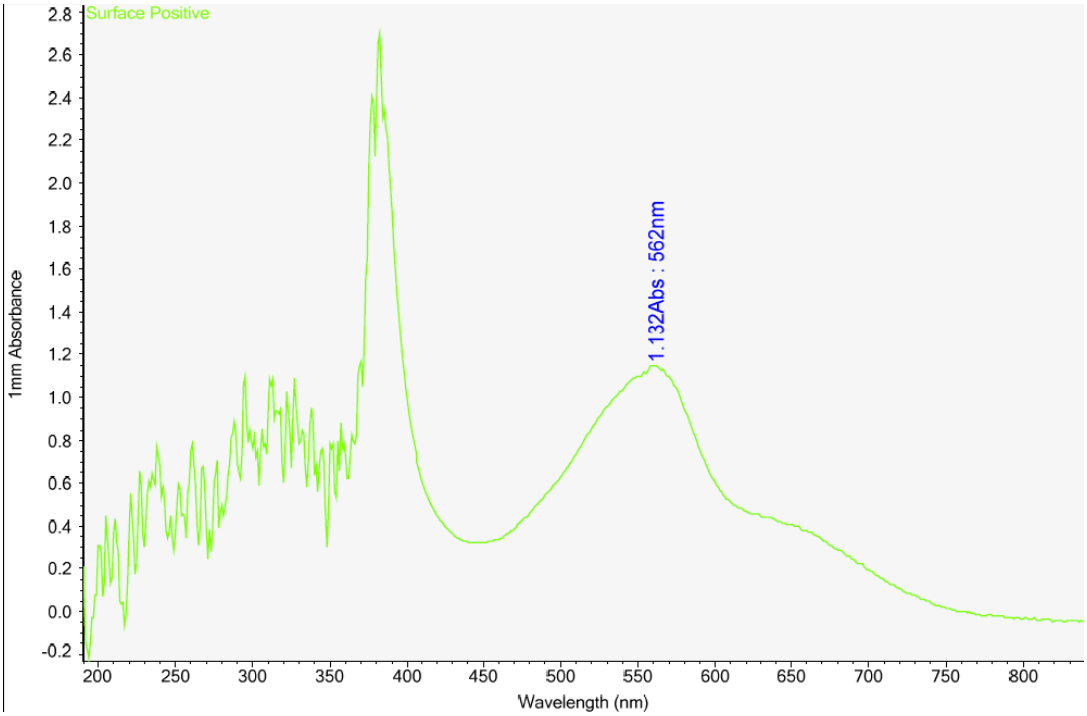


Figure 63: Surface Positive Result

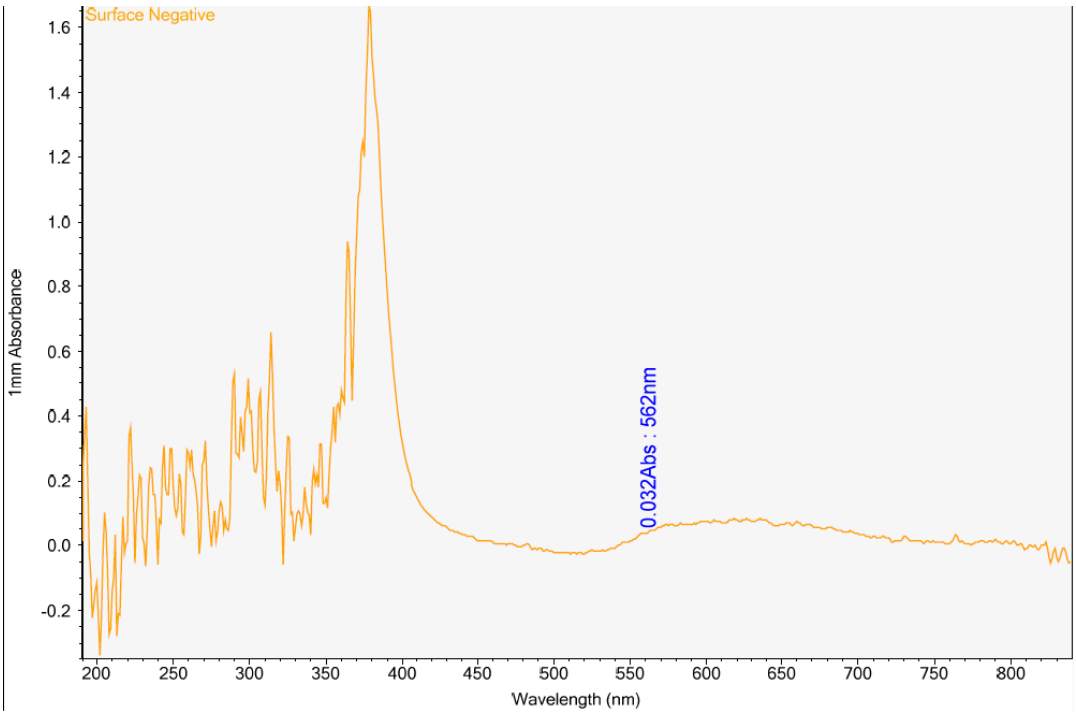


Figure 64: Surface Negative result

The absorbance for the sample that gave a positive test result is quite similar to that of the control BSA sample that was run earlier. This gives an idea that the concentration of protein material on this particular sample was a lot higher than the other samples that also provided a positive test result. This can be validated by the fact that there was lot higher presence of proteins on the surface that was handled by bare hands. External influence of the environment and the non-isolation of the surface can also be a factor that resulted in a higher absorbance reading.

The next step in the project was to quantify and analyse the amount of DNA that might be present in our samples. The skin cell samples run earlier were run through DNA extraction after the colour test had been performed on them. Qiagen DNA Amplification kit was used to do this and the protocol supplied by the manufacturer was performed. Quantiplex was performed on the extracted samples to determine the amount of DNA that might be present.

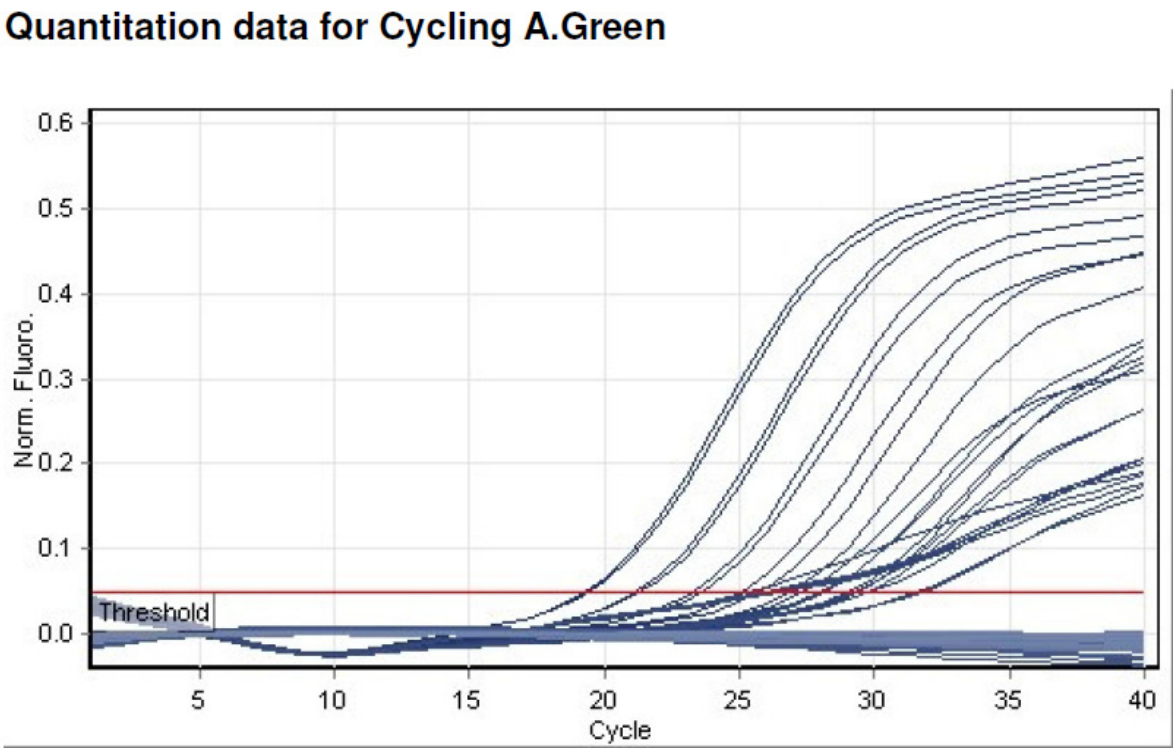


Figure 65: Quantitation Data describing the fluorescence of our samples

A standard curve is also provided by the machine itself which can be used to check the validity of our results.

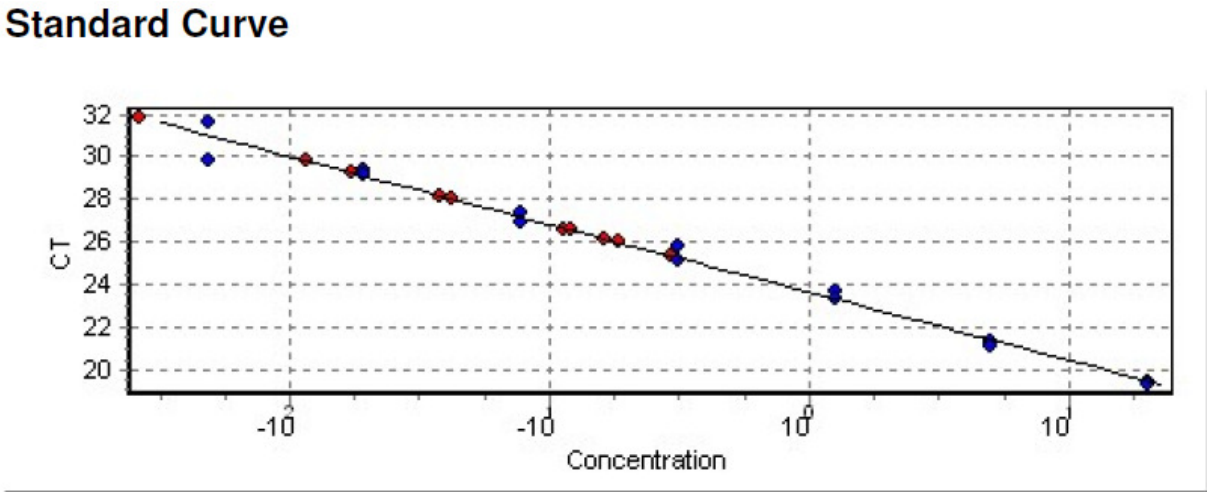


Figure 66: Standard Curve for Quantitation for samples and standards

The concentration values determined are presented below. These values were taken for two samples of skin cells. One represents a positive test result when run through the colour test and the other provided a negative result.

No.	Name	Type	Ct	Given Concentration (ng/µl)	Calculated Concentration (ng/µl)
9	Control DNA Z1, 0.078125 ng/µl	Standard	27.36	0.781	0.6761
10	Control DNA Z1, 0.078125 ng/µl	Standard	26.95	0.781	0.9132
11	Control DNA Z1, 0.01953125 ng/µl	Standard	29.43	0.195	0.1516
12	Control DNA Z1, 0.01953125 ng/µl	Standard	29.12	0.195	0.19
13	Control DNA Z1, 0.0048828125 ng/µl	Standard	31.6	0.0048	0.00316
14	Control DNA Z1, 0.0048828125 ng/µl	Standard	29.84	0.0048	0.0113
15	NTC	NTC	25.34		0.29192
16	NTC	NTC	26.15		0.16265
17	1a-1	Unknown	28.01		0.0424
18	1a-2	Unknown	28.18		0.03754
19	1b-1	Unknown	26.64		0.11392
20	1b-2	Unknown	26.56		0.12078
21	2a-1	Unknown	29.79		0.01172
22	2a-2	Unknown	29.24		0.01747
23	2b-1	Unknown	25.98		0.1833
24	2b-2	Unknown	31.84		0.00266

Table 21: Quantitation values for skin samples

Samples 1a and 1b was the first set of skin cells that was run using the colour test. Sample 1a provided us with a negative colour test result and this can be confirmed by the concentration values provided by the Quantiplex results. The 2 aliquots of sample 1a both showed extremely low values for DNA. Sample 1b showed a positive result in the colour test and according to the concentration values, both aliquots showed 0.1 ng/µl concentrate of DNA. Sample 2a provided negative colour test results and again can be confirmed by the concentration values. Both samples gave very low values for DNA. Sample 2b gave a positive colour test result and according to the concentration values acquired by the Quantiplex, aliquot 1 showed an almost 0.2 ng/µl concentrate of DNA. However, the second aliquot showed extremely low quantities of DNA. This could be due to errors that may have been caused during the extraction process of the sample.

One of the main reasons in doing the quantitation experiment was to see if there was enough DNA so profiling can be performed. The required concentration which is for profiling is t 0.2 ng/µl approximately. According to our obtained results, the quantity of pure DNA detected is almost enough to be run for profiling. The results can be improved by repetitive experiments on a much larger sample size. This could reduce any errors that might occur during the experimental process and can provide with more accurate results.

Another factor that needed to be established was to determine the limit of detection for our colour tests. This was performed using the samples that were used for quantitation as the concentration of those samples is already known. A 10 fold dilution was performed and analysed in the spectrophotometer.

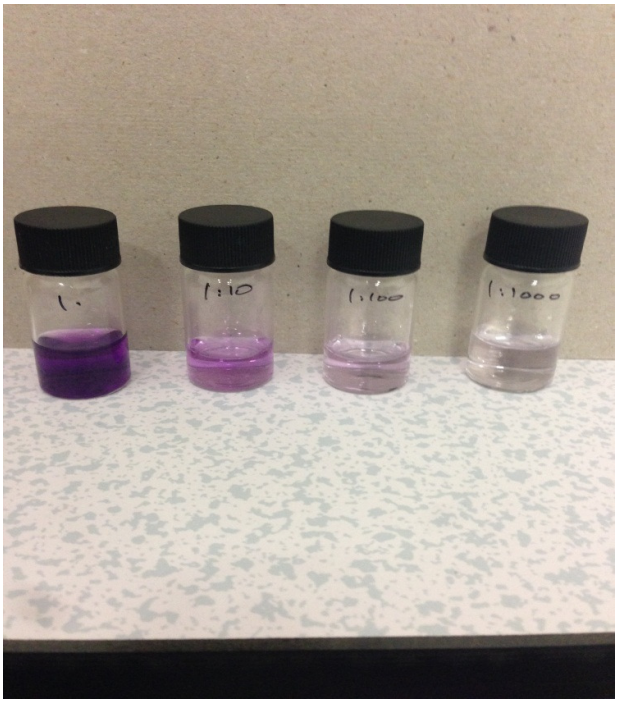


Figure 67: Dilution studies

A clear colour distinction was seen upon the addition of BCA and copper sulphate. All the samples did provide a positive result as they did have DNA present.

#	Sample ID	User Name	1(nm)	1(Abs)
1	Sample 1:1	Admin	562	0.136
2	Sample 1:10	Admin	562	0.026
3	Sample 1:100	Admin	562	0.009
4	Sample 1:1000	Admin	562	0.001

Table 22: Absorbance values for dilution samples

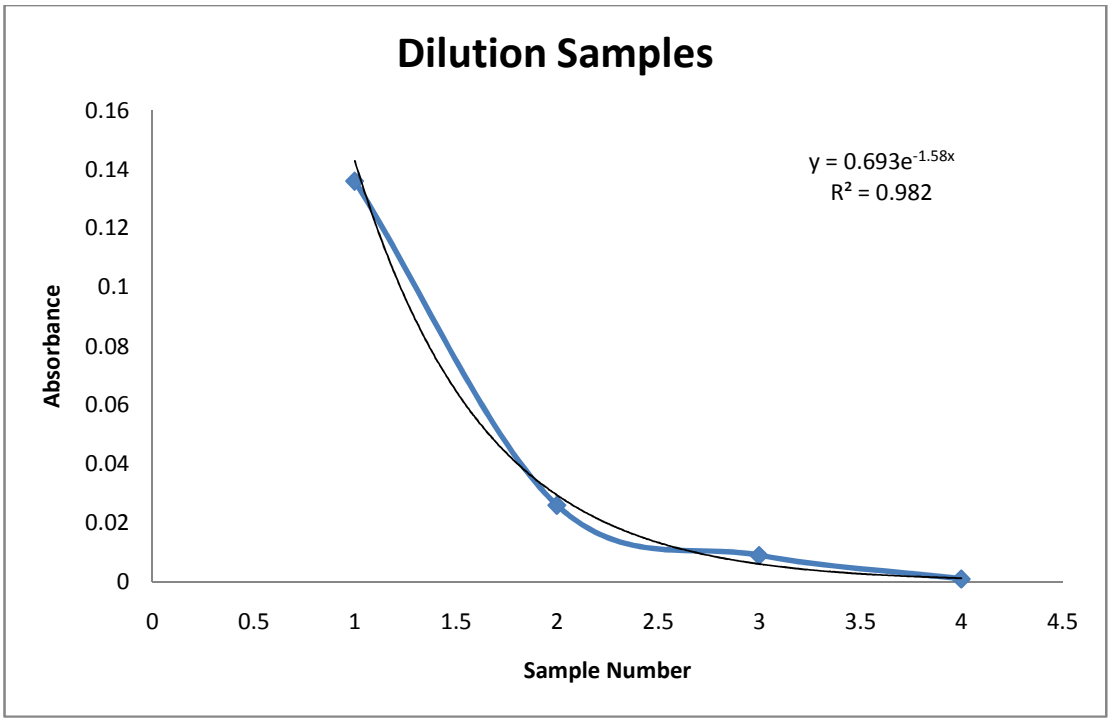


Figure 68: Dilution samples

The decrease in absorbance follows an exponential decrease. The samples seem to respond to the colour change reaction to up to a 1:100 dilution. Any sample more dilute than that does not give a noticeably change in colour. According to absorbance values, the value is extremely low as well when tested on the spectrophotometer.

In order to investigate the colour effects of other transition metal complexes on reaction with BCA and a protein, a number of samples were run having both a positive and a negative. Different transition metal complexes were run which presented with different coloured solutions in aqueous compounds. The concentration of all the solutions was kept constant at 4% by weight in a water solution.

Transition metal complex	Initial Colour	Development time	Final Colour
Chromium Oxide	Green Solution	80 minutes	Green
Vanadium (V) Oxide	Orange solution	80 minutes	Faded Orange
Cobalt (II) Oxide	Greyish Black solution	80 minutes	Greyish Black
Copper (II) Hydroxide	Dark Greenish Blue Solution	80 minutes	Violet
Chromium (III) Sulphate	Aquamarine solution	80 minutes	Light green

Table 23: Colour test using different Transition metal complexes

As can be seen from the table above, the only transition metal complex solution that gave a significant colour change was that of Copper Hydroxide. The appearance of the stock solution showed that the compound itself was insoluble in water and therefore when the solution was added to our sample, the vial had to be stilled and a suspension was pipetted into our container having the sample present. The colour of the solution turned from a greenish blue to a bright violet.

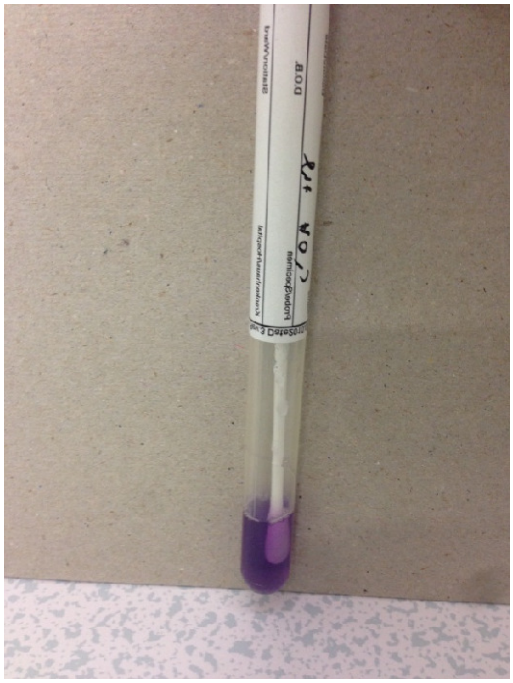


Figure 69: Copper Hydroxide Positive

The negative solution also turned colour to a very pale purple with a hint of blue. The colour changed a lot faster for this complex and due to leaving it for a full 80 minutes, the negative started to change colour as well. If the solution had been analysed after 60

minutes instead of 80, the negative colour would have been seen to remain a greenish blue and only the positive would have changed colour to violet.

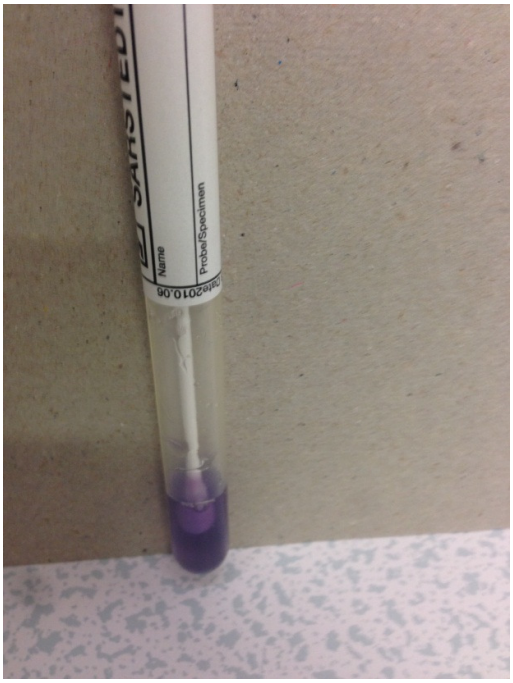


Figure 70: Copper Hydroxide Negative

The samples were also run on the spectrophotometer to see if there were any distinguishable absorbance peaks at 562 nm or were it similar to those achieved when a copper sulphate solution was used.

#	Sample ID	Username	1 (nm)	1 (Abs)
1	Copper Hydroxide +ve	Admin	562	0.098
2	Copper Hydroxide-ve	Admin	562	0.068

Table 24: Average Absorbance Values for Copper Hydroxide

A graph was produced showing the absorbance values for these samples.

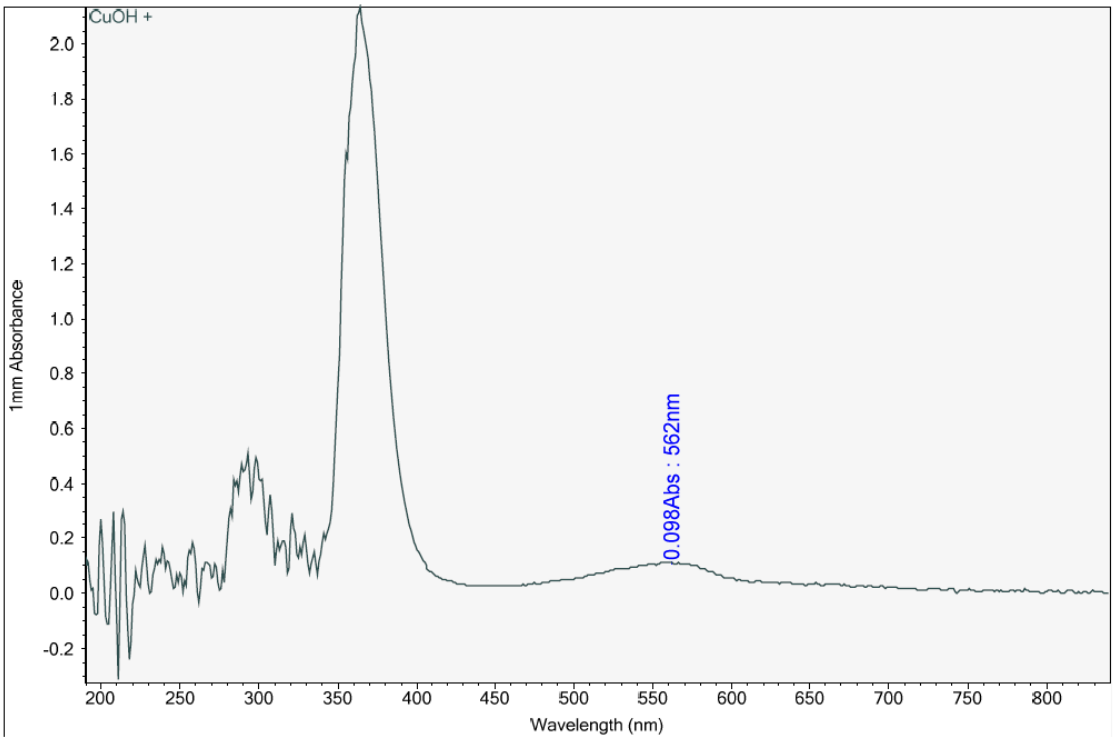


Figure 71: Absorbance for Positive Copper Hydroxide Solution

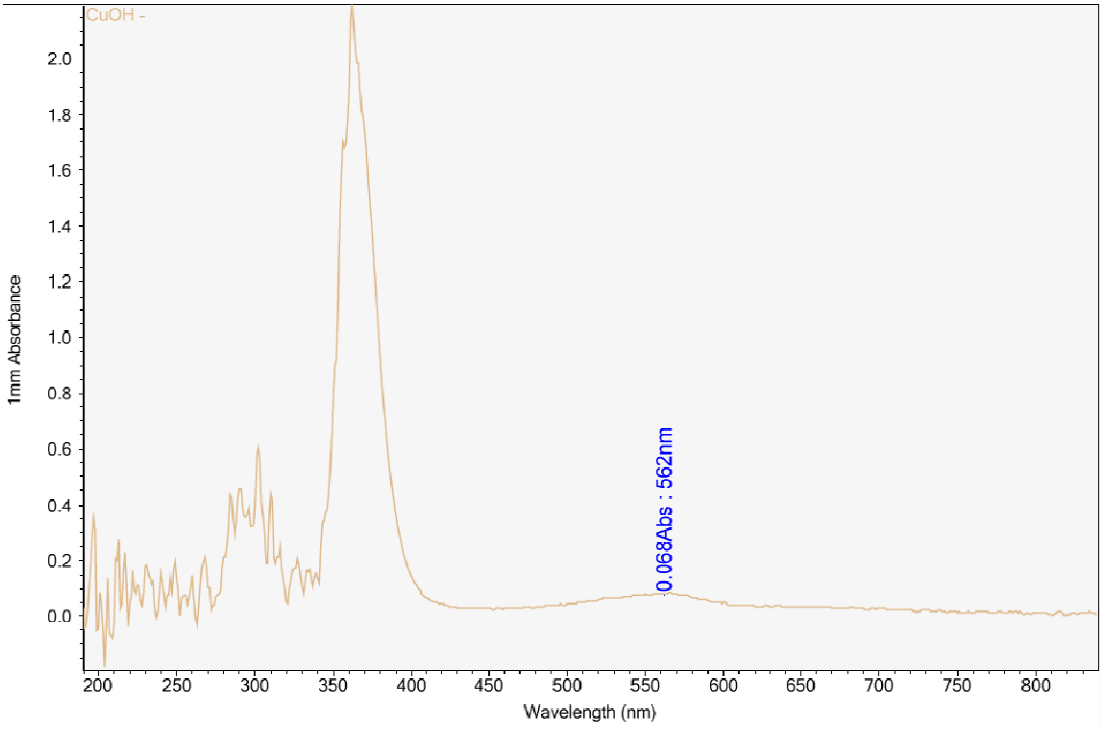


Figure 72: Absorbance of Negative Copper Hydroxide Solution

According to the results obtained from these runs, it can be seen that the absorbance value for the positive is a lot higher when compared to the reaction undergone with copper sulphate. Both the samples were taken from the same source i.e. skin swabs from the same person and left to develop for the same time. The only difference is that during the development time, the negative solution also presented with a colour change. If the run had been analysed after 60 minutes, the values might have been lower. Copper hydroxide can therefore also be used for this particular reaction and can add a certain novelty to our process as almost all other protein identification/quantification reactions using BCA have been performed using a copper sulphate solution instead of copper hydroxide.

4.0 Discussion

Keeping in view all the techniques used in this project, certain key points can be highlighted to the validity and the promise of these techniques in the field of bloodstain ageing.

Raman Spectroscopy is probably the most promising technique that can be used and further researched upon for estimating the age of a bloodstain. The issue that we had with this particular technique was the problem of not having the correct wavelength to measure the compounds and the changes that take place to these compounds in blood. Nonetheless, we were able to identify almost all of the known peaks of the different components in blood both human and equine. There were certain changes that were recorded by the 1064 nm Raman Spectrometer about the dissociation and degradation of blood over a certain period of time. By using the 785 nm wavelength, we were able to confirm our results from the 1064 nm for the identifying peaks of compounds such as porphyrin, fibrin and chromogen compounds that are all a part of blood and more specifically haemoglobin. We also noted some changes in the peaks data for aged blood where certain peaks shifted towards the right side and formed new peaks. This is related

to the degradation of complex molecules in blood which result in changes to the peaks of the same sample. This is crucial information in regards to bloodstain ageing as over time, depending on the surrounding conditions, blood will go through numerous chemical changes and a technique that is able to record and identify these changes can be essential is solving this mystery. The non-invasive nature of this technique along with the quickness in obtaining the results also adds more strength to this technique. A lot of techniques currently being employed usually take a lot longer to ascertain results and end up destroying the sample itself. The portability of this instrument is also a key factor in making this technique extremely useful as it can easily be taken to crime scenes and results can be obtained there and then. This eliminates or rather reduces the risk of contamination that can come about in the packaging and transport of evidentiary material.

Small Angle X-Ray Scattering (SAXS) is unfortunately not a very suitable technique when it comes to the analysis of blood over time. One of the biggest drawbacks to this technique is the sheer size of the machine itself. This makes is near to impossible for this machine to be used on crime scenes along with the time it takes to prepare the samples for testing. The technique itself can provide detailed description on the structure of different complexes that are present in the sample but the amount of time needed for mathematical analysis on the data to reach those results is a factor that reduces the practicality of this technique. The data interpretation is not only time consuming but also very complicated thus making it unfavourable for regular use. The invasiveness of the technique itself is also something under question as it is still not quite clear on how the sample reacts to being attacked by the X-Ray beam for the amount of time needed to get a reading. Another important issue regarding this technique is the expense that comes with it in running the samples.

Colorimetric analysis is a much simpler and a much more cost effective technique that can be used for bloodstain ageing. The technique requires minimal setup and can provide detailed results regarding structures and compounds that can be present in a sample. According to the results obtained from our experiments, it can be seen that the technique provides good and reliable information in the colour changes that take place over time in a blood sample. These changes can then be correlated to chemical changes taking place in the blood system over time which include the degradation of haemoglobin and changes that occur on proteins due to the influence of the surrounding environmental conditions. The only drawback to this technique is the influence of surface colours on the results as discussed earlier when samples were run on blood. As far as samples on white surfaces are concerned, the technique can provide solid information regarding the age of a bloodstain.

From the results obtained after performing the colour reactions for protein identification, it can be determined that the technique is viable for use in-situ to identify the presence of proteins. There still needs to be a lot of validation works that needs to be done. The process seems to be safe for use on samples that contain DNA as demonstrated by the skin cell experiment. The samples were first run through the colour test procedures and then went through extraction and DNA quantitation to provide us with the DNA values. Non-invasiveness of this technique is an extremely important advantage. Currently, the process seems to provide a positive test result for almost all proteins therefore the specificity of this technique can be under question.

5.0 Future Work

There is a lot of potential for further research on both Raman Spectroscopy as well as Colorimetric Analysis. In regards to Raman Spectroscopy, runs can be carried out using a much more appropriate wavelength such as 785nm or 535nm as suggested by the

majority of journals cited. This wavelength seems to provide the most accurate results and the least amount of background fluorescence interference. A much larger variety of samples including different genders and races can also be tested to verify any discrepancies that may arise due to these factors. Another key issue that needs to be addressed about this technique is the fact that a lot of background noise arises when the samples are run. These can be reduced or in some cases completely negated by using different algorithms that have been already developed for certain software. This can not only provide us with more accurate results but also can provide better contrast when trying to determine the age of a stain. The same can be applied for colorimetric analysis and the use of algorithms can be used to remove background surface colour so only the colour and corresponding colour change in the stain is able to be recorded.

Another key area that can be worked on is the use of this technique in trying to identify and age other biological stains such as semen and saliva. The non-invasiveness and the portability of these techniques make them ideal instruments to be used directly on crime scenes. This can not only save time but also reduce the risk of contamination along with packaging costs for samples to be collected and transferred to forensic laboratories.

For protein identification studies, in order to make our technique a lot more human specific, fluorescent probes can be attached to our samples that only interact to specific amino acid chains that are present in human skin cells. More specifically, these probes can be used to only be active in keratins present in the epidermis of human skin. This can have a lot of applications in terms of forensic investigations. Another important experiment that can be performed is surface type studies. There is still no information regarding the reactivity of this test on organic surfaces such as wood. There might be some interference in the results from the proteins present on these surfaces.

6.0 Conclusions

All the techniques presented in this project showed promising results for both the identification of blood as well as the identification of proteins. This report consists of results that re-instate the fact that blood can be identified using a Raman Spectrometer and that the difference in wavelength of the instrument used to that of used in past research does play a significant role in terms of reading. However the values for different peaks can be utilised and the spectrums provided show these identifying peaks to be present. The age estimation experiment can also be carried out by using this instrument as the presentation of new peaks as well as slightly altered peaks can indicate chemical changes that take place over time in the blood system. In regards to Small angle X-Ray scattering, there is still a lot of work that needs to be done in order to confirm its application in blood stain ageing. The preliminary results show that it can be used if there is a change in structure of the primary protein but there is still a need for more samples to be run and more interpretation to be performed in order to get all the necessary information. Colorimetric analysis is a promising tool that can be used as it fits the criteria of being non-invasive and having little to know preparation of the sample. For the technique to be more successfully used, it needs some sort of spectroscopic technique to run with it in order to see chemically any changes that occur in the blood over time. It can provide useful information regarding the physical appearance of blood along with any absorbance changes but still needs to go through another technique that can provide the chemical information regarding the changes. Protein identification is extremely important as it can provide sufficient evidence of biological materials being present at a crime scene which in some cases may not be visible. Some more work needs to be done in order to validate the results that have been presented. Initial testing shows the presence of DNA in our samples and the next logical step would be to try and acquire a genetic profile to see if the DNA present is viable for the process.

All in all, the tests showed promising results and more work needs to be done in order to perfect these techniques for the purposes state in this report.

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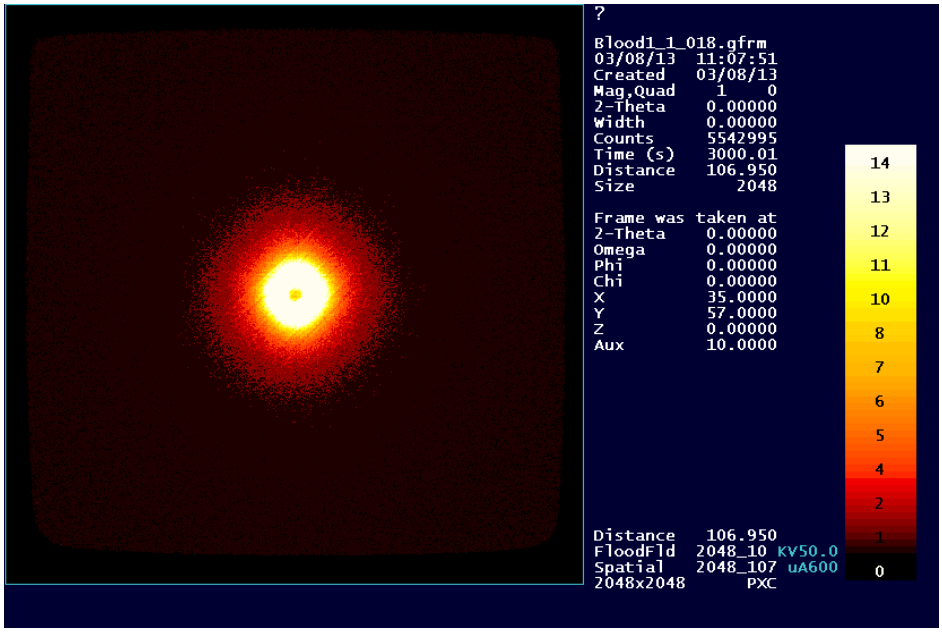
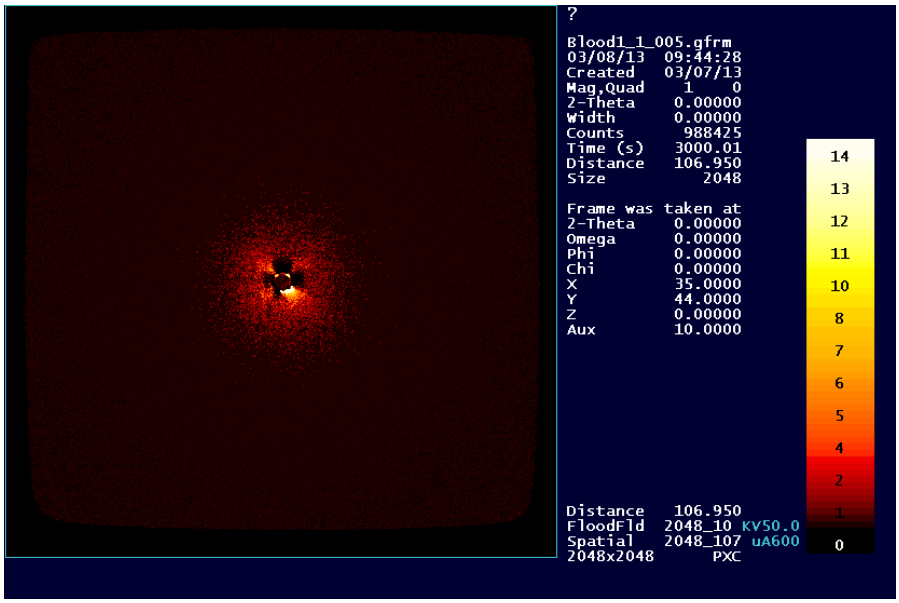
8.0 Appendix

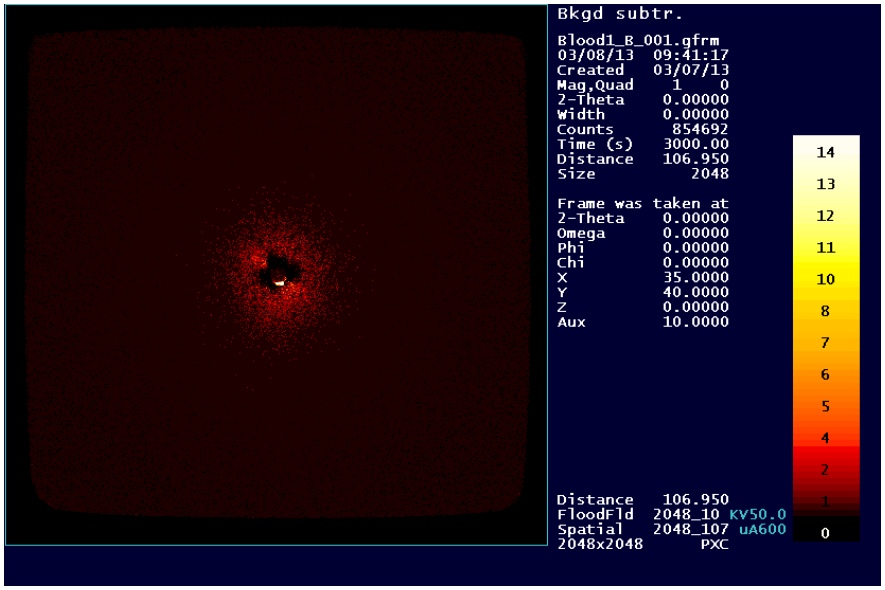
Small Angle X-Ray Scattering



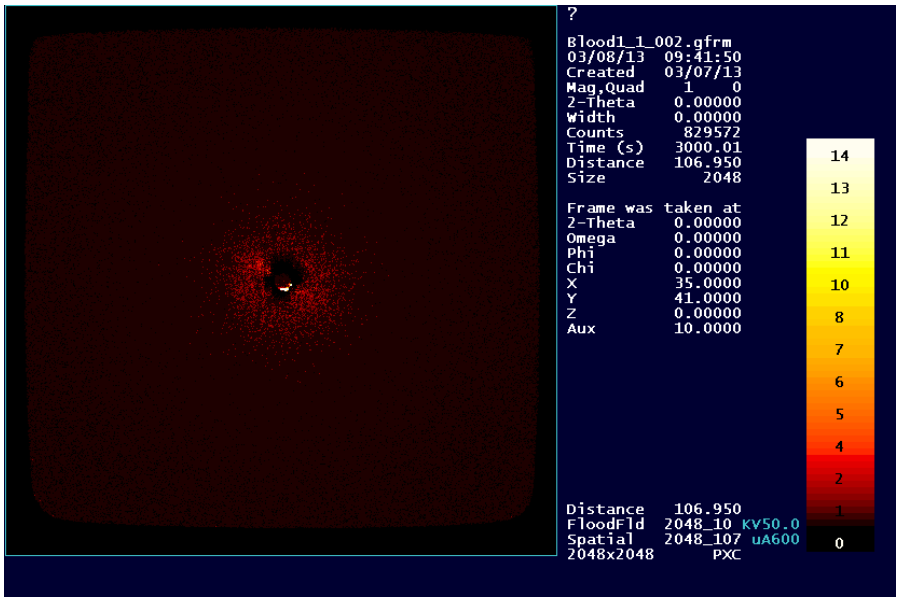
SAXS Pattern of Human Haemoglobin



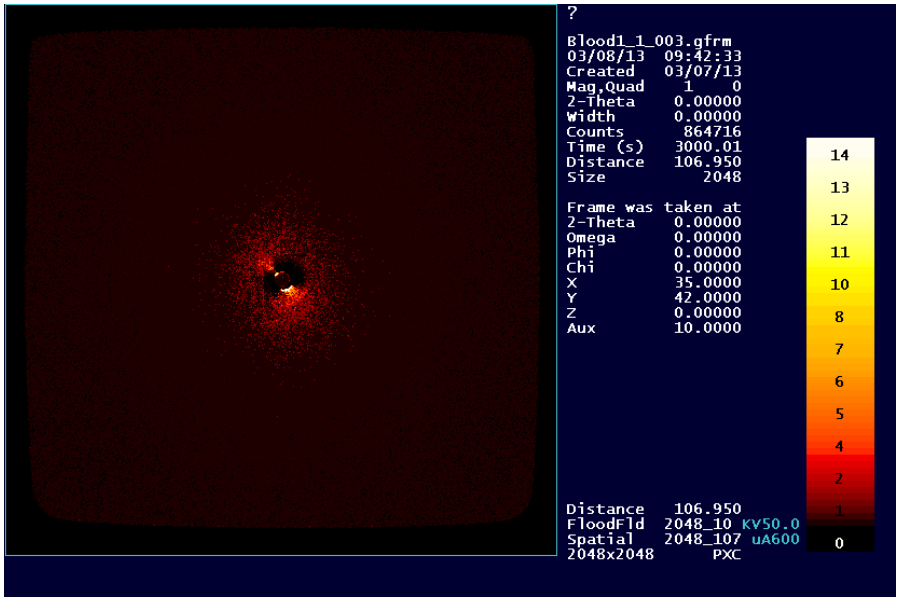
SAXS Pattern of Human Haemoglobin after 12 hours



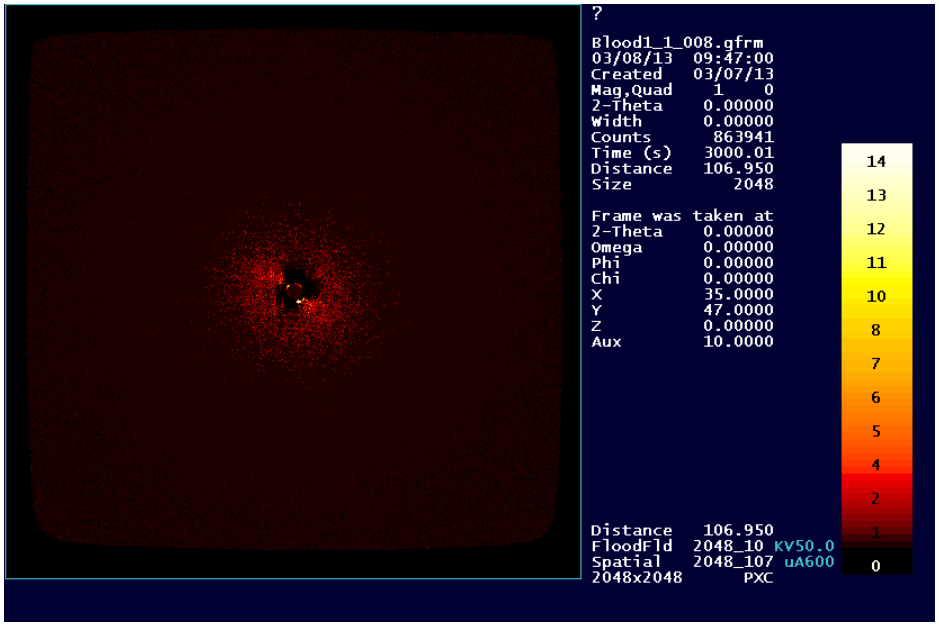
SAXS Pattern of Human Haemoglobin after 24 hours



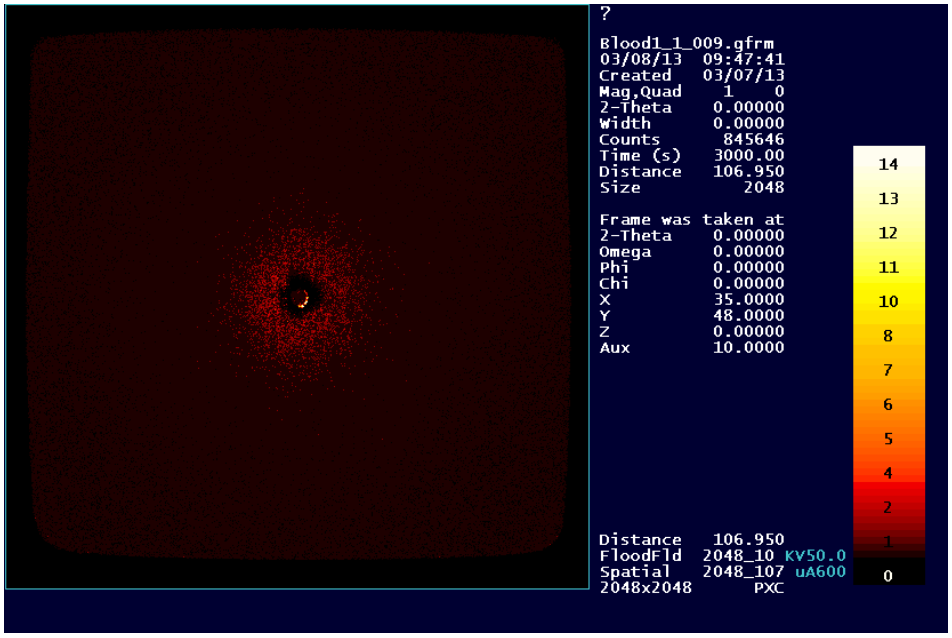
SAXS Pattern of Human Haemoglobin after 26 hours



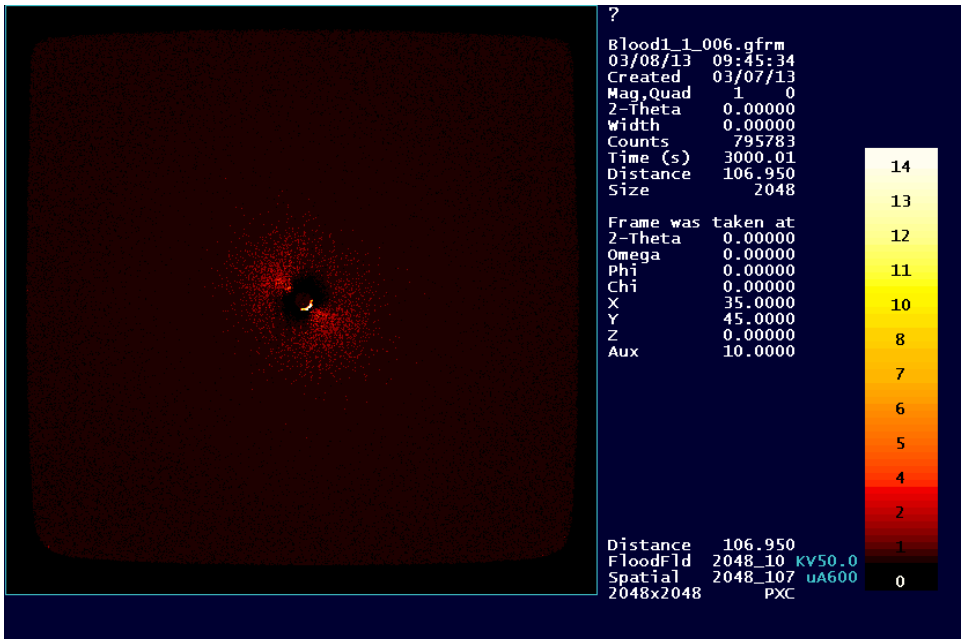
SAXS Pattern of Human Haemoglobin after 30 hours



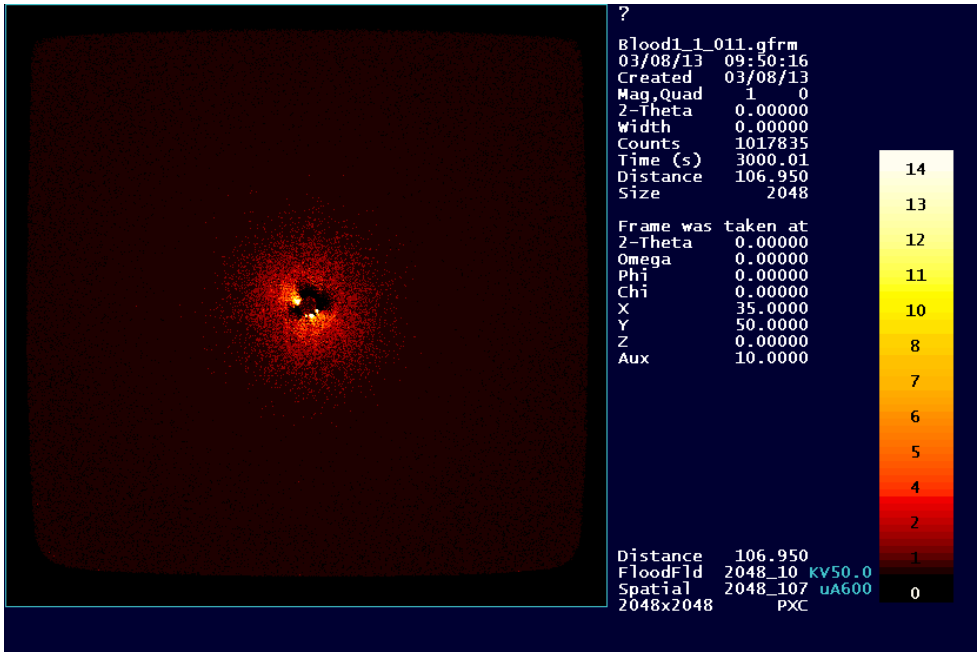
SAXS Pattern of Human Haemoglobin after 32 hours



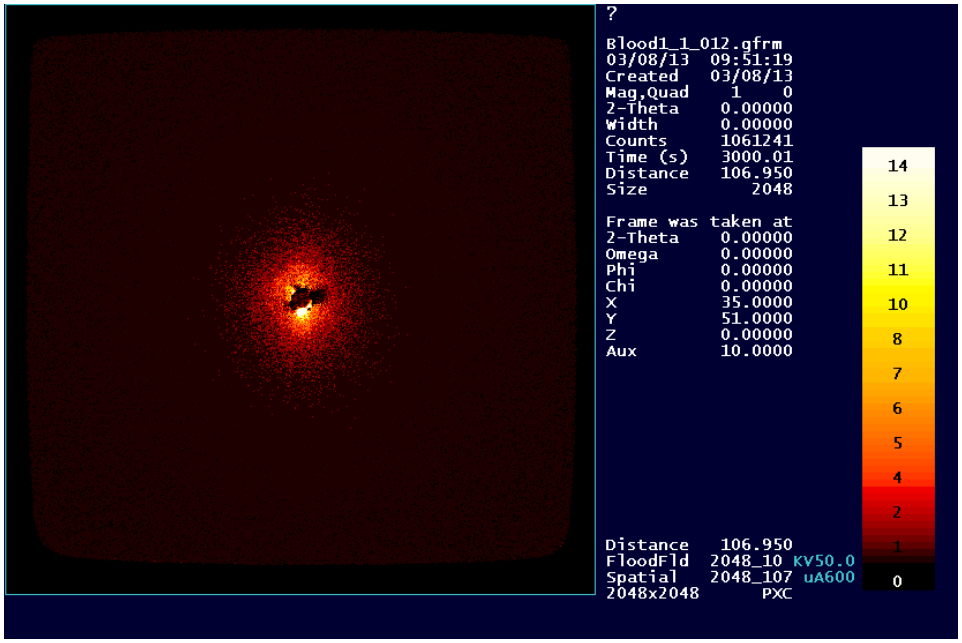
SAXS Pattern of Human Haemoglobin after 34 hours



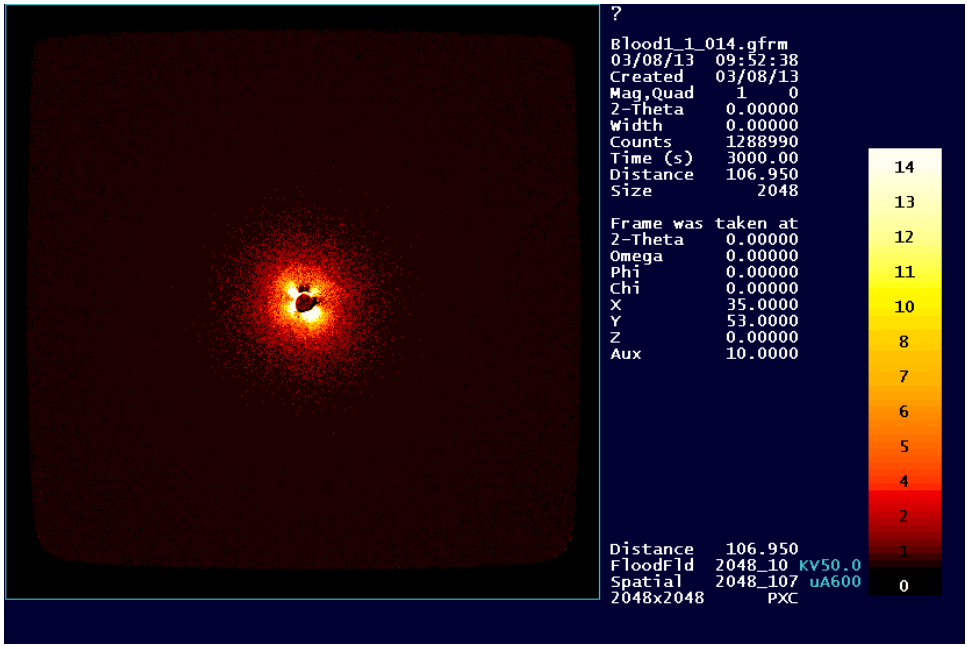
SAXS Pattern of Human Haemoglobin after 36 hours



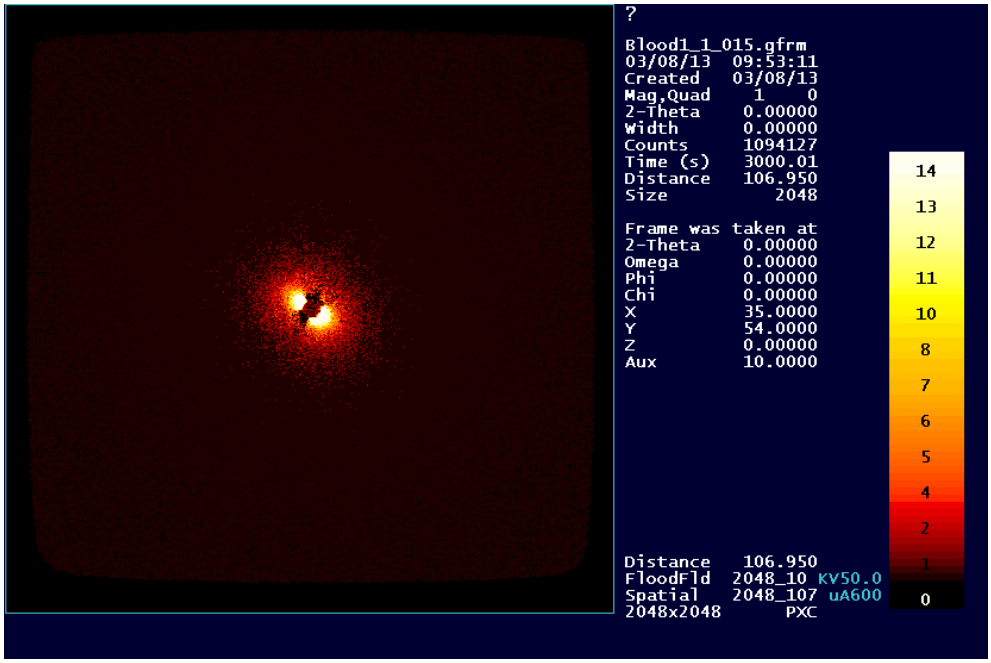
SAXS Pattern of Human Haemoglobin after 48 hours



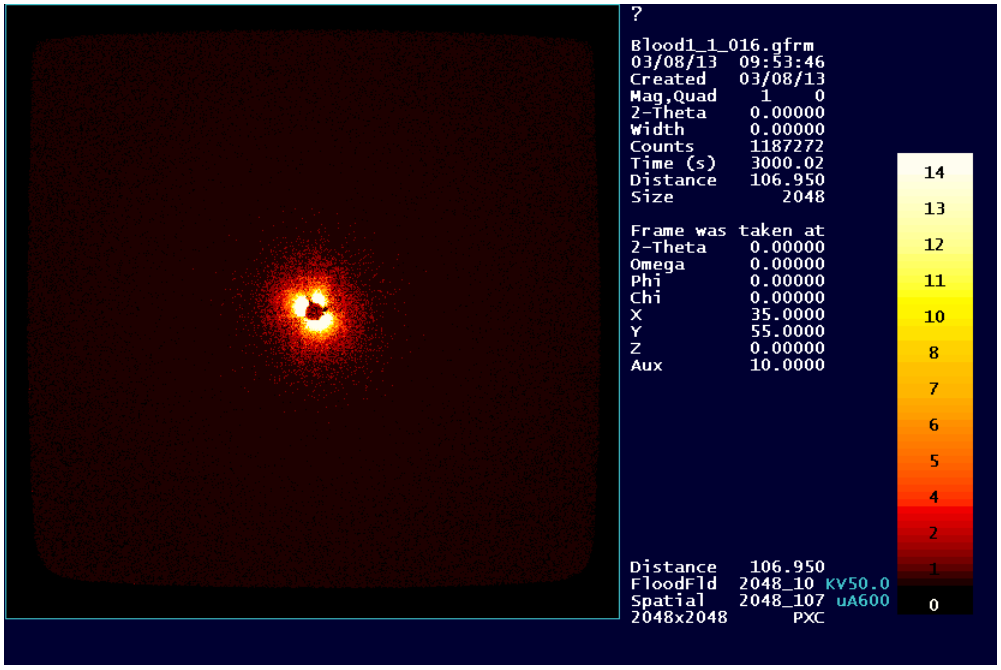
SAXS Pattern of Human Haemoglobin after 52 hours



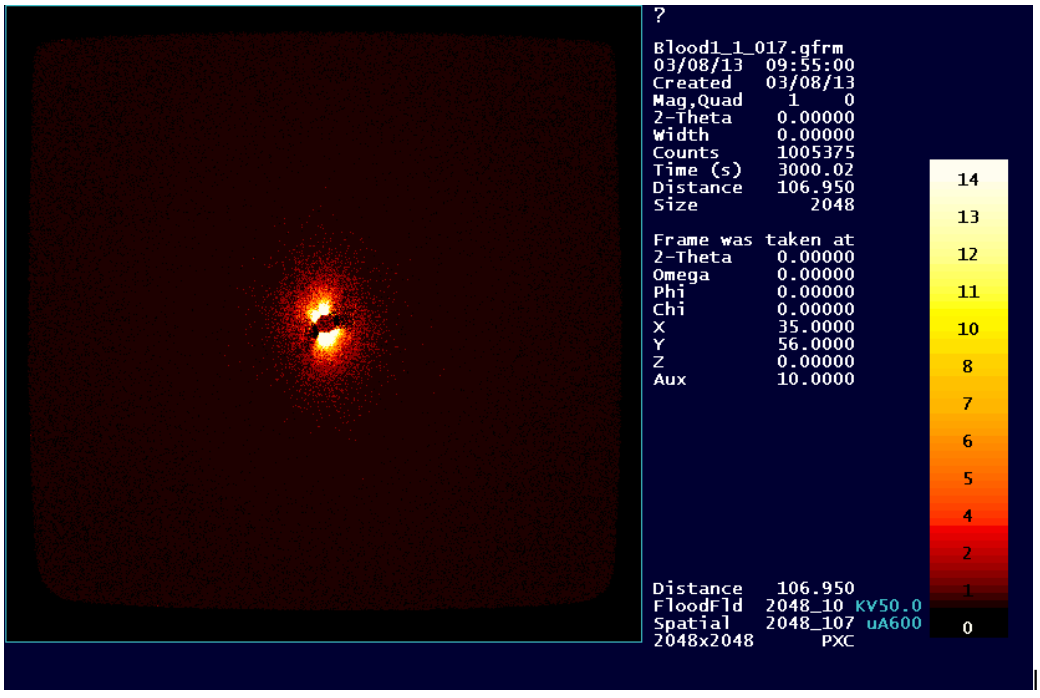
SAXS Pattern of Human Haemoglobin after 52 hours



SAXS Pattern of Human Haemoglobin after 56 hours



SAXS Pattern of Human Haemoglobin after 60 hours



SAXS Pattern of Human Haemoglobin after 64 hours

Numerical Data Regarding SAXS Run 1:

q	S^2	ln intensity Horse Blood	ln intensity Human Hb	ln intensity BSA
0.000	0	6.684611728	5.075173815	5.075173815
0.014	0.000202357	7.091995465	5.714670656	5.491305268
0.028	0.000809428	6.896799055	5.650668246	5.24547346
0.043	0.001821213	7.146188859	5.91402538	5.351064288
0.057	0.003237713	7.790447821	5.984704844	5.15395791
0.071	0.005058925	10.23549399	8.355491827	6.496638365
0.085	0.007284852	10.71220412	8.934137152	8.071535011
0.100	0.009915491	10.85883612	9.740008374	8.604606506
0.114	0.012950844	10.37798145	9.351047435	7.507674132
0.128	0.016390909	10.08435656	9.39407743	7.575746397
0.142	0.020235686	9.818222803	9.324673453	7.616450215
0.156	0.024485175	9.63322098	9.259512997	7.661273832
0.171	0.029139375	9.501093651	9.219275626	7.62656509
0.185	0.034198286	9.391822872	9.147114323	7.675002628
0.199	0.039661907	9.329591513	9.126723299	7.672002468
0.213	0.045530237	9.24975497	9.095275696	7.690145912
0.228	0.051803275	9.200499137	9.060999283	7.734007507
0.242	0.058481021	9.173299063	9.047882907	7.774018978
0.256	0.065563475	9.120106942	9.007108288	7.797437607
0.270	0.073050634	9.098558677	8.982080176	7.858874864
0.285	0.080942499	9.065762025	8.971644315	7.901026493
0.299	0.089239068	9.053200447	8.955196894	7.970494364
0.313	0.09794034	9.022854016	8.916333326	8.028623827
0.327	0.107046315	9.010238097	8.900080657	8.07050974
0.341	0.11655699	8.985001909	8.893549997	8.148706788
0.356	0.126472366	8.977817831	8.884511827	8.210267507
0.370	0.13679244	8.962039256	8.868385379	8.275245279
0.384	0.147517211	8.934780684	8.848807876	8.334048366
0.398	0.158646679	8.928324055	8.851104185	8.386216123
0.413	0.170180842	8.919976582	8.840255746	8.458896297
0.427	0.182119698	8.899462371	8.821861789	8.495892345
0.441	0.194463246	8.88387673	8.804501195	8.547675888
0.455	0.207211484	8.864913868	8.788827829	8.595800645
0.469	0.220364412	8.854987238	8.785012054	8.637918942
0.484	0.233922026	8.831833695	8.77281679	8.6854665
0.498	0.247884326	8.821038896	8.757063412	8.735918795
0.512	0.26225131	8.803120356	8.744411243	8.765732919
0.526	0.277022977	8.797710793	8.73828456	8.794542203
0.541	0.292199323	8.773562433	8.723512092	8.818449898
0.555	0.307780349	8.765751046	8.708796652	8.847060306
0.569	0.323766051	8.742216615	8.693007456	8.860175095
0.583	0.340156427	8.730896779	8.69081474	8.876157505
0.597	0.356951476	8.706052575	8.678304994	8.879766225
0.612	0.374151196	8.689957422	8.656507003	8.88275907
0.626	0.391755585	8.671758992	8.651592615	8.886487945
0.640	0.409764639	8.654426208	8.635440876	8.872284538
0.654	0.428178358	8.636223626	8.622203069	8.858973234
0.669	0.446996739	8.615658545	8.601855262	8.827315222
0.683	0.46621978	8.597350887	8.583766445	8.816082636
0.697	0.485847478	8.57239015	8.566664764	8.783267512
0.711	0.505879831	8.558106432	8.553087314	8.745637046
0.725	0.526316836	8.523810894	8.527636806	8.713303522

0.740	0.547158492	8.511446535	8.506828827	8.662127113
0.754	0.568404795	8.481041771	8.491186344	8.613299219
0.768	0.590055742	8.452618524	8.457214156	8.566366374
0.782	0.612111332	8.42363118	8.433106723	8.514215716
0.797	0.634571562	8.402982211	8.408234086	8.463292265
0.811	0.657436428	8.367944507	8.38086246	8.399861128
0.825	0.680705929	8.342230662	8.350131099	8.348380948
0.839	0.70438006	8.305347939	8.317221232	8.274780771
0.853	0.72845882	8.270453106	8.278621234	8.210113984
0.868	0.752942205	8.22851101	8.239707397	8.143036956
0.882	0.777830213	8.196872778	8.205452601	8.079230869
0.896	0.80312284	8.152709307	8.161076546	8.008804781
0.910	0.828820083	8.114878	8.12456928	7.939866793
0.925	0.85492194	8.06060796	8.076015162	7.876295772
0.939	0.881428406	8.021142402	8.018296621	7.807705812
0.953	0.908339479	7.968852275	7.974174718	7.731058932
0.967	0.935655156	7.918257043	7.914272802	7.658608304
0.982	0.963375432	7.86833695	7.871959396	7.599061166
0.996	0.991500306	7.801792114	7.801952966	7.526911067
1.010	1.020029773	7.745180556	7.743444586	7.466430265
1.024	1.048963829	7.690593968	7.679113713	7.403189774
1.038	1.078302472	7.625993597	7.61280202	7.336019774
1.053	1.108045698	7.562981463	7.550853636	7.265833498
1.067	1.138193503	7.48952248	7.469715554	7.207469713
1.081	1.168745883	7.436407559	7.430216003	7.1578469
1.095	1.199702835	7.357711813	7.342110384	7.08397478
1.110	1.231064355	7.279755247	7.250117033	7.029397504
1.124	1.262830439	7.207107364	7.195432988	6.97301345
1.138	1.295001083	7.130019147	7.109157951	6.921024699
1.152	1.327576284	7.051695598	7.031591787	6.865157557
1.166	1.360556037	6.970478227	6.955940838	6.810193794
1.181	1.393940338	6.882539754	6.890638923	6.781864268
1.195	1.427729184	6.803772395	6.815272373	6.726304607
1.209	1.46192257	6.716440549	6.715084306	6.660473315
1.223	1.496520492	6.633397696	6.643588249	6.624431048
1.238	1.531522945	6.546191872	6.554888296	6.591747072
1.252	1.566929927	6.45763843	6.498763604	6.566158177
1.266	1.602741431	6.360843923	6.40400913	6.519055461
1.280	1.638957454	6.298369581	6.344219985	6.484924901
1.294	1.675577992	6.199632571	6.250297216	6.451251233
1.309	1.71260304	6.117930144	6.201633917	6.405820071
1.323	1.750032593	6.01018546	6.129700576	6.368585772
1.337	1.787866647	5.962385464	6.078362857	6.354835074
1.351	1.826105197	5.894878223	6.017751585	6.33622222
1.366	1.864748239	5.798317763	5.977385569	6.30951581
1.380	1.903795768	5.767982385	5.901677327	6.273726762
1.394	1.943247779	5.683733589	5.863840875	6.238156765
1.408	1.983104267	5.618670482	5.812374274	6.223762903
1.422	2.023365228	5.571292555	5.787667514	6.21805935
1.437	2.064030656	5.543187474	5.753318637	6.19656389
1.451	2.105100547	5.507782096	5.73027653	6.166717916
1.465	2.146574896	5.485239972	5.715630344	6.152370963
1.479	2.188453697	5.428799107	5.672626468	6.138800064

1.494	2.230736946	5.429071605	5.63530184	6.12691196
1.508	2.273424637	5.397043904	5.630775909	6.097351063
1.522	2.316516765	5.376223368	5.64980045	6.073908849
1.536	2.360013325	5.375057837	5.657777307	6.083638432
1.550	2.403914312	5.388573569	5.631955523	6.046900597
1.565	2.44821972	5.346043284	5.629046191	6.021402673
1.579	2.492929544	5.346244091	5.612844387	6.007427161
1.593	2.538043778	5.360039804	5.642279814	5.971520115
1.607	2.583562417	5.380734197	5.619416853	5.967042694
1.622	2.629485455	5.389494914	5.632638856	5.958952895
1.636	2.675812887	5.390269765	5.63661202	5.928388255
1.650	2.722544707	5.390501757	5.623059434	5.908075473
1.664	2.76968091	5.414875492	5.637325011	5.896437034
1.678	2.817221489	5.43061799	5.633767177	5.865859855
1.693	2.865166439	5.44376262	5.659596199	5.873813559
1.707	2.913515754	5.445659532	5.621734899	5.821526807
1.721	2.962269428	5.445648076	5.64319285	5.818072002
1.735	3.011427456	5.466017694	5.671311596	5.802844079
1.750	3.06098983	5.474043479	5.67011483	5.796154865
1.764	3.110956546	5.473784313	5.656659881	5.772825908
1.778	3.161327597	5.493692693	5.649670098	5.750350235
1.792	3.212102977	5.490075624	5.655506081	5.729792479
1.806	3.263282679	5.496195757	5.655641543	5.72081154
1.821	3.314866698	5.523172524	5.652694966	5.716559789
1.835	3.366855027	5.546306077	5.645144803	5.691601345
1.849	3.419247661	5.526323405	5.659841451	5.679635021
1.863	3.472044591	5.535741097	5.662451938	5.665155971
1.878	3.525245813	5.512373056	5.653290989	5.646091443
1.892	3.578851319	5.53471227	5.620466099	5.636909526
1.906	3.632861104	5.542010646	5.639806622	5.634225741
1.920	3.68727516	5.541233925	5.637604046	5.607419647
1.934	3.74209348	5.564213195	5.647674785	5.600960623
1.949	3.797316059	5.520735006	5.639245148	5.572163663
1.963	3.85294289	5.528237104	5.617499371	5.556656723
1.977	3.908973965	5.518900146	5.587095145	5.541122618
1.991	3.965409277	5.498657849	5.585985096	5.511626587
2.006	4.022248821	5.495924046	5.569980165	5.497586503
2.020	4.079492589	5.50909158	5.542166874	5.497616944
2.034	4.137140574	5.478416556	5.523583053	5.462059829
2.048	4.195192769	5.451921827	5.520152626	5.463917212
2.062	4.253649167	5.459095879	5.490438958	5.413711279
2.077	4.312509761	5.433131668	5.481910698	5.422400232
2.091	4.371774543	5.436085719	5.471405997	5.395447026
2.105	4.431443507	5.430976653	5.464861889	5.404521649
2.119	4.491516645	5.406008826	5.431685315	5.38942464
2.134	4.55199395	5.419119861	5.426858231	5.375834845
2.148	4.612875414	5.362812877	5.422557307	5.349863824
2.162	4.674161031	5.369774223	5.41208492	5.331987724
2.176	4.735850792	5.362736268	5.389143773	5.304660124
2.190	4.79794469	5.330760137	5.356052722	5.31502133
2.205	4.860442717	5.320611402	5.349065494	5.293231289
2.219	4.923344867	5.329951209	5.334564287	5.256759581
2.233	4.986651131	5.312714794	5.340272703	5.276005242

2.247	5.050361501	5.300286556	5.335571503	5.292589936
2.262	5.11447597	5.309185825	5.299676458	5.244525337
2.276	5.17899453	5.275258436	5.272646199	5.233581397

Numerical Data Regarding SAXS Run 1:

Scattering angle (°)	q (nm-1)	Blood 1	Blood 2	Plasma
0.00	0.000	160.00	800.00	160.00
0.02	0.014	303.28	1202.30	242.57
0.04	0.028	284.48	989.10	189.71
0.06	0.043	370.19	1269.26	210.83
0.08	0.057	397.31	2417.40	173.12
0.10	0.071	4253.48	27875.24	662.91
0.12	0.085	7586.59	44900.50	3202.01
0.14	0.100	16983.68	51991.53	5456.74
0.16	0.114	11510.87	32144.01	1821.97
0.18	0.128	12017.00	23965.16	1950.32
0.20	0.142	11211.25	18365.38	2031.34
0.22	0.156	10504.02	15263.52	2124.46
0.24	0.171	10089.75	13374.35	2051.99
0.26	0.185	9387.31	11989.94	2153.83
0.28	0.199	9197.83	11266.53	2147.38
0.30	0.213	8913.08	10402.02	2186.69
0.32	0.228	8612.75	9902.07	2284.74
0.34	0.242	8500.52	9636.36	2378.01
0.36	0.256	8160.89	9137.18	2434.36
0.38	0.270	7959.17	8942.39	2588.61
0.40	0.285	7876.54	8653.87	2700.05
0.42	0.299	7748.05	8545.84	2894.29
0.44	0.313	7452.71	8290.40	3067.52
0.46	0.327	7332.56	8186.47	3198.73
0.48	0.341	7284.83	7982.46	3458.90
0.50	0.356	7219.29	7925.32	3678.53
0.52	0.370	7103.80	7801.25	3925.49
0.54	0.384	6966.08	7591.47	4163.24
0.56	0.398	6982.09	7542.61	4386.19
0.58	0.413	6906.76	7479.91	4716.85
0.60	0.427	6780.88	7328.03	4894.62
0.62	0.441	6664.17	7214.71	5154.76
0.64	0.455	6560.54	7079.18	5408.90
0.66	0.469	6535.55	7009.26	5641.58
0.68	0.484	6456.33	6848.83	5916.30

0.70	0.498	6355.42	6775.30	6222.45
0.72	0.512	6275.52	6654.98	6410.76
0.74	0.526	6237.19	6619.07	6598.13
0.76	0.541	6145.73	6461.15	6757.78
0.78	0.555	6055.95	6410.88	6953.92
0.80	0.569	5961.08	6261.76	7045.72
0.82	0.583	5948.03	6191.28	7159.23
0.84	0.597	5874.08	6039.36	7185.11
0.86	0.612	5747.42	5942.93	7206.65
0.88	0.626	5719.25	5835.76	7233.57
0.90	0.640	5627.61	5735.48	7131.55
0.92	0.654	5553.61	5632.02	7037.25
0.94	0.669	5441.75	5517.38	6817.96
0.96	0.683	5344.20	5417.29	6741.80
0.98	0.697	5253.58	5283.74	6524.16
1.00	0.711	5182.73	5208.81	6283.21
1.02	0.725	5052.49	5033.20	6083.31
1.04	0.740	4948.45	4971.35	5779.82
1.06	0.754	4871.64	4822.47	5504.38
1.08	0.768	4708.92	4687.33	5252.01
1.10	0.782	4596.76	4553.41	4985.13
1.12	0.797	4483.84	4460.35	4737.63
1.14	0.811	4362.77	4306.77	4446.45
1.16	0.825	4230.74	4197.44	4223.34
1.18	0.839	4093.77	4045.45	3923.66
1.20	0.853	3938.76	3906.72	3677.96
1.22	0.868	3788.43	3746.25	3439.35
1.24	0.882	3660.86	3629.58	3226.75
1.26	0.896	3501.95	3472.78	3007.32
1.28	0.910	3376.41	3343.85	2806.99
1.30	0.925	3216.39	3167.22	2634.10
1.32	0.939	3036.00	3044.65	2459.48
1.34	0.953	2904.96	2889.54	2278.01
1.36	0.967	2736.06	2746.98	2118.81
1.38	0.982	2622.70	2613.22	1996.32
1.40	0.996	2445.37	2444.98	1857.36
1.42	1.010	2306.40	2310.41	1748.35
1.44	1.024	2162.70	2187.67	1641.21
1.46	1.038	2023.94	2050.82	1534.59
1.48	1.053	1902.37	1925.58	1430.58
1.50	1.067	1754.11	1789.20	1349.47
1.52	1.081	1686.17	1696.64	1284.14
1.54	1.095	1543.97	1568.24	1192.70
1.56	1.110	1408.27	1450.63	1129.35
1.58	1.124	1333.33	1348.98	1067.43
1.60	1.138	1223.12	1248.90	1013.36

1.62	1.152	1131.83	1154.82	958.30
1.64	1.166	1049.37	1064.73	907.05
1.66	1.181	983.03	975.10	881.71
1.68	1.195	911.66	901.24	834.06
1.70	1.209	824.75	825.87	780.92
1.72	1.223	767.85	760.06	753.28
1.74	1.238	702.67	696.59	729.05
1.76	1.252	664.32	637.55	710.63
1.78	1.266	604.26	578.73	677.94
1.80	1.280	569.19	543.68	655.19
1.82	1.294	518.17	492.57	633.49
1.84	1.309	493.55	453.92	605.36
1.86	1.323	459.30	407.56	583.23
1.88	1.337	436.31	388.54	575.27
1.90	1.351	410.65	363.17	564.66
1.92	1.366	394.41	329.74	549.78
1.94	1.380	365.65	319.89	530.45
1.96	1.394	352.07	294.05	511.91
1.98	1.408	334.41	275.52	504.60
2.00	1.422	326.25	262.77	501.73
2.02	1.437	315.24	255.49	491.06
2.04	1.451	308.05	246.60	476.62
2.06	1.465	303.58	241.11	469.83
2.08	1.479	290.80	227.88	463.50
2.10	1.494	280.14	227.94	458.02
2.12	1.508	278.88	220.75	444.68
2.14	1.522	284.23	216.20	434.38
2.16	1.536	286.51	215.95	438.62
2.18	1.550	279.21	218.89	422.80
2.20	1.565	278.40	209.78	412.16
2.22	1.579	273.92	209.82	406.44
2.24	1.593	282.11	212.73	392.10
2.26	1.607	275.73	217.18	390.35
2.28	1.622	279.40	219.09	387.20
2.30	1.636	280.51	219.26	375.55
2.32	1.650	276.73	219.31	368.00
2.34	1.664	280.71	224.72	363.74
2.36	1.678	279.71	228.29	352.79
2.38	1.693	287.03	231.31	355.60
2.40	1.707	276.37	231.75	337.49
2.42	1.721	282.36	231.75	336.32
2.44	1.735	290.42	236.52	331.24
2.46	1.750	290.07	238.42	329.03
2.48	1.764	286.19	238.36	321.44
2.50	1.778	284.20	243.15	314.30
2.52	1.792	285.86	242.28	307.91

2.54	1.806	285.90	243.76	305.15
2.56	1.821	285.06	250.43	303.86
2.58	1.835	282.91	256.29	296.37
2.60	1.849	287.10	251.22	292.84
2.62	1.863	287.85	253.60	288.63
2.64	1.878	285.23	247.74	283.18
2.66	1.892	276.02	253.33	280.59
2.68	1.906	281.41	255.19	279.84
2.70	1.920	280.79	254.99	272.44
2.72	1.934	283.63	260.92	270.69
2.74	1.949	281.25	249.82	263.00
2.76	1.963	275.20	251.70	258.96
2.78	1.977	266.96	249.36	254.96
2.80	1.991	266.66	244.36	247.55
2.82	2.006	262.43	243.70	244.10
2.84	2.020	255.23	246.93	244.11
2.86	2.034	250.53	239.47	235.58
2.88	2.048	249.67	233.21	236.02
2.90	2.062	242.36	234.88	224.46
2.92	2.077	240.31	228.86	226.42
2.94	2.091	237.79	229.54	220.40
2.96	2.105	236.24	228.37	222.41
2.98	2.119	228.53	222.74	219.08
3.00	2.134	227.43	225.68	216.12
3.02	2.148	226.46	213.32	210.58
3.04	2.162	224.10	214.81	206.85
3.06	2.176	219.02	213.31	201.27
3.08	2.190	211.89	206.59	203.37
3.10	2.205	210.41	204.51	198.99
3.12	2.219	207.38	206.43	191.86
3.14	2.233	208.57	202.90	195.59
3.16	2.247	207.59	200.39	198.86
3.18	2.262	200.27	202.19	189.53
3.20	2.276	194.93	195.44	187.46

Logged Data for the same run:

Ln(Intensity)		
Blood 1	Blood 2	Plasma
5.0752	6.6846	5.0752
5.7147	7.0920	5.4913

5.6507	6.8968	5.2455
5.9140	7.1462	5.3511
5.9847	7.7904	5.1540
8.3555	10.2355	6.4966
8.9341	10.7122	8.0715
9.7400	10.8588	8.6046
9.3510	10.3780	7.5077
9.3941	10.0844	7.5757
9.3247	9.8182	7.6165
9.2595	9.6332	7.6613
9.2193	9.5011	7.6266
9.1471	9.3918	7.6750
9.1267	9.3296	7.6720
9.0953	9.2498	7.6901
9.0610	9.2005	7.7340
9.0479	9.1733	7.7740
9.0071	9.1201	7.7974
8.9821	9.0986	7.8589
8.9716	9.0658	7.9010
8.9552	9.0532	7.9705
8.9163	9.0229	8.0286
8.9001	9.0102	8.0705
8.8935	8.9850	8.1487
8.8845	8.9778	8.2103
8.8684	8.9620	8.2752
8.8488	8.9348	8.3340
8.8511	8.9283	8.3862
8.8403	8.9200	8.4589
8.8219	8.8995	8.4959
8.8045	8.8839	8.5477
8.7888	8.8649	8.5958
8.7850	8.8550	8.6379
8.7728	8.8318	8.6855
8.7571	8.8210	8.7359
8.7444	8.8031	8.7657
8.7383	8.7977	8.7945
8.7235	8.7736	8.8184
8.7088	8.7658	8.8471
8.6930	8.7422	8.8602
8.6908	8.7309	8.8762
8.6783	8.7061	8.8798
8.6565	8.6900	8.8828
8.6516	8.6718	8.8865
8.6354	8.6544	8.8723
8.6222	8.6362	8.8590
8.6019	8.6157	8.8273

8.5838	8.5974	8.8161
8.5667	8.5724	8.7833
8.5531	8.5581	8.7456
8.5276	8.5238	8.7133
8.5068	8.5114	8.6621
8.4912	8.4810	8.6133
8.4572	8.4526	8.5664
8.4331	8.4236	8.5142
8.4082	8.4030	8.4633
8.3809	8.3679	8.3999
8.3501	8.3422	8.3484
8.3172	8.3053	8.2748
8.2786	8.2705	8.2101
8.2397	8.2285	8.1430
8.2055	8.1969	8.0792
8.1611	8.1527	8.0088
8.1246	8.1149	7.9399
8.0760	8.0606	7.8763
8.0183	8.0211	7.8077
7.9742	7.9689	7.7311
7.9143	7.9183	7.6586
7.8720	7.8683	7.5991
7.8020	7.8018	7.5269
7.7434	7.7452	7.4664
7.6791	7.6906	7.4032
7.6128	7.6260	7.3360
7.5509	7.5630	7.2658
7.4697	7.4895	7.2075
7.4302	7.4364	7.1578
7.3421	7.3577	7.0840
7.2501	7.2798	7.0294
7.1954	7.2071	6.9730
7.1092	7.1300	6.9210
7.0316	7.0517	6.8652
6.9559	6.9705	6.8102
6.8906	6.8825	6.7819
6.8153	6.8038	6.7263
6.7151	6.7164	6.6605
6.6436	6.6334	6.6244
6.5549	6.5462	6.5917
6.4988	6.4576	6.5662
6.4040	6.3608	6.5191
6.3442	6.2984	6.4849
6.2503	6.1996	6.4513
6.2016	6.1179	6.4058
6.1297	6.0102	6.3686

6.0784	5.9624	6.3548
6.0178	5.8949	6.3362
5.9774	5.7983	6.3095
5.9017	5.7680	6.2737
5.8638	5.6837	6.2382
5.8124	5.6187	6.2238
5.7877	5.5713	6.2181
5.7533	5.5432	6.1966
5.7303	5.5078	6.1667
5.7156	5.4852	6.1524
5.6726	5.4288	6.1388
5.6353	5.4291	6.1269
5.6308	5.3970	6.0974
5.6498	5.3762	6.0739
5.6578	5.3751	6.0836
5.6320	5.3886	6.0469
5.6290	5.3460	6.0214
5.6128	5.3462	6.0074
5.6423	5.3600	5.9715
5.6194	5.3807	5.9670
5.6326	5.3895	5.9590
5.6366	5.3903	5.9284
5.6231	5.3905	5.9081
5.6373	5.4149	5.8964
5.6338	5.4306	5.8659
5.6596	5.4438	5.8738
5.6217	5.4457	5.8215
5.6432	5.4456	5.8181
5.6713	5.4660	5.8028
5.6701	5.4740	5.7962
5.6567	5.4738	5.7728
5.6497	5.4937	5.7504
5.6555	5.4901	5.7298
5.6556	5.4962	5.7208
5.6527	5.5232	5.7166
5.6451	5.5463	5.6916
5.6598	5.5263	5.6796
5.6625	5.5357	5.6652
5.6533	5.5124	5.6461
5.6205	5.5347	5.6369
5.6398	5.5420	5.6342
5.6376	5.5412	5.6074
5.6477	5.5642	5.6010
5.6392	5.5207	5.5722
5.6175	5.5282	5.5567
5.5871	5.5189	5.5411

5.5860	5.4987	5.5116
5.5700	5.4959	5.4976
5.5422	5.5091	5.4976
5.5236	5.4784	5.4621
5.5202	5.4519	5.4639
5.4904	5.4591	5.4137
5.4819	5.4331	5.4224
5.4714	5.4361	5.3954
5.4649	5.4310	5.4045
5.4317	5.4060	5.3894
5.4269	5.4191	5.3758
5.4226	5.3628	5.3499
5.4121	5.3698	5.3320
5.3891	5.3627	5.3047
5.3561	5.3308	5.3150
5.3491	5.3206	5.2932
5.3346	5.3300	5.2568
5.3403	5.3127	5.2760
5.3356	5.3003	5.2926
5.2997	5.3092	5.2445
5.2726	5.2753	5.2336

Colorimetric Analysis

Filter Paper Run 1

DATA COLLECTED FOR 420 MINS WITH 30 MIN INTERVALS
AVERAGE VALUES FOR ALL SAMPLES (19.04.2013)

Time / mins	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
0	40.732	33.366	20.842	0	0	0
30	45.412	27.284	18.422	4.68	-6.082	-2.42
60	44.850	23.156	16.622	4.118	-10.21	-4.22
90	44.728	21.820	16.082	3.996	-11.546	-4.76
120	44.596	21.264	15.808	3.864	-12.102	-5.034
150	44.530	20.816	15.620	3.798	-12.55	-5.222
180	44.446	20.378	15.454	3.714	-12.988	-5.388
210	44.576	19.952	15.306	3.844	-13.414	-5.536
240	44.374	19.698	15.146	3.642	-13.668	-5.696
270	44.408	19.328	15.030	3.676	-14.038	-5.812
300	44.264	19.192	14.936	3.532	-14.174	-5.906
330	44.392	18.908	14.900	3.66	-14.458	-5.942
360	44.544	18.576	14.808	3.812	-14.79	-6.034
390	44.426	18.318	14.644	3.694	-15.048	-6.198
420	44.446	18.142	14.608	3.714	-15.224	-6.234

log L*	log a*	log b*	log dL*	log da*	log db*
1.609936	1.523304	1.318939			
1.657171	1.435908	1.265337	0.047235	-0.0874	-0.0536
1.651762	1.364664	1.220683	0.041827	-0.15864	-0.09826
1.650579	1.338855	1.20634	0.040644	-0.18445	-0.1126
1.649296	1.327645	1.198877	0.03936	-0.19566	-0.12006
1.648653	1.318397	1.193681	0.038717	-0.20491	-0.12526
1.647833	1.309162	1.189041	0.037897	-0.21414	-0.1299
1.649101	1.299986	1.184862	0.039165	-0.22332	-0.13408
1.647129	1.294422	1.180298	0.037193	-0.22888	-0.13864
1.647461	1.286187	1.176959	0.037525	-0.23712	-0.14198
1.646051	1.28312	1.174234	0.036115	-0.24018	-0.14471
1.647305	1.276646	1.173186	0.037369	-0.24666	-0.14575
1.648789	1.268952	1.170496	0.038853	-0.25435	-0.14844
1.647637	1.262878	1.16566	0.037701	-0.26043	-0.15328
1.647833	1.258685	1.164591	0.037897	-0.26462	-0.15435

Standard deviation							
log L*	log a*	log b*	log dL*	log da*	log db*	log a*log b	log a*/log b*
0.062034073	0.013451	0.059404				2.009145843	1.154946281
0.029618034	0.001079	0.036544	0.032416	0.012373	0.022859	1.816907254	1.134803055
0.029362404	0.013796	0.044917	0.032672	0.000345	0.014487	1.665821964	1.117950549
0.028639248	0.017373	0.046645	0.033395	0.003922	0.012759	1.615114112	1.109848535
0.02879542	0.017351	0.047294	0.033239	0.003899	0.01211	1.591682914	1.107407219
0.028748249	0.018937	0.047956	0.033286	0.005485	0.011448	1.573745822	1.104480382
0.028825274	0.020855	0.048704	0.033209	0.007404	0.0107	1.556646647	1.101023143
0.027487733	0.022518	0.048934	0.034546	0.009067	0.01047	1.54030415	1.097163008
0.028441242	0.022711	0.049584	0.033593	0.00926	0.00982	1.527803793	1.096690993
0.027953594	0.025332	0.050561	0.03408	0.011881	0.008842	1.513789243	1.092805219
0.02857196	0.023969	0.050576	0.033462	0.010518	0.008828	1.506683797	1.092729304
0.027368978	0.025573	0.049743	0.034665	0.012122	0.00966	1.49774308	1.088186615
0.025939368	0.026952	0.049535	0.036095	0.0135	0.009869	1.485303992	1.084114566
0.026310135	0.02667	0.050474	0.035724	0.013219	0.00893	1.47208608	1.083401985

0.025918016	0.026546	0.049803	0.036116	0.013094	0.0096	1.46585311	1.080796109

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
Sample 1a - 0 mins	49.85	34.86	25.29	------	------	------
Sample 1a - 30 mins	50.01	27.38	20.75	0.16	-7.48	-4.54
Sample 1a - 60 mins	49.35	24.22	19.24	-0.5	-10.64	-6.05
Sample 1a - 90 mins	49.1	23.09	18.72	-0.75	-11.77	-6.57
Sample 1a - 120 mins	48.98	22.5	18.44	-0.87	-12.36	-6.85
Sample 1a - 150 mins	48.9	22.14	18.26	-0.95	-12.72	-7.03
Sample 1a - 180 mins	48.82	21.81	18.11	-1.03	-13.05	-7.18
Sample 1a - 210 mins	48.75	21.47	17.95	-1.1	-13.39	-7.34
Sample 1a - 240 mins	48.68	21.21	17.8	-1.17	-13.65	-7.49
Sample 1a - 270 mins	48.64	20.99	17.72	-1.21	-13.87	-7.57
Sample 1a - 300 mins	48.58	20.75	17.61	-1.27	-14.11	-7.68
Sample 1a - 330 mins	48.53	20.55	17.52	-1.32	-14.31	-7.77
Sample 1a - 360 mins	48.47	20.28	17.4	-1.38	-14.58	-7.89
Sample 1a - 390 mins	48.4	19.98	17.26	-1.45	-14.89	-8.03
Sample 1a - 420 mins	48.36	19.78	17.18	-1.49	-15.08	-8.11

log L*	log a*	log b*	log dL*	log da*	log db*
1.697665	1.542327	1.402949			
1.699057	1.437433	1.317018	0.001392	-0.10489	-0.08593
1.693287	1.384174	1.284205	-0.00438	-0.15815	-0.11874
1.691081	1.363424	1.272306	-0.00658	-0.1789	-0.13064
1.690019	1.352183	1.265761	-0.00765	-0.19014	-0.13719
1.689309	1.345178	1.261501	-0.00836	-0.19715	-0.14145
1.688598	1.338656	1.257918	-0.00907	-0.20367	-0.14503
1.687975	1.331832	1.254064	-0.00969	-0.2105	-0.14888
1.687351	1.326541	1.25042	-0.01031	-0.21579	-0.15253
1.686994	1.322012	1.248464	-0.01067	-0.22031	-0.15449
1.686458	1.317018	1.245759	-0.01121	-0.22531	-0.15719
1.68601	1.312812	1.243534	-0.01165	-0.22952	-0.15941
1.685473	1.307068	1.240549	-0.01219	-0.23526	-0.1624
1.684845	1.300595	1.237041	-0.01282	-0.24173	-0.16591

1.684486	1.296226	1.235023	-0.01318	-0.2461	-0.16793

DATA COLLECTED OVER 90 MINS WITH 10 MIN INTERVALS

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
0 mins	45.96	33.56333	22.81	------	------	------
10 mins	47.00667	30.39667	20.93	1.046667	-3.16667	-1.88
20 mins	46.53667	25.28	18.41333	0.576667	-8.28333	-4.39667
30 mins	46.47667	24.09	17.99333	0.516667	-9.47333	-4.81667
40 mins	46.34333	23.20667	17.59	0.383333	-10.3567	-5.22
50 mins	46.17333	22.78	17.33	0.213333	-10.7833	-5.48
60 mins	46.16333	22.30667	17.13667	0.203333	-11.2567	-5.67333
70 mins	46.11	22.19333	17.13667	0.15	-11.37	-5.67333
80 mins	45.71	21.86333	16.78333	-0.25	-11.7	-6.02667
90 mins	45.68	21.47	16.6	-0.28	-12.0933	-6.21

log L*	log a*	log b*	log dL*	log da*	log db*
1.66238	1.525865	1.358125			
1.672159	1.482826	1.320769	0.009779	-0.04304	-0.03736
1.667795	1.402777	1.265132	0.005415	-0.12309	-0.09299
1.667235	1.381837	1.255112	0.004855	-0.14403	-0.10301
1.665987	1.365613	1.245266	0.003607	-0.16025	-0.11286
1.664391	1.357554	1.238799	0.002011	-0.16831	-0.11933
1.664297	1.348435	1.233926	0.001917	-0.17743	-0.1242
1.663795	1.346223	1.233926	0.001415	-0.17964	-0.1242
1.660011	1.339716	1.224878	-0.00237	-0.18615	-0.13325
1.659726	1.331832	1.220108	-0.00265	-0.19403	-0.13802

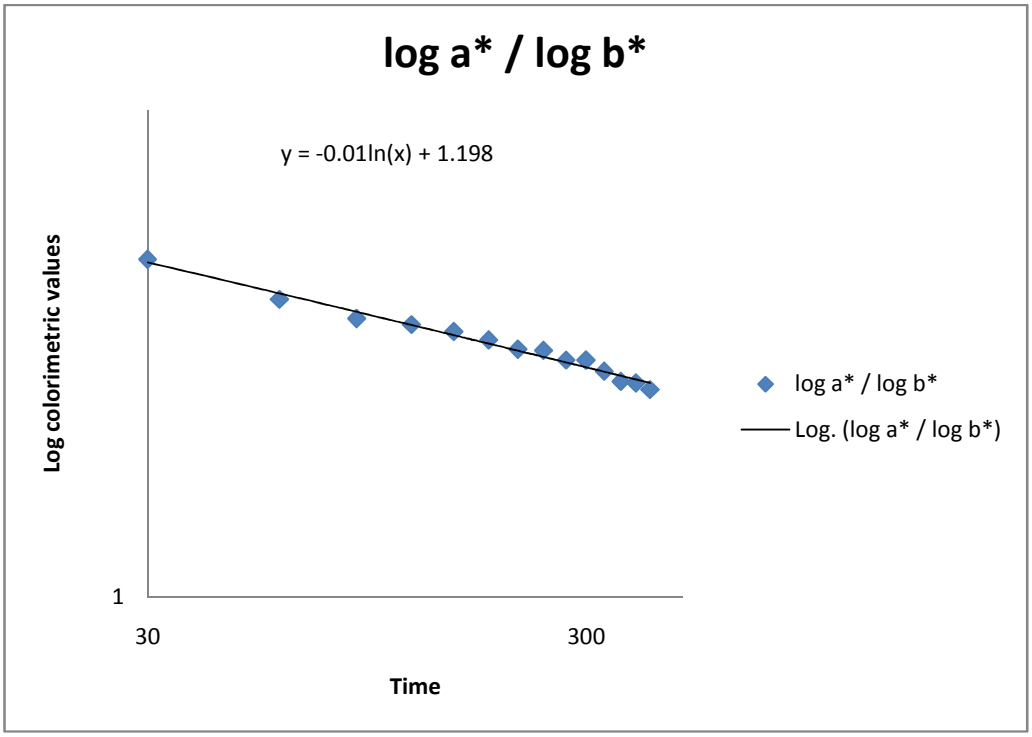
Average:

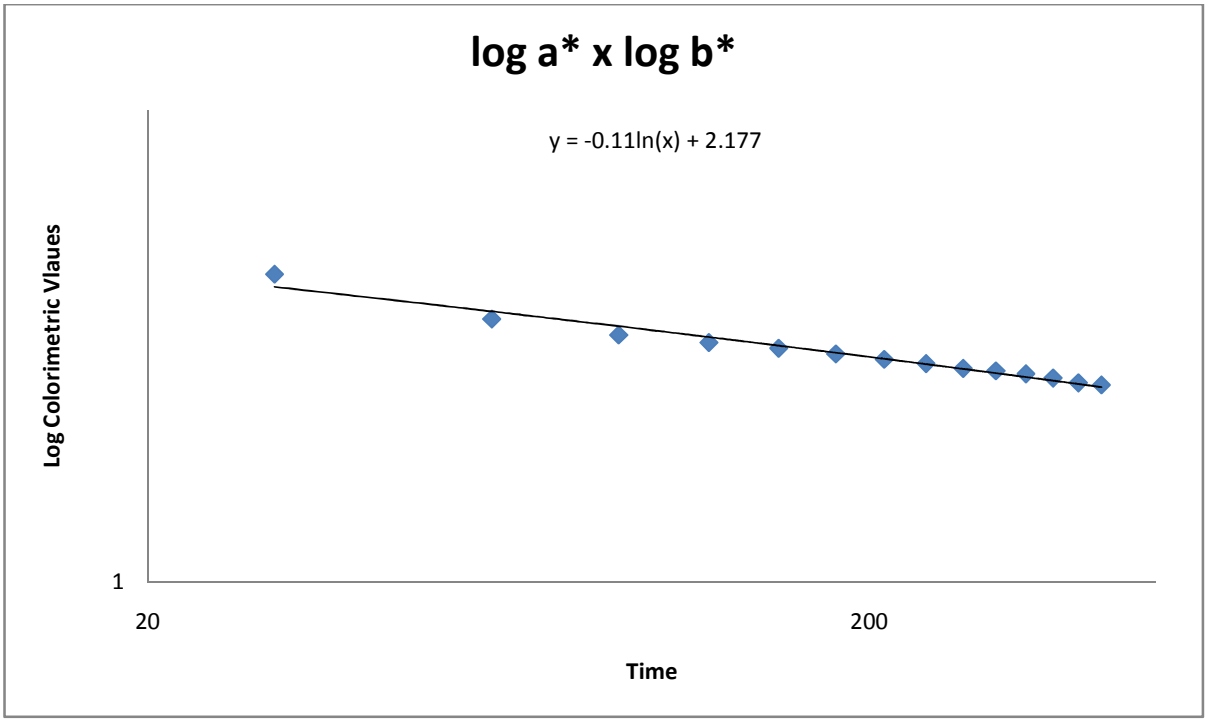
Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
Sample 1 - 0 mins	44.92	33.64	7.48	------	------	------

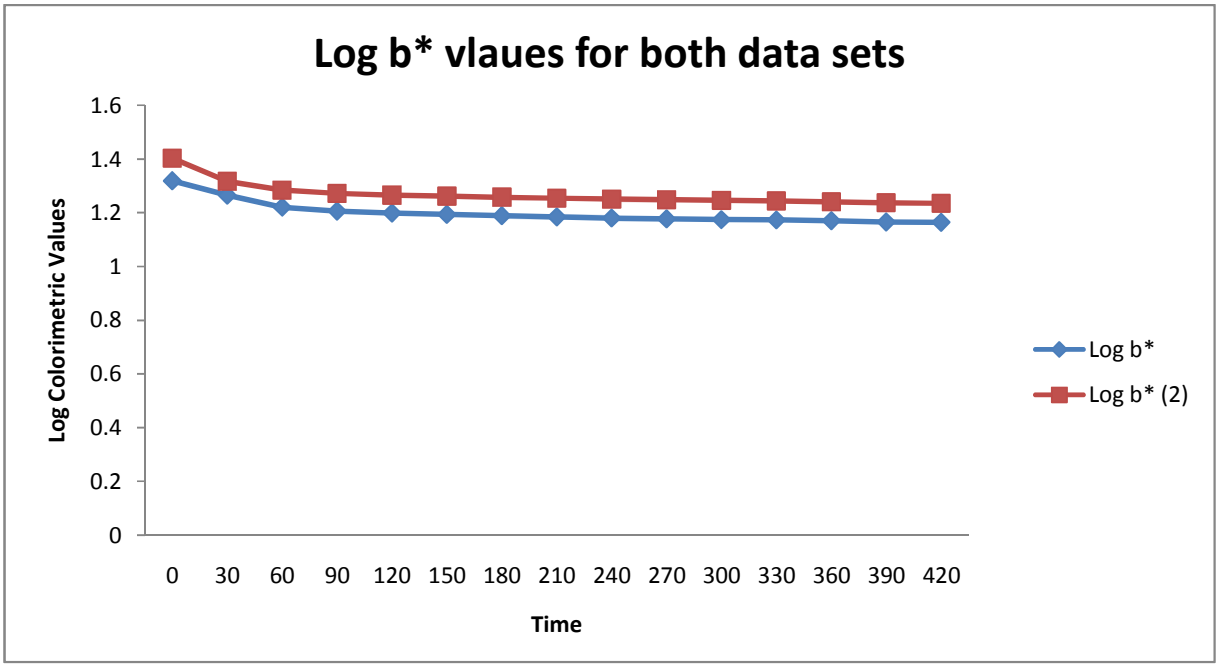
Sample 1 - 10 mins	45.08	33.74	7.51	0.15	0.05	0.03
Sample 1 - 20 mins	45.31	16.95	7.61	0.38	0.13	0.13
Sample 1 - 30 mins	45.53	16.96	7.64	0.61	0.14	0.16
Sample 1 - 40 mins	45.79	17.11	7.8	0.87	0.29	0.32
Sample 1 - 50 mins	45.74	17.23	7.8	0.81	0.41	0.32
Sample 1 - 60 mins	46.07	17.5	8.05	1.15	0.68	0.57
Sample 1 - 70 mins	46.02	17.57	8.05	1.1	0.76	0.57
Sample 1 - 80 mins	46.11	18.5	8.69	1.19	1.69	1.22
Sample 1 - 90 mins	46.3	18.67	8.81	1.38	1.85	1.34

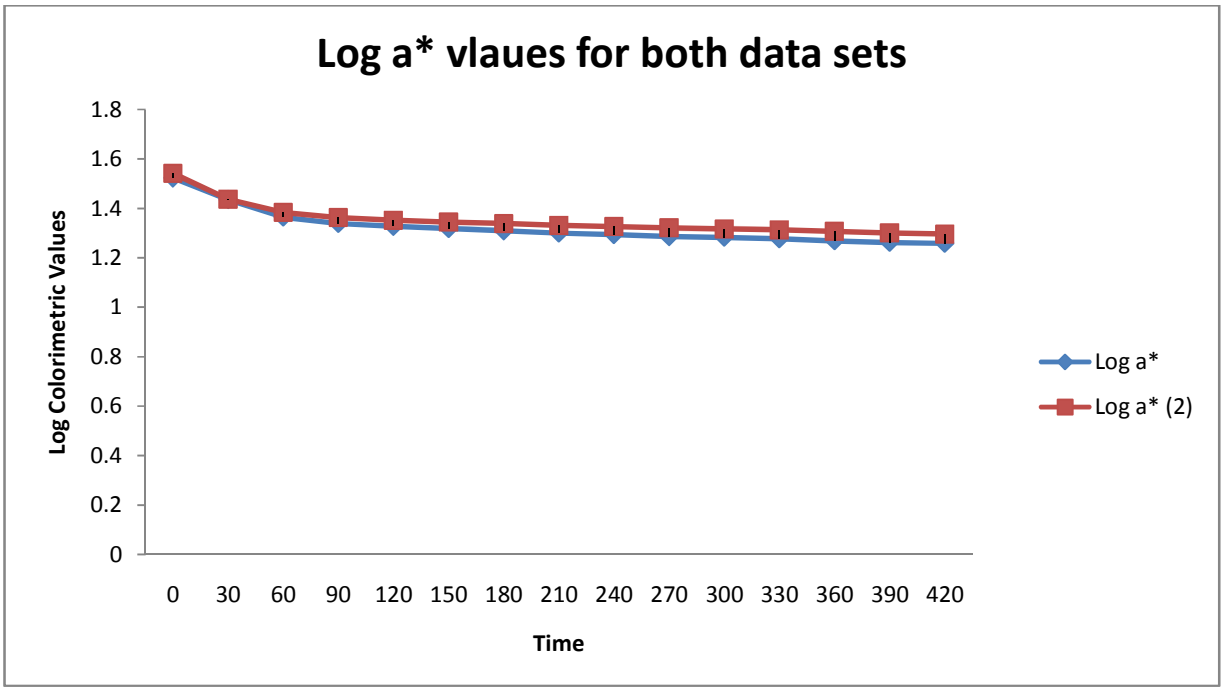
log L*	log a*	log b*	log dL*	log da*	log db*
1.65244	1.526856	0.873902			
1.653984	1.528145	0.87564	0.001544	0.001289	0.001738
1.656194	1.22917	0.881385	0.003754	-0.29769	0.007483
1.658298	1.229426	0.883093	0.005858	-0.29743	0.009192
1.660771	1.23325	0.892095	0.008331	-0.29361	0.018193
1.660296	1.236285	0.892095	0.007856	-0.29057	0.018193
1.663418	1.243038	0.905796	0.010978	-0.28382	0.031894
1.662947	1.244772	0.905796	0.010507	-0.28208	0.031894
1.663795	1.267172	0.93902	0.011355	-0.25968	0.065118
1.665581	1.271144	0.944976	0.013141	-0.25571	0.071074

Graphs for run 1


log a* / log b*
y = -0.01ln(x) + 1.198
Log colorimetric values
1
30
300
Time
log a* / log b*
Log. (log a* / log b*)


log a* x log b*
y = -0.11ln(x) + 2.177
Log Colorimetric Vlaues
1
20
200
Time





Filter Paper Run 2

Data collected at 10 minute intervals

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
Sample 1 - 0 mins	44.92	16.82	7.48	------	------	------
Sample 1 - 10 mins	45.08	16.87	7.51	0.15	0.05	0.03
Sample 1 - 20 mins	45.31	16.95	7.61	0.38	0.13	0.13
Sample 1 - 30 mins	45.53	16.96	7.64	0.61	0.14	0.16
Sample 1 - 40 mins	45.79	17.11	7.8	0.87	0.29	0.32

Sample 1 - 50 mins	45.74	17.23	7.8	0.81	0.41	0.32
Sample 1 - 60 mins	46.07	17.5	8.05	1.15	0.68	0.57
Sample 1 - 70 mins	46.02	17.57	8.05	1.1	0.76	0.57
Sample 1 - 80 mins	46.11	18.5	8.69	1.19	1.69	1.22
Sample 1 - 90 mins	46.3	18.67	8.81	1.38	1.85	1.34
Sample 1 - 100 mins	46.32	18.51	8.69	1.4	1.69	1.21
Sample 1 - 110 mins	46.64	18.15	8.46	1.71	1.34	0.98
Sample 1 - 120 mins	46.71	18.34	8.53	1.79	1.52	1.05
Sample 1 - 130 mins	47.06	18.73	8.83	2.13	1.91	1.35
Sample 1 - 140 mins	46.98	18.8	8.83	2.05	1.98	1.35
Sample 1 - 150 mins	47.16	18.93	8.92	2.24	2.11	1.44
Sample 1 - 160 mins	46.86	18.58	8.7	1.94	1.77	1.22
Sample 1 - 170 mins	47.4	19.24	9.16	2.48	2.42	1.68
Sample 1 - 180 mins	47.63	19.53	9.34	2.71	2.72	1.86
Sample 1 - 190 mins	47.55	19.47	9.29	2.62	2.65	1.81
Sample 1 - 200 mins	47.61	19.48	9.33	2.68	2.66	1.85
Sample 1 - 210 mins	47.52	19.39	9.29	2.59	2.57	1.81
Sample 1 - 220 mins	47.61	19.51	9.35	2.69	2.69	1.87
Sample 1 - 230 mins	47.69	19.59	9.41	2.76	2.77	1.93
Sample 1 - 240 mins	47.82	19.75	9.5	2.89	2.93	2.02
Sample 1 - 250 mins	47.68	19.49	9.4	2.75	2.67	1.92
Sample 1 - 260 mins	48.05	19.95	9.71	3.13	3.13	2.23
Sample 1 - 270 mins	48.19	20.04	9.82	3.27	3.22	2.34
Sample 1 - 280 mins	47.9	19.79	9.59	2.98	2.97	2.11
Sample 1 - 290 mins	48.24	20.08	9.88	3.32	3.27	2.4
Sample 1 - 300 mins	48.08	19.9	9.76	3.15	3.08	2.28
Sample 1 - 310 mins	48.14	19.96	9.73	3.21	3.14	2.25
Sample 1 - 320 mins	48.24	20.05	9.87	3.32	3.23	2.39
Sample 1 - 330 mins	49.05	20.85	10.76	4.13	4.03	3.28
Sample 1 - 340 mins	48.6	20.3	10.14	3.68	3.48	2.67
Sample 1 - 350 mins	48.59	20.26	10.14	3.67	3.44	2.66
Sample 1 - 360 mins	48.73	20.3	10.31	3.81	3.48	2.83

Data Collected at 1 hour interval

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
Sample 1 - 0 mins	44.92	16.82	7.48	------	------	------
Sample 1 - 60 mins	46.07	17.5	8.05	1.15	0.68	0.57
Sample 1 - 120 mins	46.71	18.34	8.53	1.79	1.52	1.05
Sample 1 - 180 mins	47.63	19.53	9.34	2.71	2.72	1.86
Sample 1 - 240 mins	47.82	19.75	9.5	2.89	2.93	2.02
Sample 1 - 300 mins	48.08	19.9	9.76	3.15	3.08	2.28
Sample 1 - 360 mins	48.73	20.3	10.31	3.81	3.48	2.83

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
Sample 2 - 0 mins	44.92	16.82	7.48	------	------	------
Sample 2 - 1 hour	42.69	24.72	11.86	-2.24	7.9	4.39
Sample 2 - 2 hours	45.45	23.92	12.19	0.53	7.1	4.71
Sample 2 - 3 hours	48.64	23.73	13.03	3.72	6.91	5.55
Sample 2 - 4 hours	52.45	21.7	13.24	7.53	4.88	5.77
Sample 2 - 5 hours	52.54	14.47	10.83	7.61	-2.35	3.36
Sample 2 - 6 hours	52.33	13.42	10.74	7.4	-3.4	3.26

Average for both sets of data

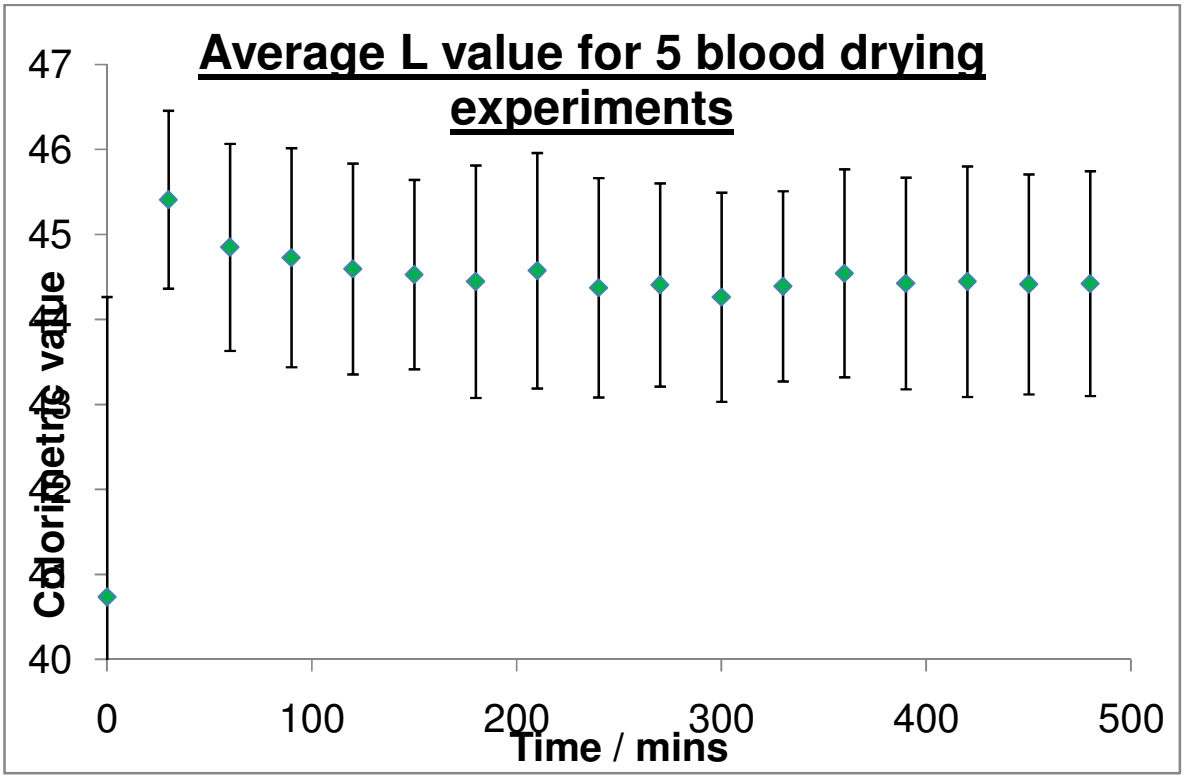
Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
0 hours	44.92	16.82	7.48			
1 hour	44.38	21.11	9.955	-0.545	4.29	2.48
2 hours	46.08	21.13	10.36	1.16	4.31	2.88
3 hours	48.135	21.63	11.185	3.215	4.815	3.705
4 hours	50.135	20.725	11.37	5.21	3.905	3.895
5 hours	50.31	17.185	10.295	5.38	0.365	2.82
6 hours	50.53	16.86	10.525	5.605	0.04	3.045

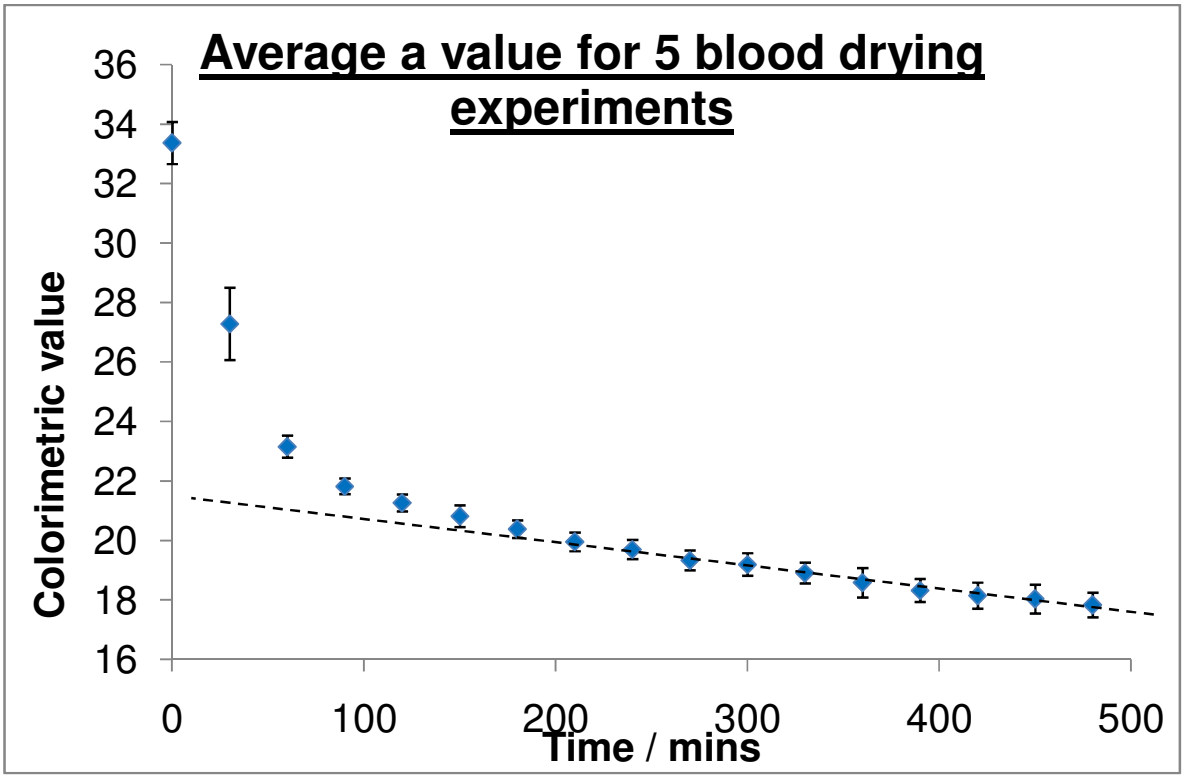
AVERAGE VALUES FOR ALL SAMPLES

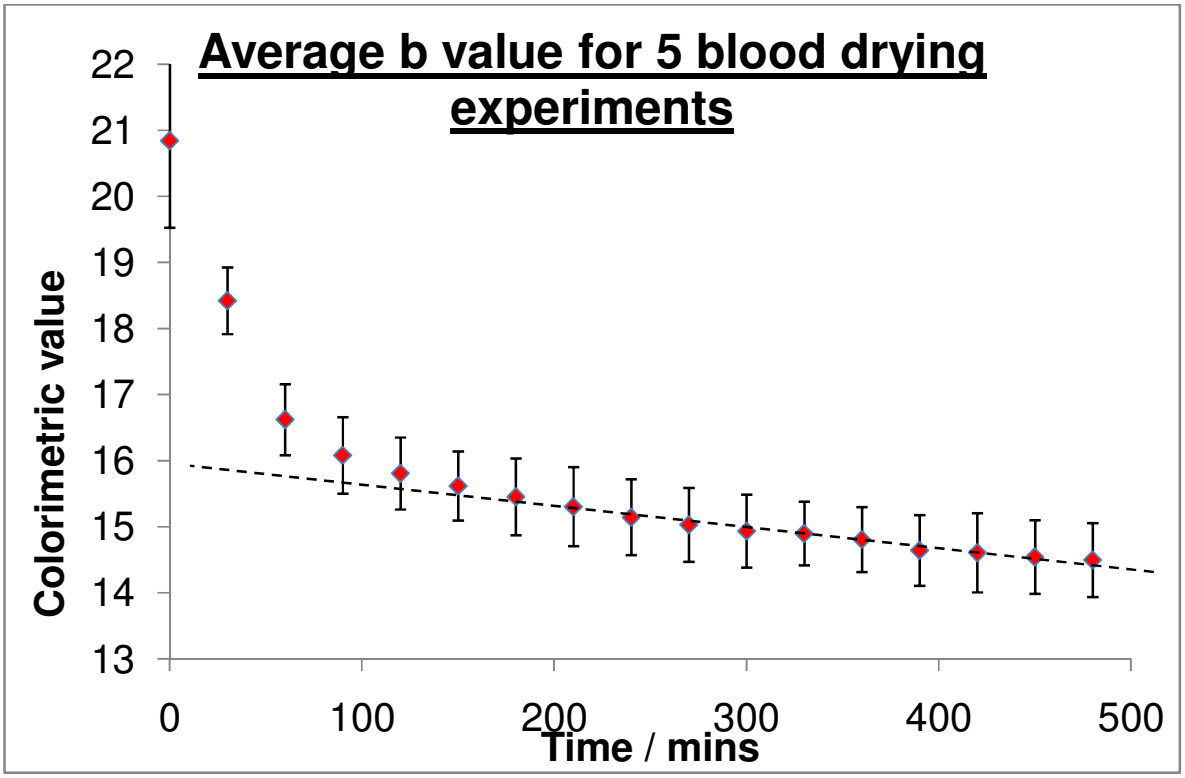
Time / mins	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)	da/db	da * db
0	40.732	33.366	20.842	0	0	0		
30	45.412	27.284	18.422	4.68	-6.082	-2.42	2.5132	14.718
60	44.850	23.156	16.622	4.118	-10.21	-4.22	2.4194	43.086
90	44.728	21.820	16.082	3.996	-11.546	-4.76	2.4256	54.959
120	44.596	21.264	15.808	3.864	-12.102	-5.034	2.4041	60.921
150	44.530	20.816	15.620	3.798	-12.55	-5.222	2.4033	65.536
180	44.446	20.378	15.454	3.714	-12.988	-5.388	2.4105	69.979

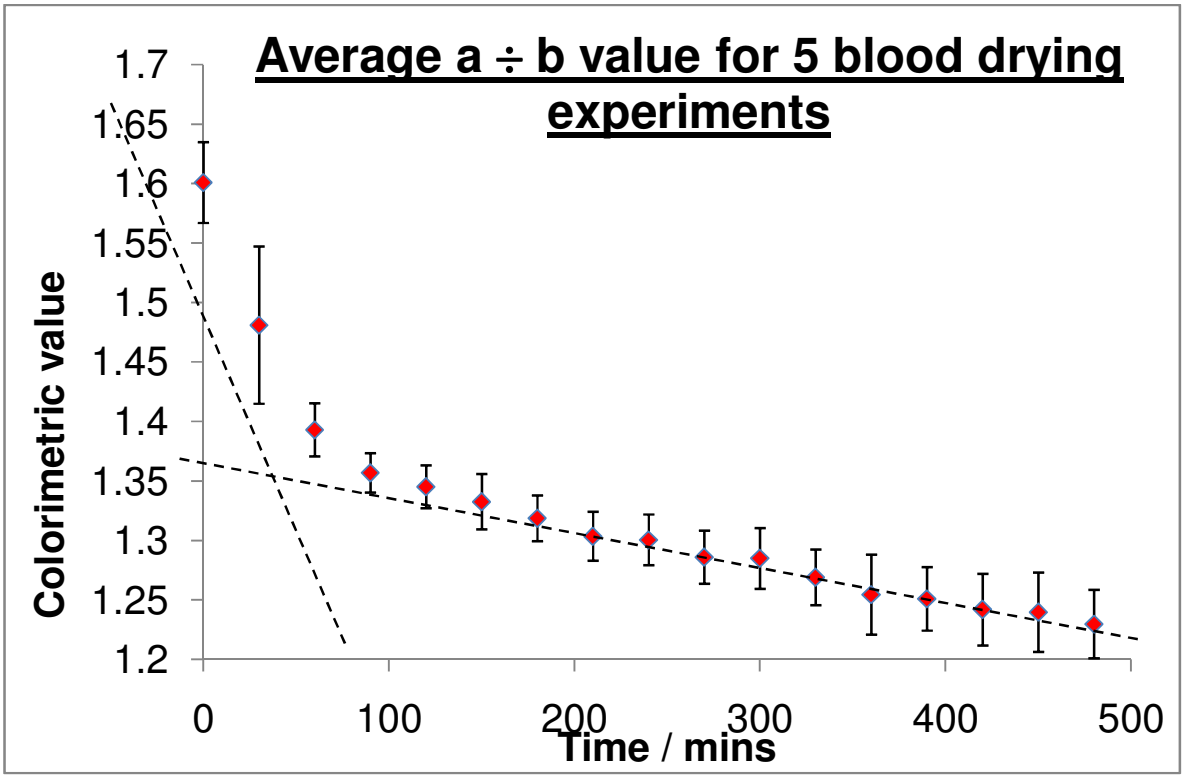
210	44.576	19.952	15.306	3.844	-13.414	-5.536	2.4230	74.260
240	44.374	19.698	15.146	3.642	-13.668	-5.696	2.3996	77.853
270	44.408	19.328	15.030	3.676	-14.038	-5.812	2.4153	81.589
300	44.264	19.192	14.936	3.532	-14.174	-5.906	2.3999	83.712
330	44.392	18.908	14.900	3.66	-14.458	-5.942	2.4332	85.909
360	44.544	18.576	14.808	3.812	-14.79	-6.034	2.4511	89.243
390	44.426	18.318	14.644	3.694	-15.048	-6.198	2.4279	93.268
420	44.446	18.142	14.608	3.714	-15.224	-6.234	2.4421	94.906
450	44.414	18.030	14.544	3.682	-15.336	-6.298	2.4351	96.586
480	44.424	17.828	14.498	3.692	-15.538	-6.344	2.4492	98.573

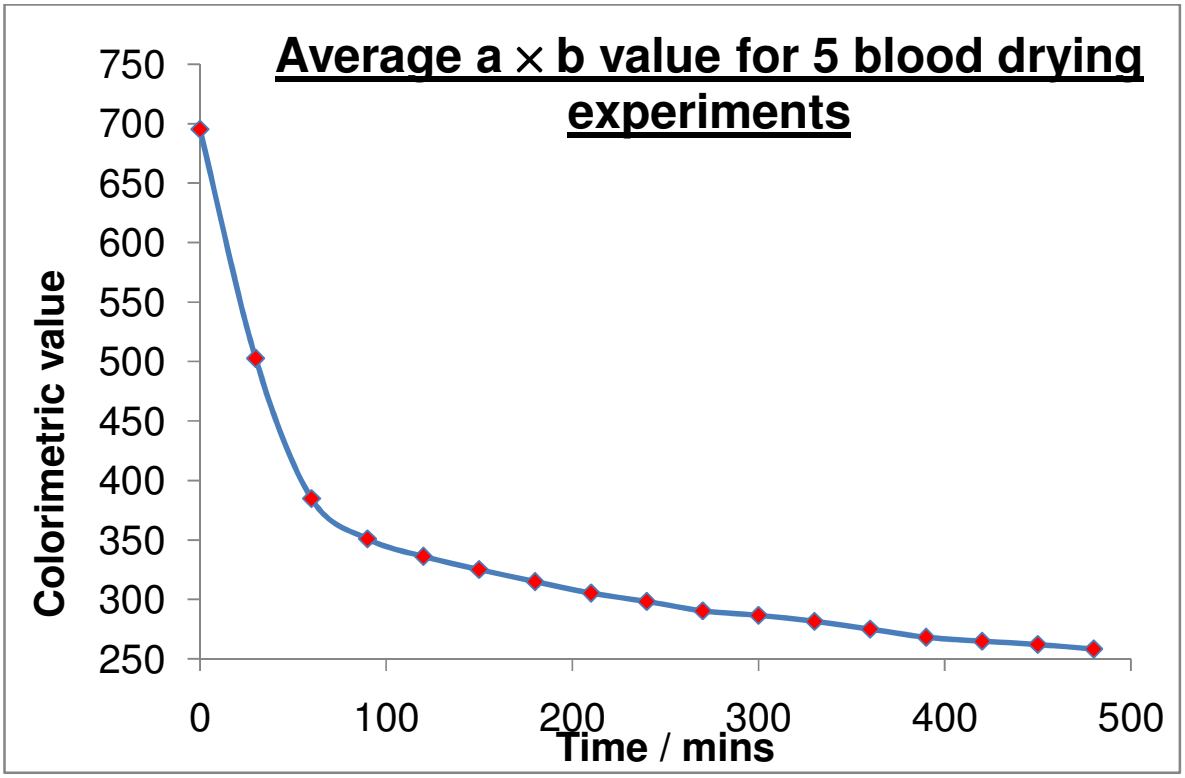
Graphs for run 2

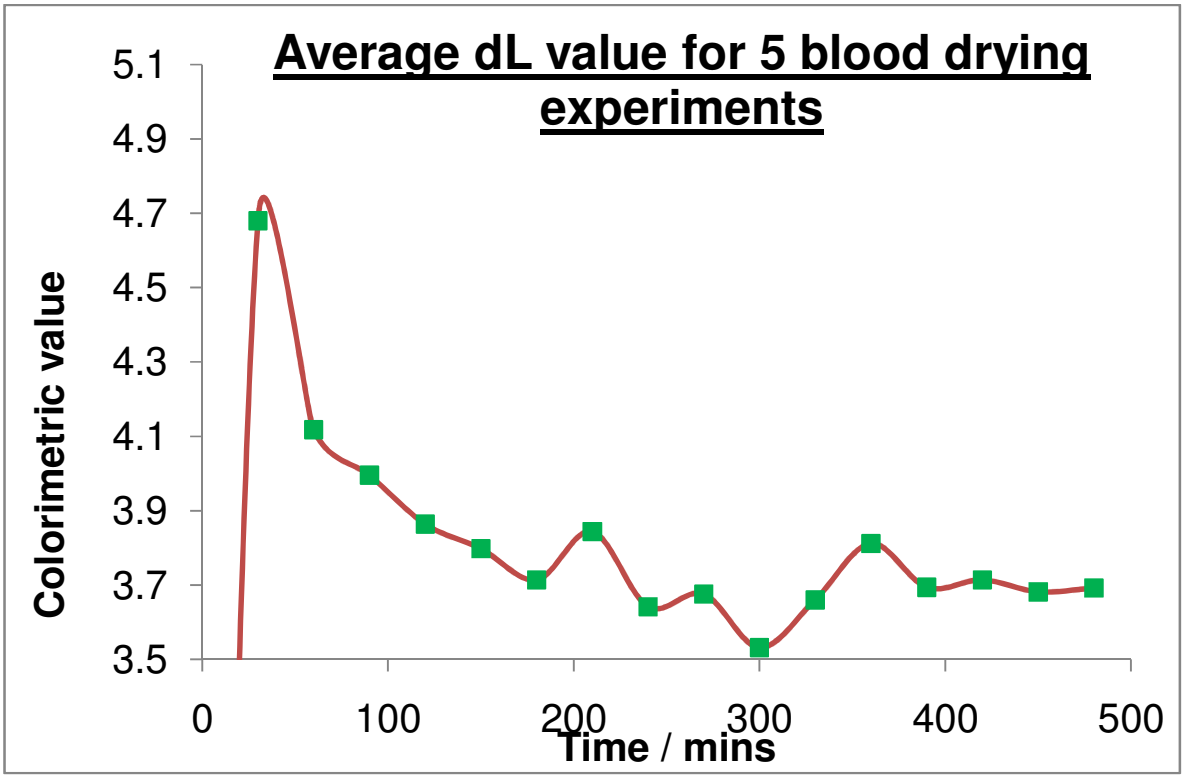


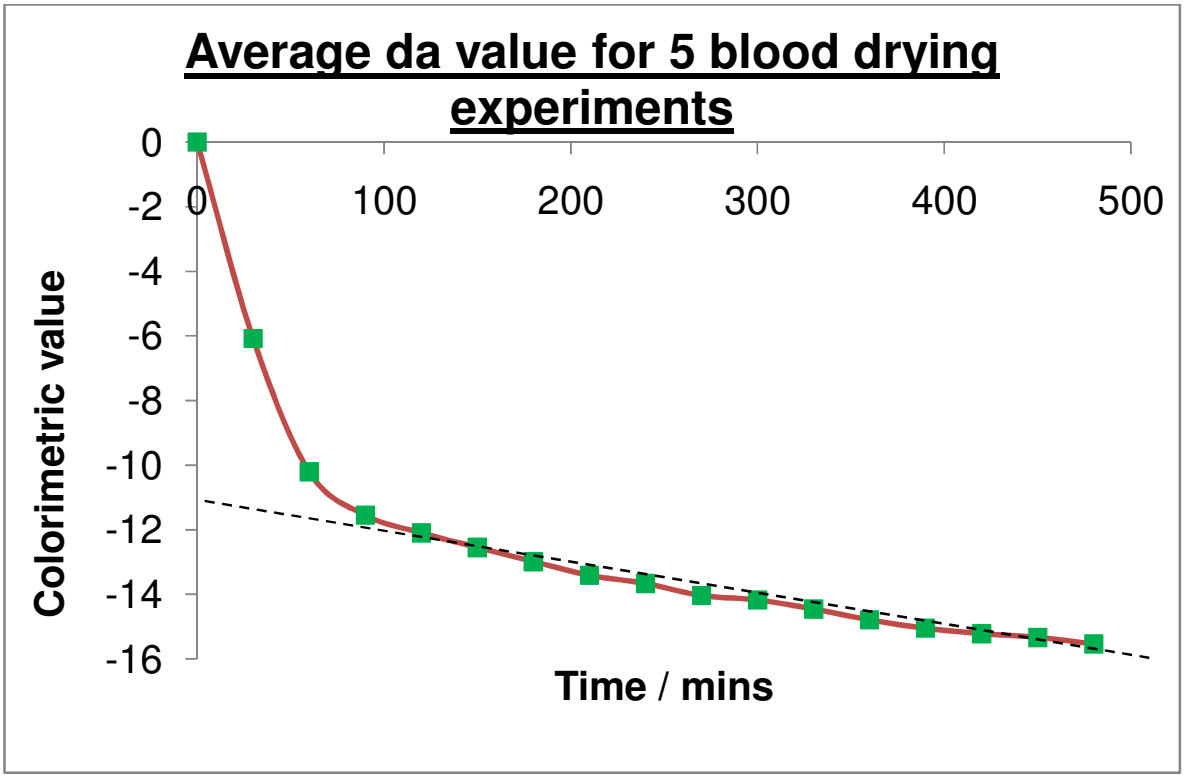

Average a value for 5 blood drying experiments
Colorimetric value
36
34
32
30
28
26
24
22
20
18
16
0
100
200
300
400
500
Time / mins

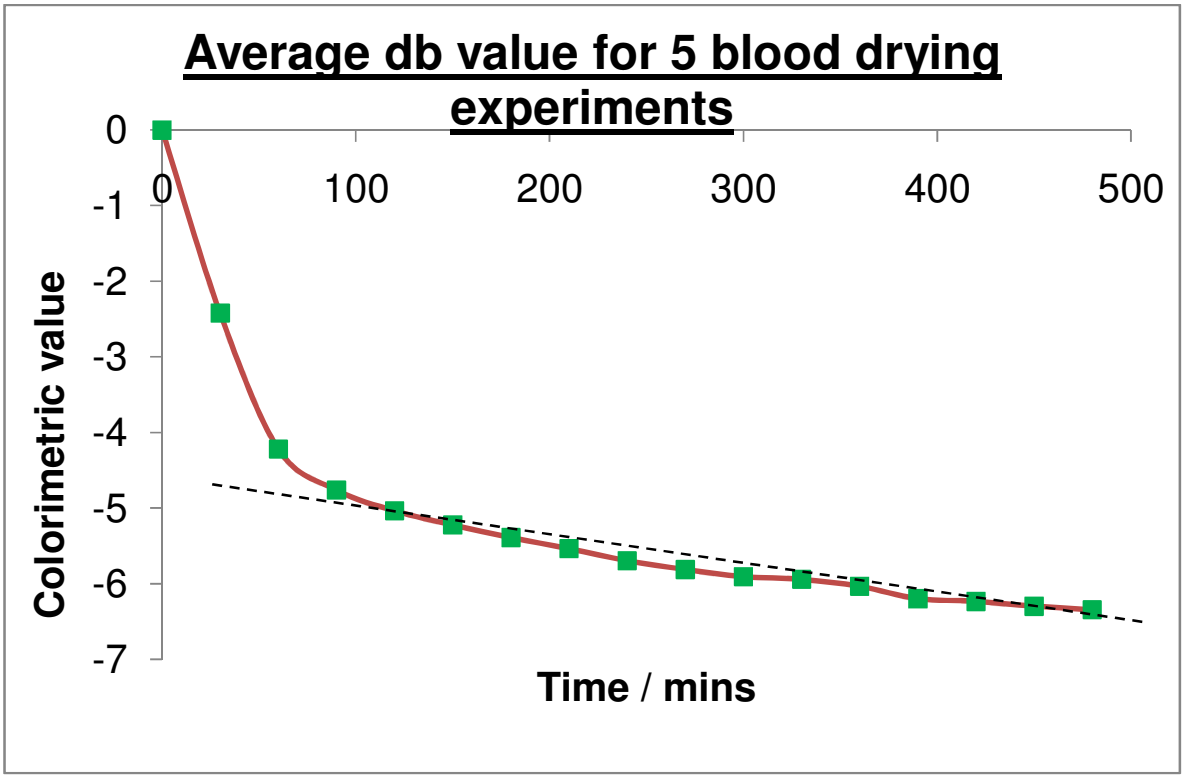

Average b value for 5 blood drying experiments
Colorimetric value
22
21
20
19
18
17
16
15
14
13
0
100
200
300
400
500
Time / mins

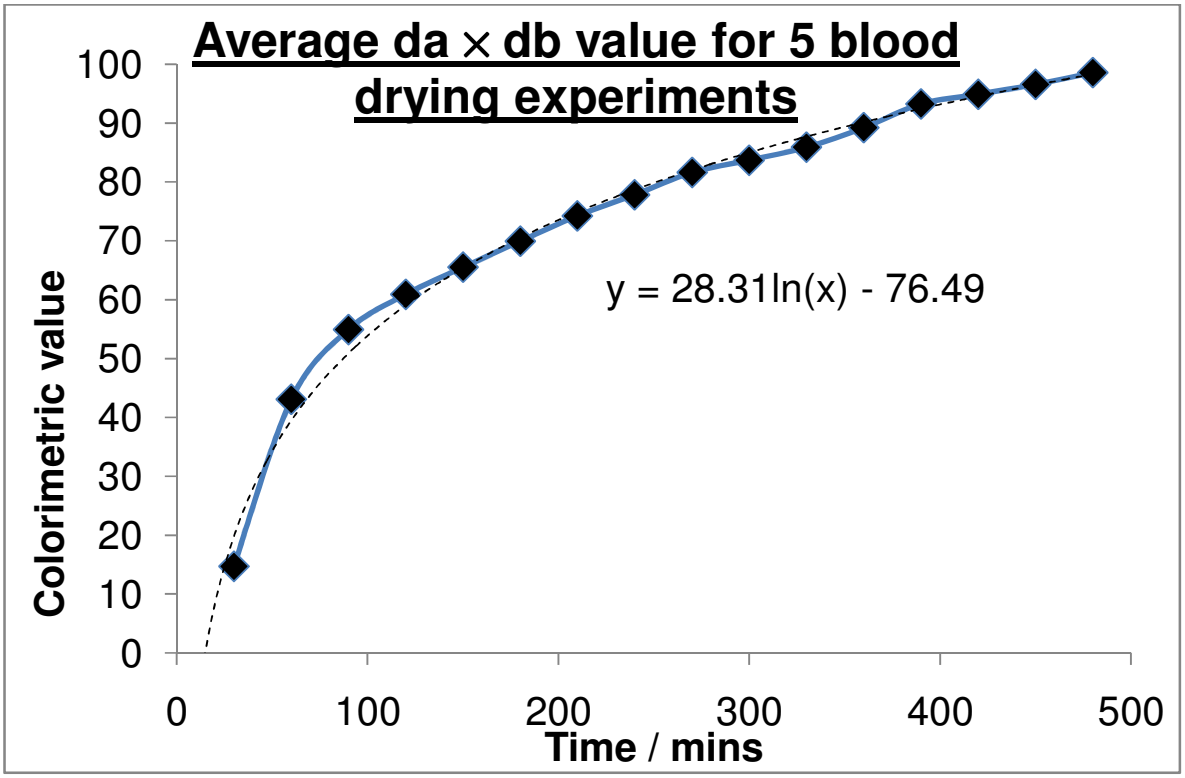

Average a ÷ b value for 5 blood drying experiments
Colorimetric value
1.7
1.65
1.6
1.55
1.5
1.45
1.4
1.35
1.3
1.25
1.2
0
100
200
300
400
500
Time / mins


Average a × b value for 5 blood drying experiments
Colorimetric value
750
700
650
600
550
500
450
400
350
300
250
0
100
200
300
400
500
Time / mins


Average dL value for 5 blood drying experiments
Colorimetric value
5.1
4.9
4.7
4.5
4.3
4.1
3.9
3.7
3.5
0
100
200
300
400
500
Time / mins


Average da value for 5 blood drying experiments
Colorimetric value
0
-2
-4
-6
-8
-10
-12
-14
-16
0
100
200
300
400
500
Time / mins





Filter Paper Run 3:

SAMPLE 1a

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
Sample 1a - 0 mins	34.71	32.63	18.79	------	------	------
Sample 1a - 30 mins	44.31	29.18	18.81	9.6	-3.44	0.02
Sample 1a - 60 mins	43.41	23.39	16.15	8.69	-9.24	-2.64
Sample 1a - 90 mins	43	22.04	15.49	8.29	-10.58	-3.3
Sample 1a - 120 mins	42.77	21.5	15.19	8.06	-11.13	-3.6
Sample 1a - 150 mins	43.01	20.94	15.11	8.3	-11.68	-3.69
Sample 1a - 180 mins	42.73	20.5	14.84	8.02	-12.13	-3.95
Sample 1a - 210 mins	42.54	20.11	14.58	7.82	-12.52	-4.21
Sample 1a - 240 mins	42.69	19.86	14.6	7.98	-12.76	-4.19
Sample 1a - 270 mins	42.72	19.51	14.49	8.01	-13.11	-4.3
Sample 1a - 300 mins	42.51	19.4	14.33	7.8	-13.23	-4.46
Sample 1a - 330 mins	42.83	18.97	14.36	8.12	-13.66	-4.43
Sample 1a - 360 mins	42.48	18.82	14.15	7.76	-13.81	-4.64
Sample 1a - 390 mins	42.48	18.61	14.03	7.77	-14.01	-4.77
Sample 1a - 420 mins	42.36	18.41	13.94	7.65	-14.22	-4.85
Sample 1a - 450 mins	42.34	18.38	13.88	7.63	-14.24	-4.91
Sample 1a - 480 mins	42.26	18.15	13.78	7.55	-14.48	-5.01

SAMPLE 1b

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
Sample 1b - 0 mins	40.51	33.12	20.6	------	------	------
Sample 1b - 30 mins	45.58	27.76	18.68	5.07	-5.36	-1.92
Sample 1b - 60 mins	44.85	22.7	16.48	4.34	-10.42	-4.12
Sample 1b - 90 mins	44.57	21.55	15.96	4.06	-11.57	-4.64
Sample 1b - 120 mins	44.53	20.92	15.7	4.02	-12.2	-4.9
Sample 1b - 150 mins	44.57	20.34	15.51	4.06	-12.78	-5.09
Sample 1b - 180 mins	44.4	19.94	15.28	3.89	-13.18	-5.32
Sample 1b - 210 mins	44.49	19.54	15.19	3.98	-13.58	-5.41
Sample 1b - 240 mins	44.37	19.28	15.02	3.86	-13.84	-5.58
Sample 1b - 270 mins	44.43	18.91	14.93	3.92	-14.21	-5.67
Sample 1b - 300 mins	44.3	18.7	14.8	3.79	-14.42	-5.8
Sample 1b - 330 mins	44.39	18.39	14.74	3.88	-14.73	-5.86
Sample 1b - 360 mins	45.1	17.83	14.74	4.59	-15.29	-5.86
Sample 1b - 390 mins	44.91	17.74	14.7	4.4	-15.38	-5.9

Sample 1b - 420 mins	44.89	17.53	14.6	4.38	-15.59	-6
Sample 1b - 450 mins	44.83	17.35	14.47	4.32	-15.77	-6.13
Sample 1b - 480 mins	44.84	17.19	14.48	4.33	-15.93	-6.12

SAMPLE 1c

Data Name	**L*(D65)**	**a*(D65)**	**b*(D65)**	**dL*(D65)**	**da*(D65)**	**db*(D65)**
Sample 1c - 0 mins	43.41	34.38	22.35	------	------	------
Sample 1c - 30 mins	46.93	26.69	18.8	3.52	-7.69	-3.55
Sample 1c - 60 mins	46.44	23.63	17.45	3.03	-10.75	-4.9
Sample 1c - 90 mins	46.36	22.16	16.89	2.95	-12.22	-5.46
Sample 1c - 120 mins	46.11	21.61	16.59	2.7	-12.77	-5.76
Sample 1c - 150 mins	45.95	21.34	16.41	2.54	-13.04	-5.94
Sample 1c - 180 mins	46.29	20.75	16.28	2.88	-13.63	-6.07
Sample 1c - 210 mins	46.25	20.38	16.14	2.84	-14	-6.21
Sample 1c - 240 mins	46.01	20.14	15.98	2.6	-14.24	-6.37
Sample 1c - 270 mins	45.92	19.77	15.82	2.51	-14.61	-6.53
Sample 1c - 300 mins	45.68	19.7	15.72	2.27	-14.68	-6.63
Sample 1c - 330 mins	45.82	19.32	15.63	2.41	-15.06	-6.72
Sample 1c - 360 mins	45.62	19.18	15.48	2.21	-15.2	-6.87
Sample 1c - 390 mins	45.84	18.74	15.44	2.43	-15.64	-6.91
Sample 1c - 420 mins	46.04	18.65	15.45	2.63	-15.73	-6.9
Sample 1c - 450 mins	45.77	18.6	15.33	2.36	-15.78	-7.02
Sample 1c - 480 mins	45.81	18.24	15.27	2.4	-16.14	-7.08

SAMPLE 1d

Data Name	**L*(D65)**	**a*(D65)**	**b*(D65)**	**dL*(D65)**	**da*(D65)**	**db*(D65)**
Sample 1d - 0 mins	42.77	32.92	21.38	------	------	------
Sample 1d - 30 mins	45.7	26.1	18.17	2.93	-6.82	-3.21
Sample 1d - 60 mins	45.59	22.9	16.84	2.82	-10.02	-4.54
Sample 1d - 90 mins	45.54	21.64	16.43	2.77	-11.28	-4.95
Sample 1d - 120 mins	45.27	21.15	16.08	2.5	-11.77	-5.3
Sample 1d - 150 mins	45.13	20.7	15.84	2.36	-12.22	-5.54
Sample 1d - 180 mins	45.16	20.29	15.8	2.39	-12.63	-5.58
Sample 1d - 210 mins	45.36	19.82	15.62	2.59	-13.1	-5.76

Sample 1d - 240 mins	45.16	19.56	15.45	2.39	-13.36	-5.93
Sample 1d - 270 mins	45.02	19.33	15.35	2.25	-13.59	-6.03
Sample 1d - 300 mins	45.1	19	15.24	2.33	-13.92	-6.14
Sample 1d - 330 mins	44.97	18.78	15.08	2.2	-14.14	-6.3
Sample 1d - 360 mins	45.05	18.5	15.03	2.28	-14.42	-6.35
Sample 1d - 390 mins	44.76	18.29	14.75	1.99	-14.63	-6.63
Sample 1d - 420 mins	44.84	18.23	14.89	2.07	-14.69	-6.49
Sample 1d - 450 mins	44.98	17.97	14.82	2.21	-14.95	-6.56
Sample 1d - 480 mins	44.89	17.85	14.75	2.12	-15.07	-6.63

SAMPLE 1e

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
Sample 1e - 0 mins	42.26	33.78	21.09	------	------	------
Sample 1e - 30 mins	44.54	26.69	17.65	2.28	-7.09	-3.44
Sample 1e - 60 mins	43.96	23.16	16.19	1.7	-10.62	-4.9
Sample 1e - 90 mins	44.17	21.71	15.64	1.91	-12.07	-5.45
Sample 1e - 120 mins	44.3	21.14	15.48	2.04	-12.64	-5.61
Sample 1e - 150 mins	43.99	20.76	15.23	1.73	-13.02	-5.86
Sample 1e - 180 mins	43.65	20.41	15.07	1.39	-13.37	-6.02
Sample 1e - 210 mins	44.24	19.91	15	1.98	-13.87	-6.09
Sample 1e - 240 mins	43.64	19.65	14.68	1.38	-14.13	-6.41
Sample 1e - 270 mins	43.95	19.12	14.56	1.69	-14.66	-6.53
Sample 1e - 300 mins	43.73	19.16	14.59	1.47	-14.62	-6.5
Sample 1e - 330 mins	43.95	19.08	14.69	1.69	-14.7	-6.4
Sample 1e - 360 mins	44.47	18.55	14.64	2.21	-15.23	-6.45
Sample 1e - 390 mins	44.14	18.21	14.3	1.88	-15.57	-6.79
Sample 1e - 420 mins	44.1	17.89	14.16	1.84	-15.89	-6.93
Sample 1e - 450 mins	44.15	17.85	14.22	1.89	-15.93	-6.87
Sample 1e - 480 mins	44.32	17.71	14.21	2.06	-16.07	-6.88

AVERAGE VALUES FOR ALL SAMPLES

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
0 mins	40.732	33.366	20.842	------	------	------
30 mins	45.412	27.284	18.422	4.68	-6.082	-2.42

60 mins	44.85	23.156	16.622	4.118	-10.21	-4.22
90 mins	44.728	21.82	16.082	3.996	-11.546	-4.76
120 mins	44.596	21.264	15.808	3.864	-12.102	-5.034
150 mins	44.53	20.816	15.62	3.798	-12.55	-5.222
180 mins	44.446	20.378	15.454	3.714	-12.988	-5.388
210 mins	44.576	19.952	15.306	3.844	-13.414	-5.536
240 mins	44.374	19.698	15.146	3.642	-13.668	-5.696
270 mins	44.408	19.328	15.03	3.676	-14.038	-5.812
300 mins	44.264	19.192	14.936	3.532	-14.174	-5.906
330 mins	44.392	18.908	14.9	3.66	-14.458	-5.942
360 mins	44.544	18.576	14.808	3.812	-14.79	-6.034
390 mins	44.426	18.318	14.644	3.694	-15.048	-6.198
420 mins	44.446	18.142	14.608	3.714	-15.224	-6.234
450 mins	44.414	18.03	14.544	3.682	-15.336	-6.298
480 mins	44.424	17.828	14.498	3.692	-15.538	-6.344

Cotton Cloth Run:

SAMPLE 1a

Data Name	**L*(D65)**	**a*(D65)**	**b*(D65)**	**dL*(D65)**	**da*(D65)**	**db*(D65)**
Sample 1a- 0 mins	49.66	33.45	23.84	------	------	------
Sample 1a - 10 mins	49.6	27.85	20.52	-0.06	-5.6	-3.33
Sample 1a - 20 mins	49.53	24.74	19.27	-0.13	-8.71	-4.57
Sample 1a - 30 mins	49.03	24.08	18.85	-0.62	-9.37	-5
Sample 1a - 40 mins	49.08	23.32	18.56	-0.57	-10.13	-5.29
Sample 1a - 50 mins	48.5	22.76	18.05	-1.15	-10.69	-5.79
Sample 1a - 60 mins	48.95	22.49	18.16	-0.7	-10.96	-5.69
Sample 1a - 70 mins	48.98	22.67	18.35	-0.68	-10.78	-5.49
Sample 1a - 80 mins	48.48	22.09	17.84	-1.18	-11.36	-6
Sample 1a - 90 mins	48.6	21.64	17.66	-1.05	-11.81	-6.18

SAMPLE 1b

Data Name	**L*(D65)**	**a*(D65)**	**b*(D65)**	**dL*(D65)**	**da*(D65)**	**db*(D65)**
Sample 1b - 0 mins	44.06	33.5	22.29	------	------	------
Sample 1b - 10 mins	46.14	31.71	21.33	-3.52	-1.75	-2.52
Sample 1b - 20	45.48	25.42	18.09	-4.18	-8.03	-5.75

mins						
Sample 1b - 30 mins	45.61	23.91	17.61	-4.04	-9.55	-6.23
Sample 1b - 40 mins	45.26	23.11	17.2	-4.4	-10.34	-6.64
Sample 1b - 50 mins	45.63	22.83	17.3	-4.03	-10.62	-6.54
Sample 1b - 60 mins	45.14	22.15	16.78	-4.52	-11.3	-7.06
Sample 1b - 70 mins	44.99	21.83	16.6	-4.66	-11.62	-7.24
Sample 1b - 80 mins	44.54	21.87	16.39	-5.11	-11.58	-7.45
Sample 1b - 90 mins	44.54	21.54	16.28	-5.11	-11.91	-7.56

SAMPLE 1c

Data Name	**L*(D65)**	**a*(D65)**	**b*(D65)**	**dL*(D65)**	**da*(D65)**	**db*(D65)**
Sample 1c - 0 mins	44.16	33.74	22.3	------	------	------
Sample 1c - 10 mins	45.28	31.63	20.94	-4.38	-1.82	-2.9
Sample 1c - 20 mins	44.6	25.68	17.88	-5.06	-7.77	-5.96
Sample 1c - 30 mins	44.79	24.28	17.52	-4.86	-9.17	-6.32
Sample 1c - 40 mins	44.69	23.19	17.01	-4.97	-10.26	-6.83
Sample 1c - 50 mins	44.39	22.75	16.64	-5.27	-10.7	-7.2
Sample 1c - 60 mins	44.4	22.28	16.47	-5.26	-11.17	-7.38
Sample 1c - 70 mins	44.36	22.08	16.46	-5.29	-11.37	-7.38
Sample 1c - 80 mins	44.11	21.63	16.12	-5.55	-11.82	-7.72
Sample 1c - 90 mins	43.9	21.23	15.86	-5.75	-12.22	-7.98

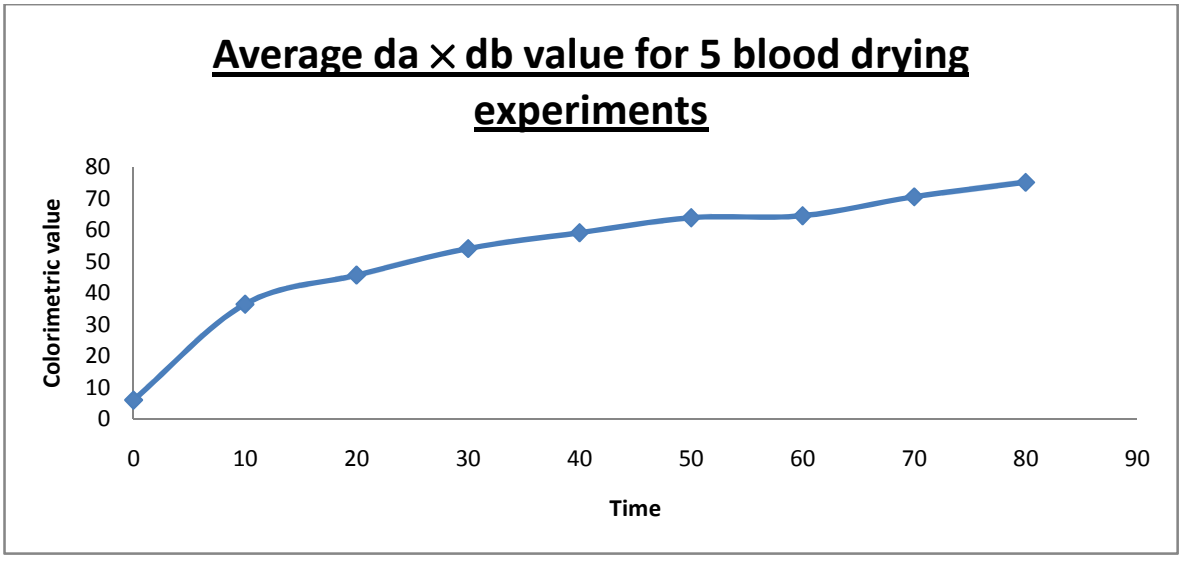
AVERAGE VALUES

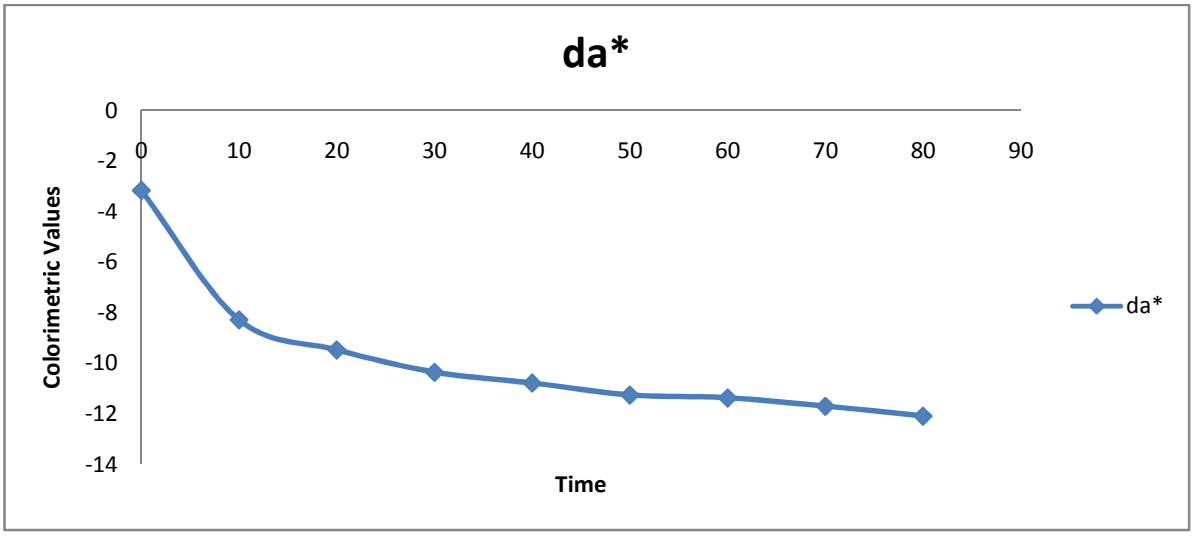
Data Name	**L*(D65)**	**a*(D65)**	**b*(D65)**	**dL*(D65)**	**da*(D65)**	**db*(D65)**
0	45.96	33.56333	22.81	------	------	------
10	47.00667	30.39667	20.93	1.046667	-3.16667	-1.88
20	46.53667	25.28	18.41333	0.576667	-8.28333	-4.39667
30	46.47667	24.09	17.99333	0.516667	-9.47333	-4.81667
40	46.34333	23.20667	17.59	0.383333	-10.3567	-5.22
50	46.17333	22.78	17.33	0.213333	-10.7833	-5.48
60	46.16333	22.30667	17.13667	0.203333	-11.2567	-5.67333
70	46.11	22.19333	17.13667	0.15	-11.37	-5.67333

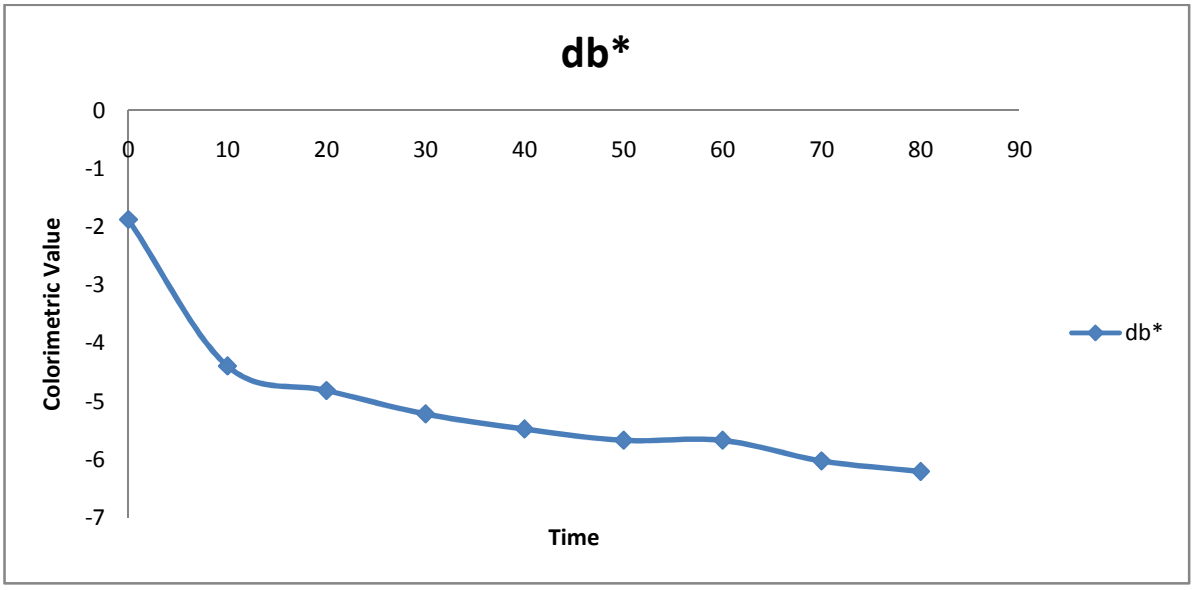
80	45.71	21.86333	16.78333	-0.25	-11.7	-6.02667
90	45.68	21.47	16.6	-0.28	-12.0933	-6.21

Data Name	sdev L*	sdev a*	sdev b*	sdev dL*	a/b	da*db	da/db
0	3.204684	0.155027	0.89202		1.471431		
10	2.286686	2.205841	0.405093	2.286686	1.452301	5.953333	1.684397163
20	2.629379	0.485386	0.749289	2.629379	1.372918	36.41906	1.884003033
30	2.24894	0.185203	0.743259	2.24894	1.338829	45.62989	1.966782007
40	2.387097	0.105987	0.845399	2.39283	1.31931	54.0618	1.98403576
50	2.108182	0.043589	0.705479	2.113701	1.314484	59.09267	1.967761557
60	2.441523	0.171561	0.899685	2.44723	1.301692	63.86282	1.98413631
70	2.505374	0.431316	1.053107	2.499647	1.295079	64.5058	2.004112808
80	2.408506	0.230072	0.925005	2.406083	1.302681	70.512	1.941371681
90	2.548961	0.213776	0.941701	2.548961	1.293373	75.0996	1.947396672

Graphs of this run:







Cotton Cloth Run 2:

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
Sample 1a - 0 mins	35.69	36.79	19.01	------	------	------
Sample 1a - 30 mins	47.26	27.01	18.12	11.57	-9.78	-0.9
Sample 1a - 60 mins	46.86	21.2	16.03	11.17	-15.59	-2.98
Sample 1a - 90 mins	46.63	20.17	15.61	10.94	-16.62	-3.4
Sample 1a - 120 mins	46.59	19.46	15.36	10.9	-17.33	-3.65
Sample 1a - 150 mins	46.6	19.2	15.22	10.91	-17.59	-3.79
Sample 1a - 180 mins	48.08	18.05	14.92	12.39	-18.74	-4.1
Sample 1a -210 mins	46.4	16.18	14.77	10.71	-20.61	-4.24
Sample 1a - 240	47.91	17.22	14.65	12.22	-19.57	-4.37

mins						
Sample 1a - 270 mins	48.28	16.99	14.62	12.58	-19.8	-4.39
Sample 1a - 300 mins	46.51	17.75	14.68	10.82	-19.04	-4.33
Sample 1a - 330 mins	47.95	16.91	14.4	12.26	-19.88	-4.61
Sample 1a - 360 mins	46.61	17.24	14.51	10.92	-19.55	-4.5
Sample 1a - 490 mins	46.43	17.07	14.45	10.74	-19.72	-4.57
Sample 1a - 420 mins	47.71	16.36	14.42	12.02	-20.43	-4.59
Sample 1a - 450 mins	48.02	15.97	14.3	12.33	-20.82	-4.71

Data Name	**L*(D65)**	**a*(D65)**	**b*(D65)**	**dL*(D65)**	**da*(D65)**	**db*(D65)**
Sample 1b - 0 mins	33.5	39.31	20.37	------	------	------
Sample 1b- 30 mins	44.53	26.6	17.33	11.03	-12.71	-3.04
Sample 1b - 60 mins	45.01	22.41	15.86	11.51	-16.9	-4.51
Sample 1b - 90 mins	44.64	21.47	15.39	11.14	-17.84	-4.98
Sample 1b - 120 mins	44.55	20.57	15.06	11.05	-18.74	-5.31
Sample 1b - 150 mins	44.41	20.35	14.92	10.91	-18.96	-5.45
Sample 1b - 180 mins	44.47	19.92	14.81	10.97	-19.39	-5.56
Sample 1b -210 mins	41.08	18.69	14.28	7.58	-20.62	-6.09
Sample 1b - 240 mins	41.68	19.48	13.46	8.18	-19.83	-6.91
Sample 1b - 270 mins	44.17	18.94	14.39	10.67	-20.37	-5.98
Sample 1b - 300 mins	44.41	18.78	14.41	10.91	-20.53	-5.96
Sample 1b - 330 mins	44.26	18.54	14.29	10.76	-20.77	-6.08
Sample 1b - 360 mins	44.2	18.24	14.16	10.7	-21.07	-6.21
Sample 1b - 390 mins	43.82	18.24	14.07	10.32	-21.07	-6.3
Sample 1b - 420 mins	43.73	18.03	13.97	10.23	-21.28	-6.4
Sample 1b - 450 mins	44.32	17.49	13.95	10.82	-21.82	-6.42

Data Name	**L*(D65)**	**a*(D65)**	**b*(D65)**	**dL*(D65)**	**da*(D65)**	**db*(D65)**
Sample 1c - 0 mins	34.6	38.88	20.26	------	------	------
Sample 1c - 30 mins	44.01	25.57	16.88	9.41	-13.31	-3.38

Sample 1c - 60 mins	43.6	22.99	15.83	9	-15.89	-4.43
Sample 1c - 90 mins	43.82	21.65	15.32	9.22	-17.23	-4.94
Sample 1c - 120 mins	43.28	21.05	14.99	8.68	-17.83	-5.27
Sample 1c - 150 mins	43.35	20.76	14.93	8.75	-18.12	-5.33
Sample 1c - 180 mins	43.59	20.27	14.8	8.99	-18.61	-5.46
Sample 1c -210 mins	41.2	19.33	14.11	6.6	-19.55	-6.15
Sample 1c - 240 mins	43.26	19.29	14.37	8.66	-19.59	-5.89
Sample 1c - 270 mins	42.98	19.4	14.36	8.38	-19.48	-5.9
Sample 1c - 300 mins	42.99	19.29	14.31	8.39	-19.59	-5.95
Sample 1c - 330 mins	42.96	18.76	14.17	8.36	-20.12	-6.09
Sample 1c - 360 mins	43.11	18.94	14.26	8.51	-19.94	-6
Sample 1c - 390 mins	42.88	18.46	14.06	8.28	-20.42	-6.2
Sample 1c - 420 mins	43.43	17.89	13.85	8.83	-20.99	-6.41
Sample 1c - 450 mins	43.41	17.79	13.86	8.81	-21.09	-6.4

Data Name	**L*(D65)**	**a*(D65)**	**b*(D65)**	**dL*(D65)**	**da*(D65)**	**db*(D65)**
Sample 1d - 0 mins	36.05	36.28	19.58	------	------	------
Sample 1d - 30 mins	46.11	23.9	16.94	10.06	-12.38	-2.64
Sample 1d - 60 mins	45.68	21.36	15.93	9.63	-14.92	-3.65
Sample 1d - 90 mins	45.32	20.37	15.5	9.27	-15.91	-4.08
Sample 1d - 120 mins	45.28	19.66	15.23	9.23	-16.62	-4.35
Sample 1d - 150 mins	45.44	19.44	15.16	9.39	-16.84	-4.42
Sample 1d - 180 mins	45.36	19.12	15.05	9.31	-17.16	-4.53
Sample 1d -210 mins	45.11	17.98	14.89	9.06	-18.3	-4.69
Sample 1d - 240 mins	45.17	18.14	14.67	9.12	-18.14	-4.91
Sample 1d - 270 mins	45.17	18	14.64	9.12	-18.28	-4.94
Sample 1d - 300 mins	45.28	17.95	14.65	9.23	-18.33	-4.93
Sample 1d - 330 mins	45.07	17.75	14.5	9.02	-18.53	-5.08
Sample 1d - 360 mins	44.95	17.51	14.49	8.9	-18.77	-5.09
Sample 1d - 390	44.99	17.34	14.37	8.94	-18.94	-5.21

mins						
Sample 1d - 420 mins	44.97	17.05	14.29	8.92	-19.23	-5.29
Sample 1d - 450 mins	44.98	16.89	14.21	8.93	-19.39	-5.37

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
Sample 1e - 0 mins	35.82	37.14	21.63	------	------	------
Sample 1e - 30 mins	43.29	26.48	18.51	7.47	-10.66	-3.12
Sample 1e - 60 mins	42.82	23.72	17.46	7	-13.42	-4.17
Sample 1e - 90 mins	42.37	22.56	16.72	6.55	-14.58	-4.91
Sample 1e - 120 mins	42.6	21.8	16.76	6.78	-15.34	-4.87
Sample 1e - 150 mins	42.34	21.47	16.49	6.52	-15.67	-5.14
Sample 1e - 180 mins	42.28	21.14	16.4	6.46	-16	-5.23
Sample 1e -210 mins	42.33	19.55	15.93	6.51	-17.59	-5.7
Sample 1e - 240 mins	42	20	15.85	6.18	-17.14	-5.78
Sample 1e - 270 mins	42.04	19.85	15.85	6.22	-17.29	-5.78
Sample 1e - 300 mins	42.35	19.85	16.01	6.53	-17.29	-5.62
Sample 1e - 330 mins	42.06	19.51	15.74	6.24	-17.63	-5.89
Sample 1e - 360 mins	42.09	19.14	15.42	6.27	-18	-6.21
Sample 1e - 390 mins	41.94	19.05	15.56	6.12	-18.09	-6.07
Sample 1e - 420 mins	42.37	18.85	15.62	6.55	-18.29	-6.01
Sample 1e - 450 mins	42.07	18.51	15.27	6.25	-18.63	-6.36

AVERAGE VALUES (cotton)

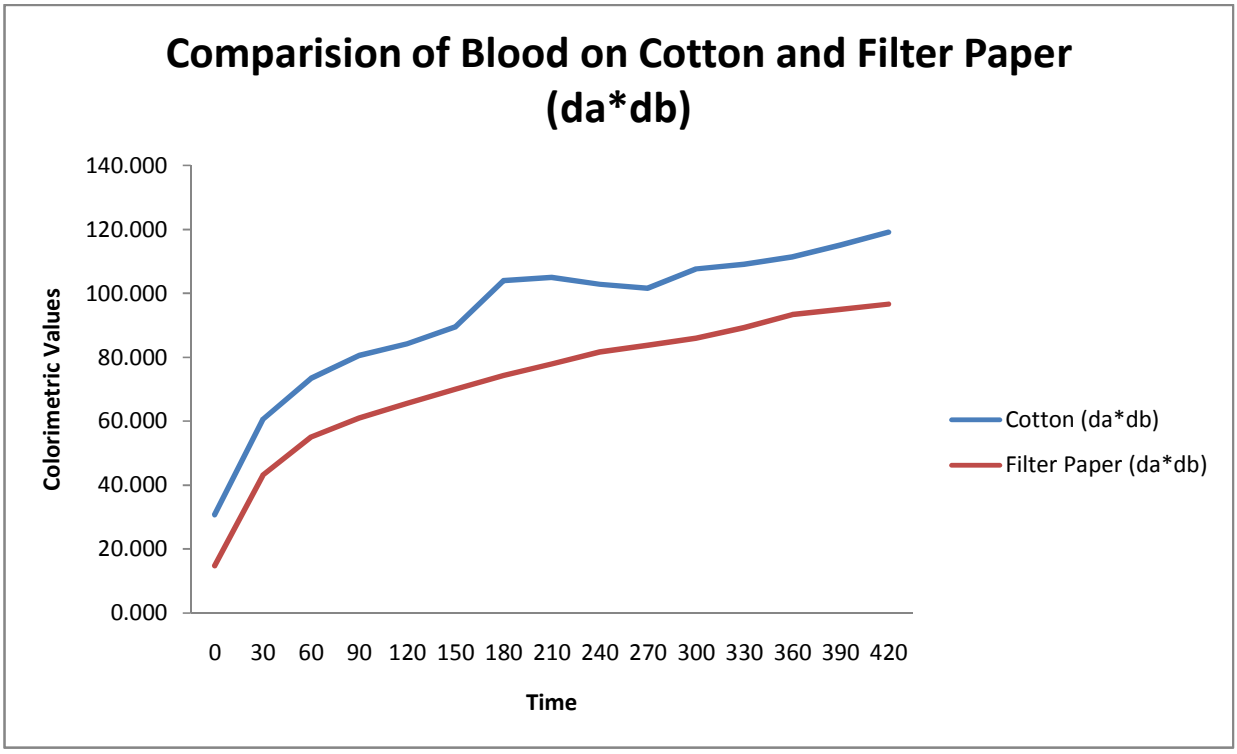
Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)	a/b	da*db	da/db
0	35.132	37.680	20.170	------	------	------	1.868		
30	45.040	25.912	17.556	9.908	-11.768	-2.614	1.476	30.762	4.502
60	44.794	22.336	16.222	9.662	-15.344	-3.948	1.377	60.578	3.887
90	44.556	21.244	15.708	9.424	-16.436	-4.462	1.352	73.337	3.684
120	44.460	20.508	15.480	9.328	-17.172	-4.690	1.325	80.537	3.661
150	44.428	20.244	15.344	9.296	-17.436	-4.826	1.319	84.146	3.613
180	44.756	19.700	15.196	9.624	-17.980	-4.974	1.296	89.433	3.615
210	43.224	18.346	14.796	8.092	-19.334	-5.374	1.240	103.901	3.598
240	44.004	18.826	14.600	8.872	-18.854	-5.570	1.289	105.017	3.385
270	44.528	18.636	14.772	9.396	-19.044	-5.398	1.262	102.800	3.528
300	44.308	18.724	14.812	9.176	-18.956	-5.358	1.264	101.566	3.538
330	44.460	18.294	14.620	9.328	-19.386	-5.550	1.251	107.592	3.493
360	44.192	18.214	14.568	9.060	-19.466	-5.602	1.250	109.04	3.475

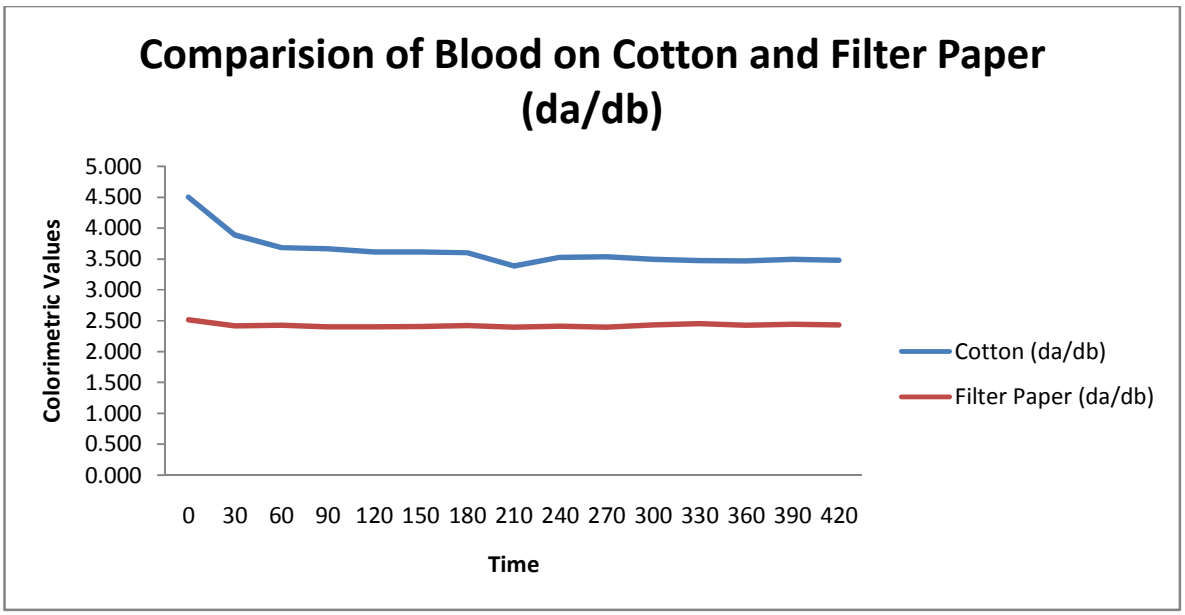
								9	
390	44.012	18.032	14.502	8.880	-19.648	-5.668	1.243	111.365	3.466
420	44.442	17.636	14.430	9.310	-20.044	-5.740	1.222	115.053	3.492
450	44.560	17.330	14.318	9.428	-20.350	-5.852	1.210	119.088	3.477

Comparison data of cotton with filter paper:

AVERAGE VALUES
(filter paper)

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)	a/b	da*db	da/db
0	40.732	33.366	20.842	------	------	------	1.600902		
30	45.412	27.284	18.422	4.68	-6.082	-2.42	1.481055	14.71844	2.51322314
60	44.85	23.156	16.622	4.118	-10.21	-4.22	1.393093	43.0862	2.41943128
90	44.728	21.82	16.082	3.996	-11.546	-4.76	1.356796	54.95896	2.425630252
120	44.596	21.264	15.808	3.864	-12.102	-5.034	1.345142	60.92147	2.404052443
150	44.53	20.816	15.62	3.798	-12.55	-5.222	1.33265	65.5361	2.403293757
180	44.446	20.378	15.454	3.714	-12.988	-5.388	1.318623	69.97934	2.410541945
210	44.576	19.952	15.306	3.844	-13.414	-5.536	1.303541	74.2599	2.423049133
240	44.374	19.698	15.146	3.642	-13.668	-5.696	1.300541	77.85293	2.399578652
270	44.408	19.328	15.03	3.676	-14.038	-5.812	1.285961	81.58886	2.415347557
300	44.264	19.192	14.936	3.532	-14.174	-5.906	1.284949	83.71164	2.399932272
330	44.392	18.908	14.9	3.66	-14.458	-5.942	1.268993	85.90944	2.433187479
360	44.544	18.576	14.808	3.812	-14.79	-6.034	1.254457	89.24286	2.451110375
390	44.426	18.318	14.644	3.694	-15.048	-6.198	1.250888	93.2675	2.427879961
420	44.446	18.142	14.608	3.714	-15.224	-6.234	1.241922	94.90642	2.442091755
450	44.414	18.03	14.544	3.682	-15.336	-6.298	1.239686	96.58613	2.435058749





Denim Run:

Data Name	L*(D65)	a*(D65)	b*(D65)	dL*(D65)	da*(D65)	db*(D65)
Blue Surface 0 mins	46.69	-4.45	-13.41	------	------	------
Sample 1a - 0 mins	23.82	1.31	1.95	-22.87	5.76	15.35
Sample 1a - 30 mins	28.32	2.96	2.8	-18.36	7.41	16.2
Sample 1a - 60 mins	29.18	1.29	2.29	-17.51	5.73	15.7
Sample 1a - 90 mins	29.09	0.54	1.92	-17.6	4.99	15.33
Sample 1a - 120	29.4	0.91	2.12	-17.28	5.36	15.53

mins						
Sample 1a - 150 mins	29.43	0.79	2.04	-17.26	5.23	15.45
Sample 1a - 180 mins	29.47	0.89	2.02	-17.22	5.34	15.43

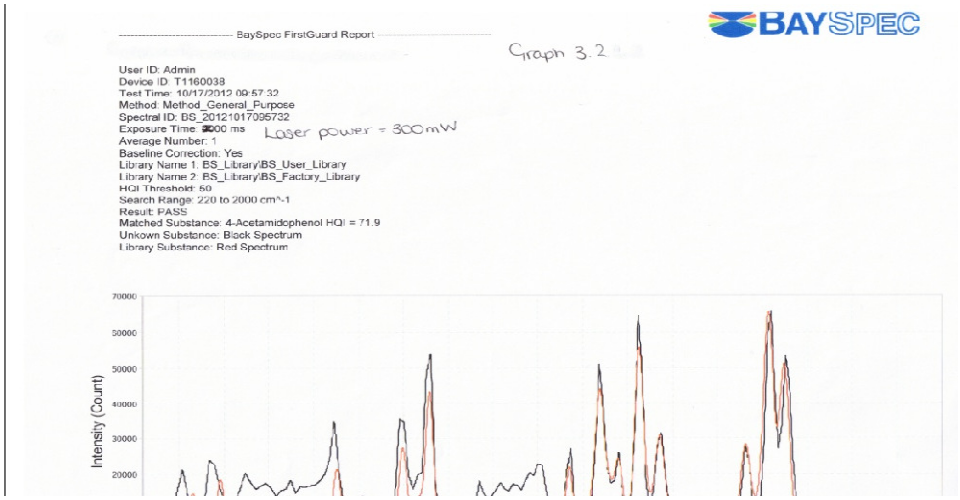
Data Name	**L*(D65)**	**a*(D65)**	**b*(D65)**	**dL*(D65)**	**da*(D65)**	**db*(D65)**
Blue Surface 0 mins	46.69	-4.45	-13.41	------	------	------
Sample 1b - 0 mins	24.05	3.15	3.11	-22.64	7.6	16.52
Sample 1b - 30 mins	28.21	2.26	2.47	-18.48	6.71	15.88
Sample 1b - 60 mins	28.47	1.82	2.4	-18.21	6.27	15.81
Sample 1b - 90 mins	28.64	1.37	2.32	-18.05	5.82	15.73
Sample 1b - 120 mins	28.78	1.19	2.16	-17.91	5.64	15.57
Sample 1b - 150 mins	30.47	2.96	3.91	-16.22	7.4	17.32
Sample 1b - 180 mins	29.03	0.95	1.37	-17.65	5.4	14.78

AVERAGE VALUES (Jeans)

Data Name	**L*(D65)**	**a*(D65)**	**b*(D65)**	**dL*(D65)**	**da*(D65)**	**db*(D65)**	**da*db**	**da/db**
0 mins	23.935	2.23	2.53	4.33	0.38	0.105	0.0399	3.619047619
30 mins	28.265	2.61	2.635	4.89	-0.675	-0.185	0.124875	3.648648649
60 mins	28.825	1.555	2.345	4.93	-1.275	-0.41	0.52275	3.109756098
90 mins	28.865	0.955	2.12	5.155	-1.18	-0.39	0.4602	3.025641026
120 mins	29.09	1.05	2.14	6.015	-0.355	0.445	-0.15798	-0.797752809
150 mins	29.95	1.875	2.975	5.315	-1.31	-0.835	1.09385	1.568862275
180 mins	29.25	0.92	1.695	-23.935	-2.23	-2.53	5.6419	0.881422925

Raman Spectroscopy:

Paracetamol Runs:

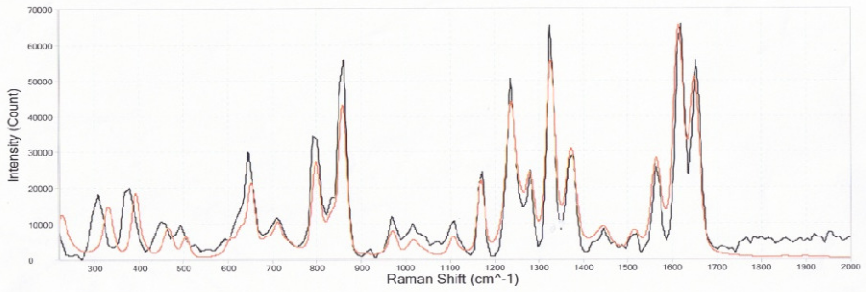


BAYSPEC

BaySpec FirstGuard Report

Graph 3.3

User ID: Admin
Device ID: T1160038
Test Time: 10/17/2012 10:00:00
Method: Method_General_Purpose
Spectral ID: BS_201210171000000
Exposure Time: 2000 ms
Average Number: 1
Laser power = 300mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 83.9
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

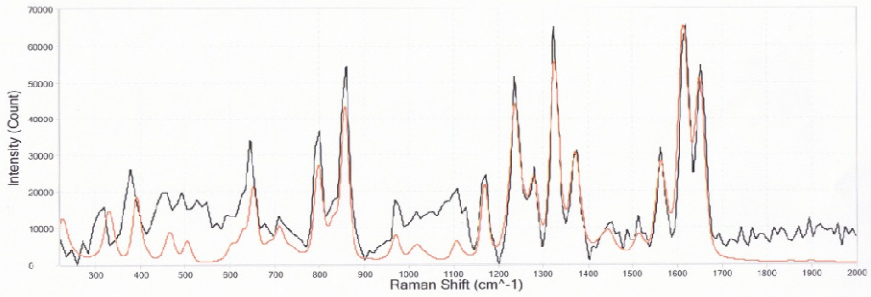


BAYSPEC

BaySpec FirstGuard Report

Graph 3.4

User ID: Admin
Device ID: T1160038
Test Time: 10/17/2012 10:01:54
Method: Method_General_Purpose
Spectral ID: BS_201210171001154
Exposure Time: 2000 ms
Average Number: 1
Laser power = 305mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 75.1
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

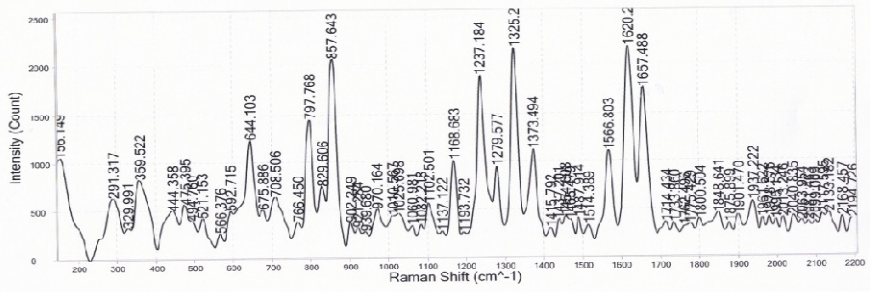


BAYSPEC

BaySpec FirstGuard Report

Graph 3.5.1

User ID: Admin
Device ID: T1160038
Test Time: 02/06/2013 11:02:29
Method: Method_General_Purpose
Spectral ID: BS_201302061102229
Exposure Time: 8000 ms
Average Number: 1
Laser power = 50mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 74.3
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

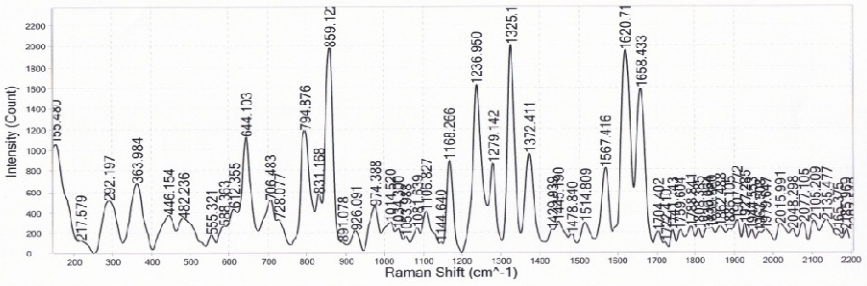


BaySpec FirstGuard Report

Graph 3.5.2

BAYSPEC

User ID: Admin
Device ID: T1160038
Test Time: 02/06/2013 11:04:10
Method: Method_General_Purpose
Spectral ID: BS_20130206110416
Exposure Time: 8000 ms
Average Number: 2
Laser power = 50mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 60
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 73.4
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

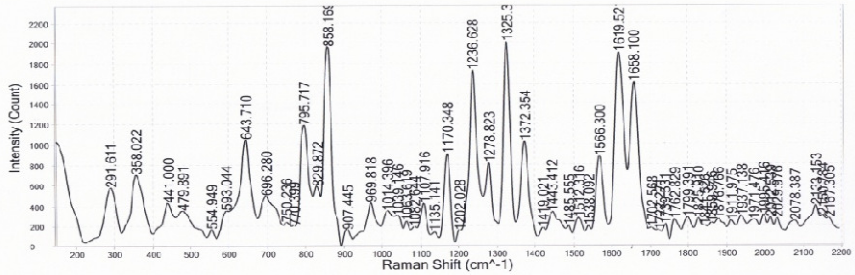


BaySpec FirstGuard Report

Graph 3.5.3

BAYSPEC

User ID: Admin
Device ID: T1160038
Test Time: 02/06/2013 11:07:28
Method: Method_General_Purpose
Spectral ID: BS_20130206110728
Exposure Time: 8000 ms
Laser Power = 50mW
Average Number: 3
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 74.6
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

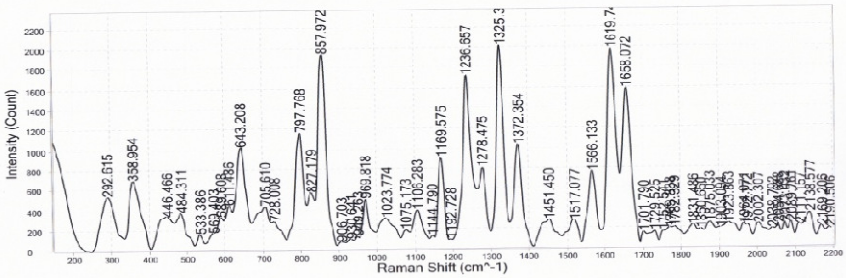


BaySpec FirstGuard Report

Graph 3.5.4

BAYSPEC

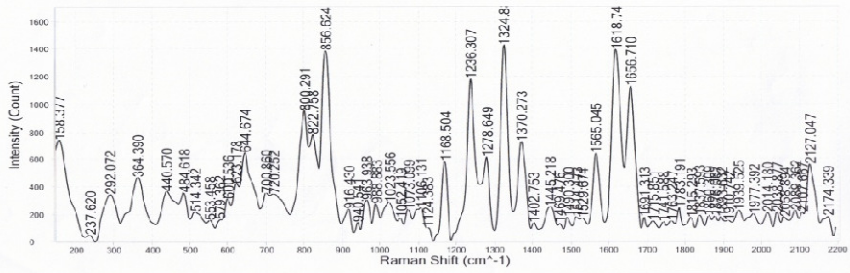
User ID: Admin
Device ID: T1160038
Test Time: 02/06/2013 11:10:45
Method: Method_General_Purpose
Spectral ID: BS_20130206111045
Exposure Time: 8000 ms
Laser Power = 50mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 75.6
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.5

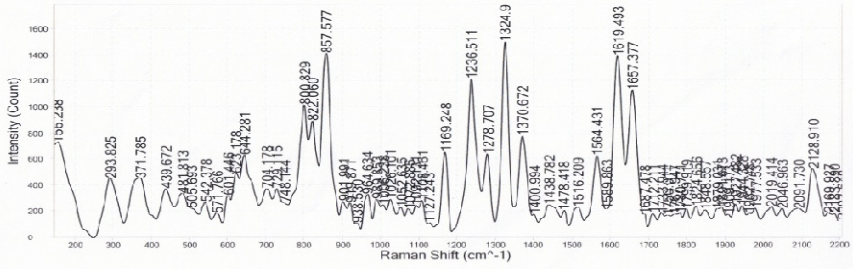
User ID: Admin
Device ID: T1160038
Test Time: 11/08/2012 11:27:44
Method: Method_General_Purpose
Spectral ID: BS_20121108112744
Exposure Time: 8000 ms
Average Number: 5
Laser Power = 50mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 40
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 72.2
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.6

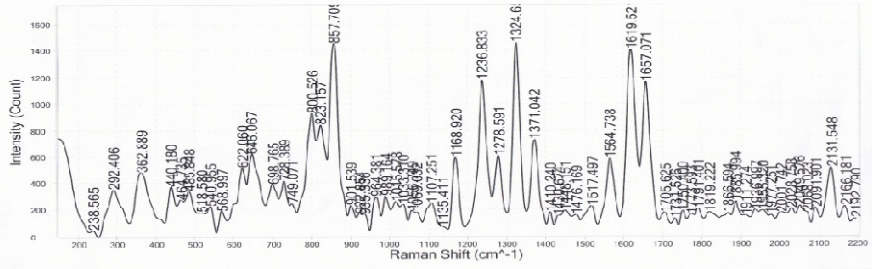
User ID: Admin
Device ID: T1160038
Test Time: 11/08/2012 11:29:56
Method: Method_General_Purpose
Spectral ID: BS_20121108112956
Exposure Time: 8000 ms
Laser Power = 50mW
Average Number: 6
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 40
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 71.0
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.7

User ID: Admin
Device ID: T1160038
Test Time: 11/08/2012 11:32:53
Method: Method_General_Purpose
Spectral ID: BS_20121108113253
Exposure Time: 8000 ms
Laser Power = 50mW
Average Number: 7
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 40
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 71.1
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

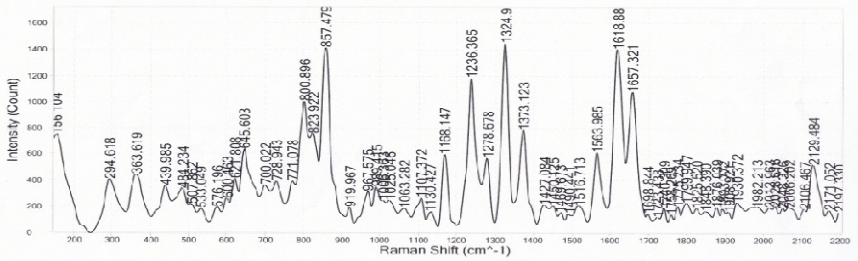


BAYSPEC

Graph 3.5.8

---------- BaySpec FirstGuard Report ----------

User ID: Admin
Device ID: T1160038
Test Time: 11/08/2012 11:18:38
Method: Method_General_Purpose
Spectral ID: BS_20121108111838
Exposure Time: 8000 ms
Average Number: 9
Laser Power = 50mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 40
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 71.1
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

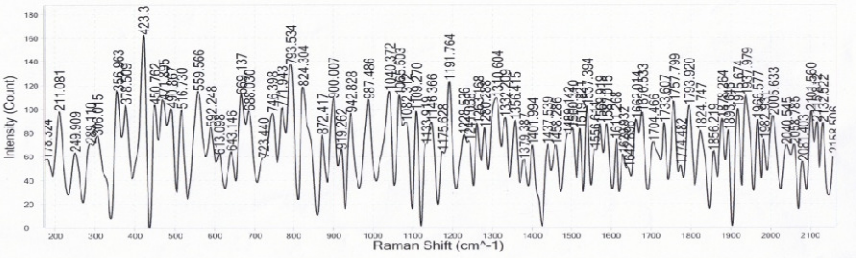


BAYSPEC

Graph 3.5.9

---------- BaySpec FirstGuard Report ----------

User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 09:53:34
Method: Method_General_Purpose
Spectral ID: BS_20121107095334
Exposure Time: 2000 ms
Laser power = 50mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: FAIL

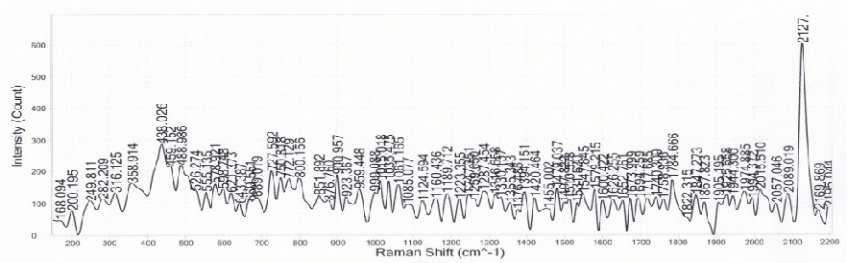


BAYSPEC

Graph 3.5.10

---------- BaySpec FirstGuard Report ----------

User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 16:16:21
Method: Method_General_Purpose
Spectral ID: BS_20121107161621
Exposure Time: 2000 ms
Laser Power = 400mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = ~~77.0~~
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

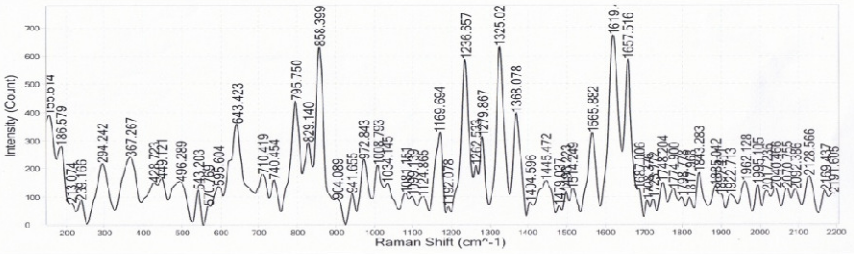


BAYSPEC

Graph 3.5.11

BaySpec FirstGuard Report

User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 13:50:53
Method: Method_General_Purpose
Spectral ID: BS_20121107135053
Exposure Time: 2000 ms
Average Number: 4
Laser power = 50mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 73.2
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

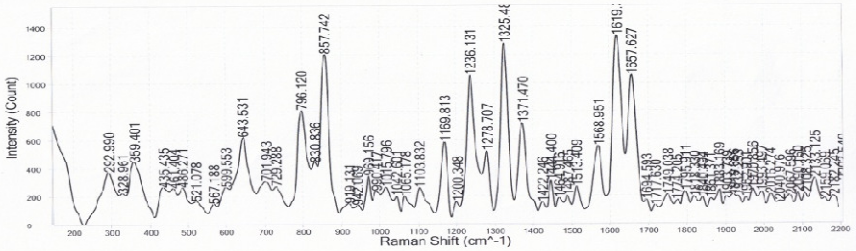


BAYSPEC

Graph 3.5.12

BaySpec FirstGuard Report

User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 14:06:30
Method: Method_General_Purpose
Spectral ID: BS_20121107140630
Exposure Time: 2000 ms
Laser power = 100mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 76.0
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

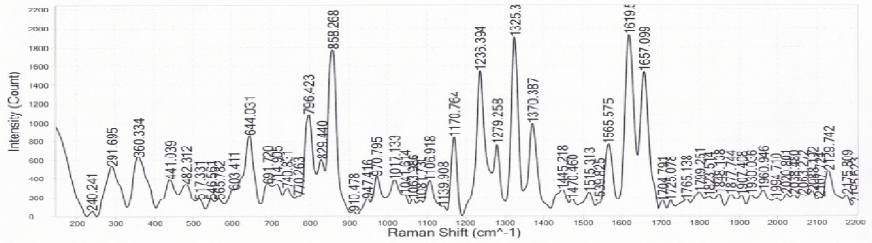


BAYSPEC

Graph 3.5.13

BaySpec FirstGuard Report

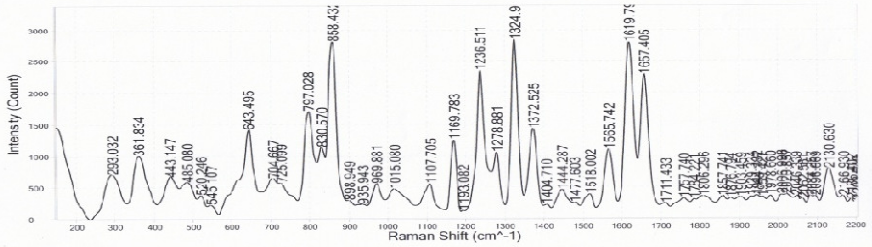
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 14:20:30
Method: Method_General_Purpose
Spectral ID: BS_20121107142030
Exposure Time: 2000 ms
Laser power = 150mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 76.9
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.15

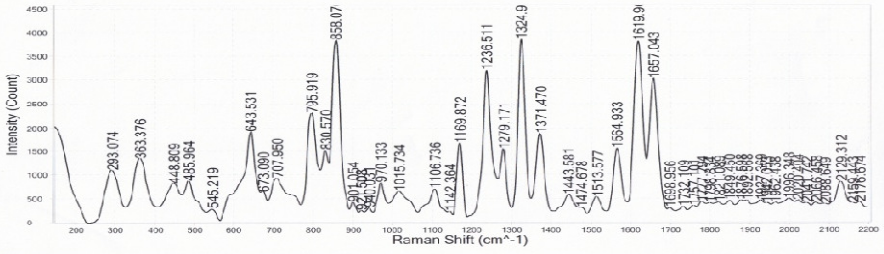
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 15:14:38
Method: Method_General_Purpose
Spectral ID: BS_20121107154438
Exposure Time: 2000 ms
Average Number: 4 Laser power = 250mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 74.4
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.16

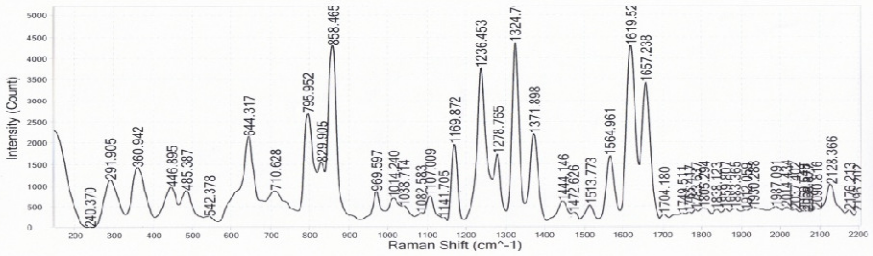
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 15:46:28
Method: Method_General_Purpose
Spectral ID: BS_20121107154628
Exposure Time: 2000 ms Laser power = 300mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 74.6
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.17

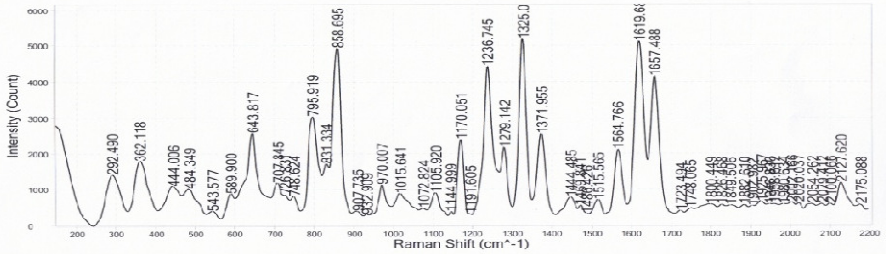
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 15:59:11
Method: Method_General_Purpose
Spectral ID: BS_20121107165011
Exposure Time: 2000 ms
Laser power = 350mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 75.9
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.18

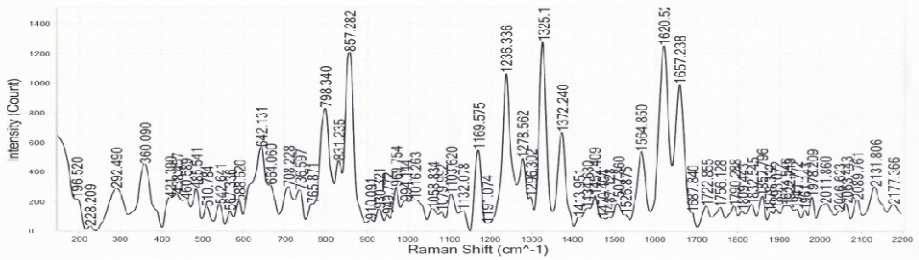
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 16:12:55
Method: Method_General_Purpose
Spectral ID: BS_20121107161255
Exposure Time: 2000 ms
Laser power = 400mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 77.0
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.19

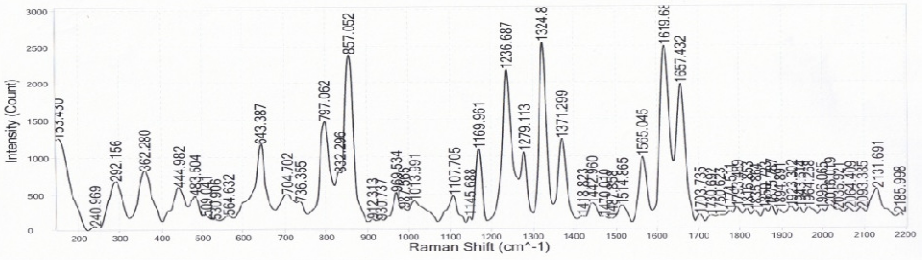
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 13:53:04
Method: Method_General_Purpose
Spectral ID: BS_20121107135304
Exposure Time: 4000 ms
Laser power = 50mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 74.4
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.20

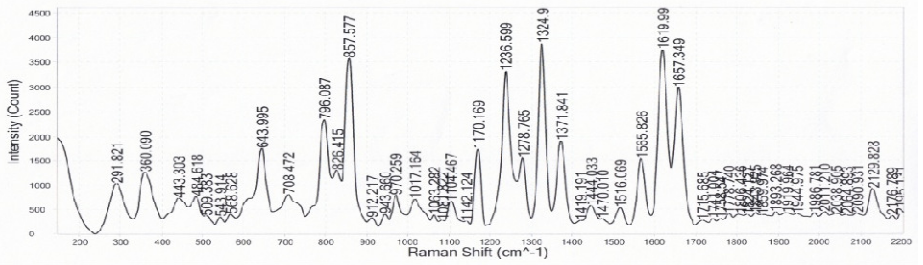
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 14:08:19
Method: Method_General_Purpose
Spectral ID: BS_20121107140819
Exposure Time: 4000 ms
Average Number: 4
Laser power = 100mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 76.9
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.21

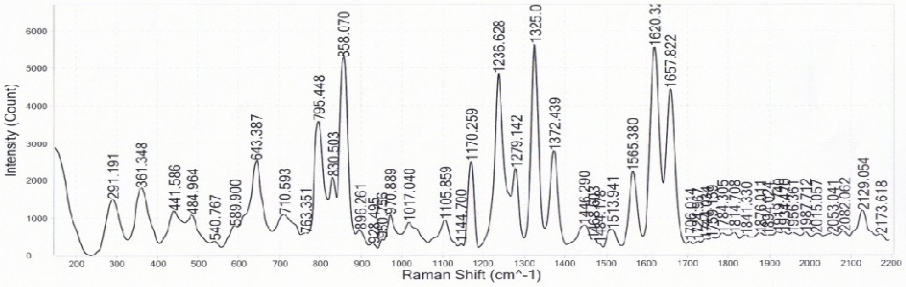
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 14:21:34
Method: Method_General_Purpose
Spectral ID: BS_20121107142134
Exposure Time: 4000 ms
Average Number: 4
Laser power = 150mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 76.7
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.22

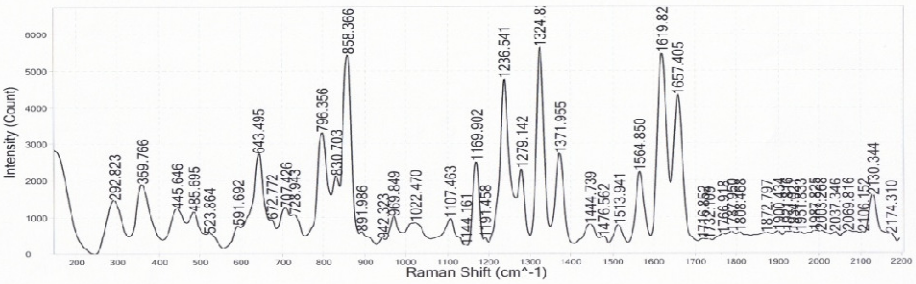
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 14:46:47
Method: Method_General_Purpose
Spectral ID: BS_20121107144647
Exposure Time: 4000 ms
Average Number: 4
Laser power = 200mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 76.2
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.23

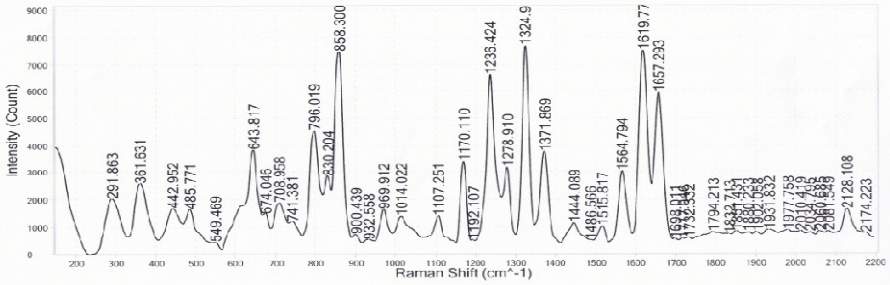
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 15:15:43
Method: Method_General_Purpose
Spectral ID: BS_20121107151543
Exposure Time: 4000 ms
Average Number: 4
Laser power = 250mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 75.9
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.24

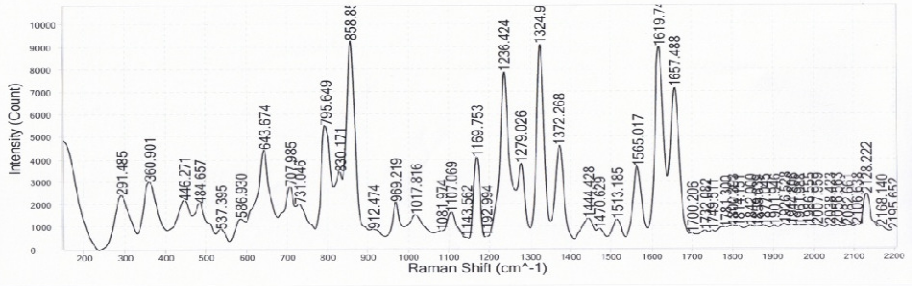
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 15:47:47
Method: Method_General_Purpose
Spectral ID: BS_20121107154747
Exposure Time: 4000 ms
Laser power = 300mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 75.3
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.25

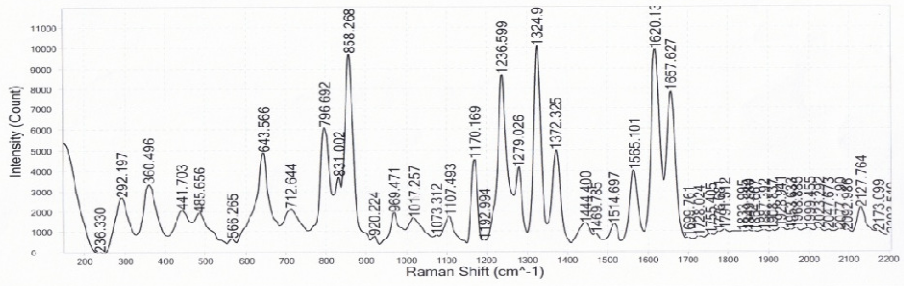
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 16:01:46
Method: Method_General_Purpose
Spectral ID: BS_20121107160148
Exposure Time: 4000 ms
Laser power = 350mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 75.2
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.26

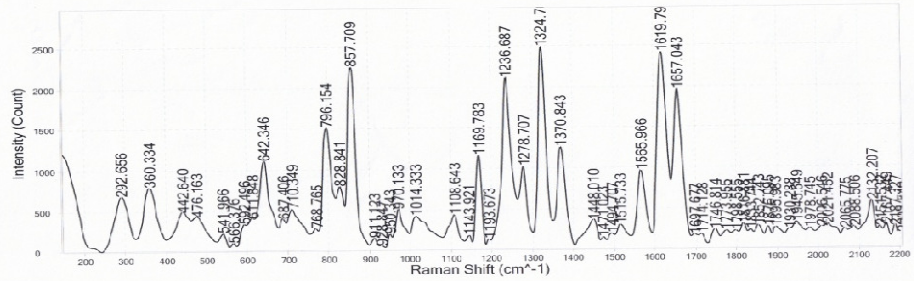
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 15:17:59
Method: Method_General_Purpose
Spectral ID: BS_20121107161750
Exposure Time: 4000 ms
Average Number: 4
Laser power = 400mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 76.1
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.27

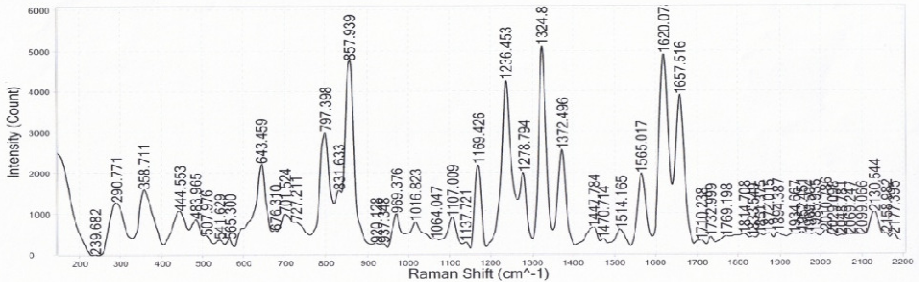
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 13:55:35
Method: Method_General_Purpose
Spectral ID: BS_20121107135535
Exposure Time: 8000 ms
Average Number: 4
Laser power = 50mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 77.4
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.28

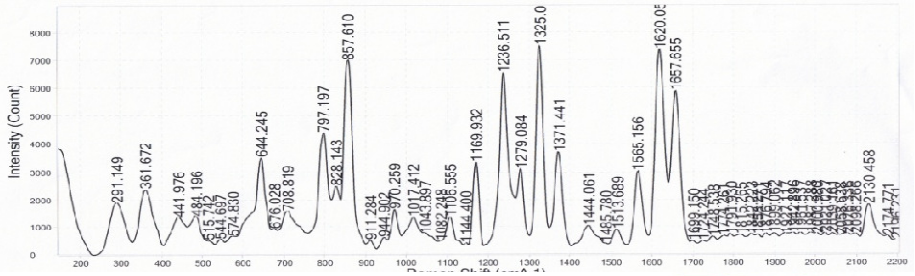
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 14:10:08
Method: Method_General_Purpose
Spectral ID: BS_20121107141008
Exposure Time: 8000 ms
Average Number: 4
Laser power = 100mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 76.9
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.29

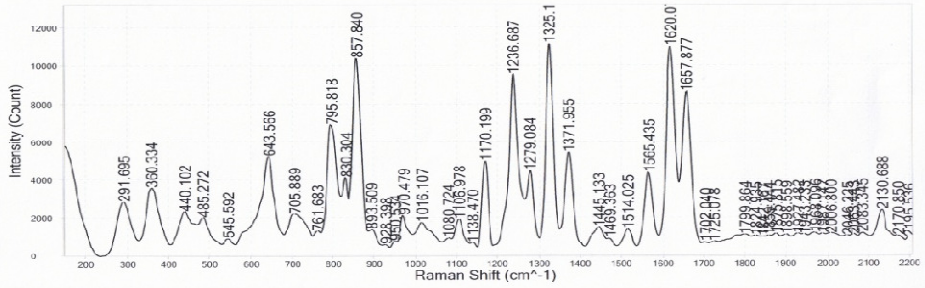
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 14:23:12
Method: Method_General_Purpose
Spectral ID: BS_20121107142312
Exposure Time: 8000 ms
Average Number: 4
Laser power = 150mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 78.9
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3 5 30

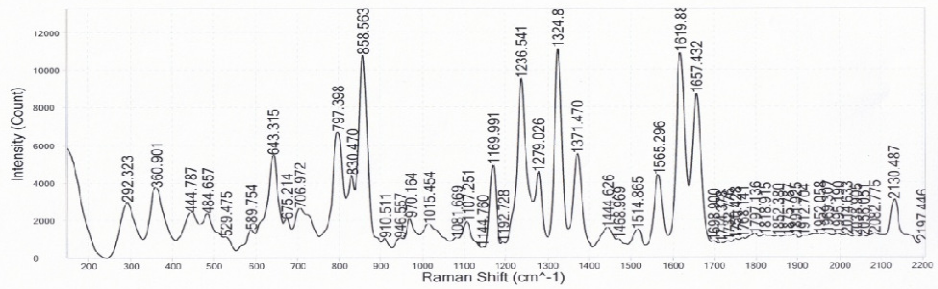
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 14:48:48
Method: Method_General_Purpose
Spectral ID: BS_20121107144848
Exposure Time: 8000 ms
Laser power = 200mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 75.6
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3 5.31

User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 15:17:23
Method: Method_General_Purpose
Spectral ID: BS_20121107151723
Exposure Time: 8000 ms
Laser power = 250mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 75.6
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

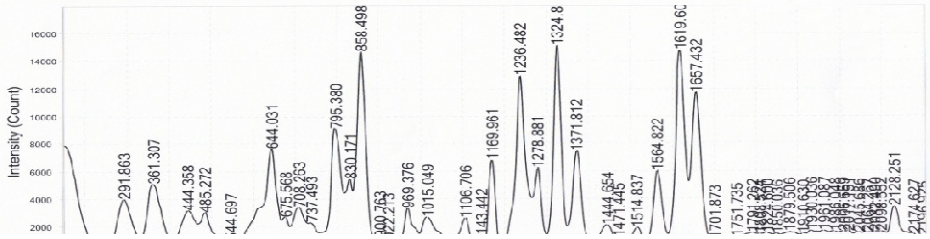


BaySpec FirstGuard Report

Graph 3.5.32

User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 15:49:30
Method: Method_General_Purpose
Spectral ID: BS_20121107154930
Exposure Time: 8000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 75.9
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

Laser power = 300mW

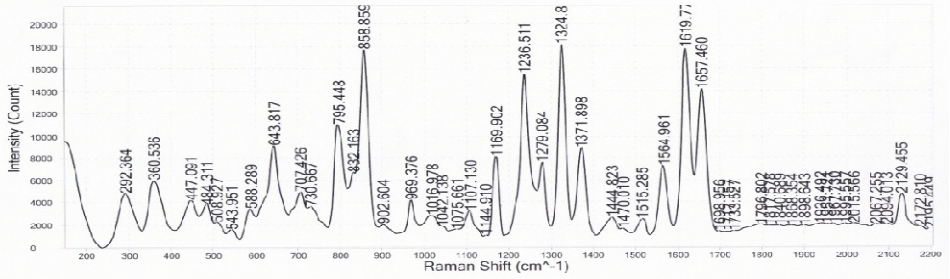


BaySpec FirstGuard Report

Graph 3.5.33

User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 16:03:32
Method: Method_General_Purpose
Spectral ID: BS_20121107160332
Exposure Time: 8000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 76.2
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

Laser power = 350mW

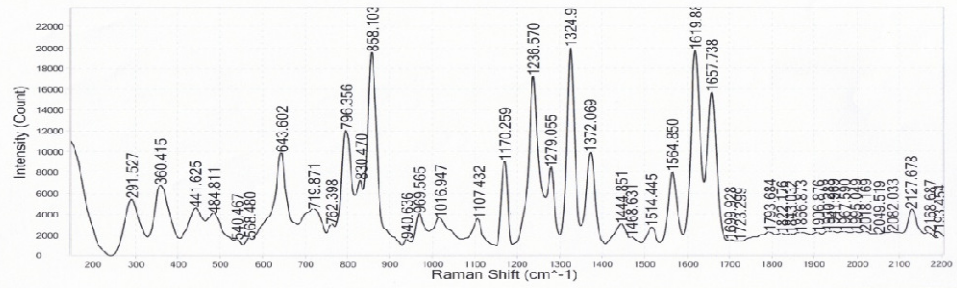


BaySpec FirstGuard Report

Graph 3.5.34

User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 16:19:38
Method: Method_General_Purpose
Spectral ID: BS_20121107161938
Exposure Time: 8000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 75.5
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

Laser power = 400mW

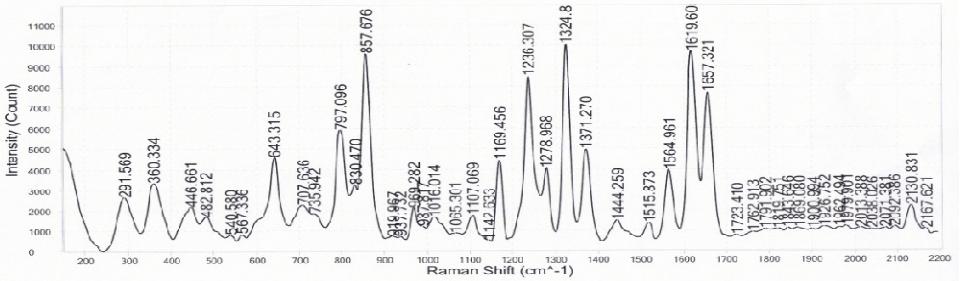


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.5.36

User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 14:12:57
Method: Method_General_Purpose
Spectral ID: BS_20121107141257
Exposure Time: 16000 ms
Average Number: 4
Laser power - 100mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 76.2
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

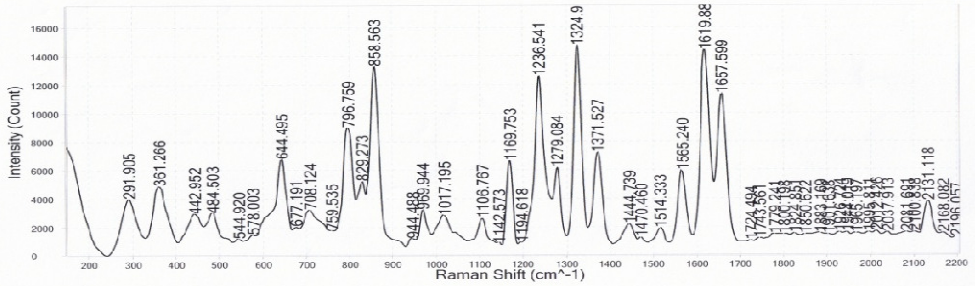


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.5.37

User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 14:27:11
Method: Method_General_Purpose
Spectral ID: BS_20121107142711
Exposure Time: 16000 ms
Average Number: 4
Laser power = 150mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 76.7
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

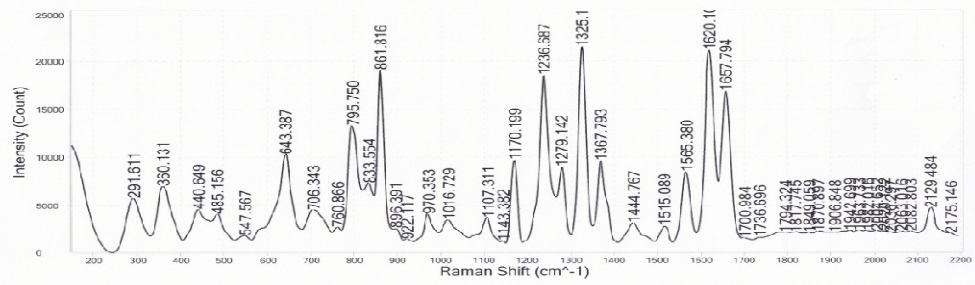


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.5.38

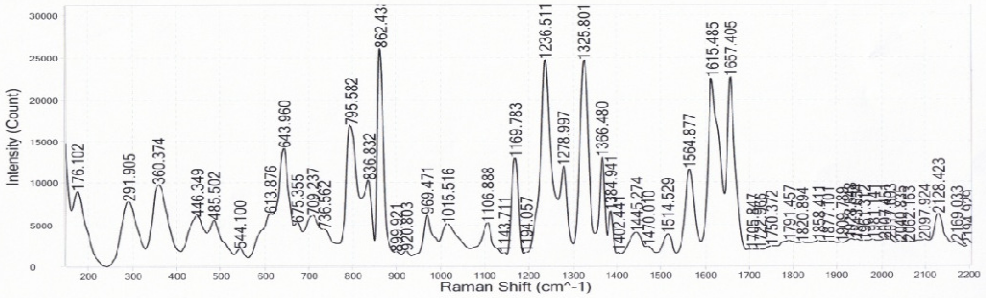
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 14:51:44
Method: Method_General_Purpose
Spectral ID: BS_20121107145144
Exposure Time: 16000 ms
Average Number: 4
Laser power = 200mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 75.4
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.40

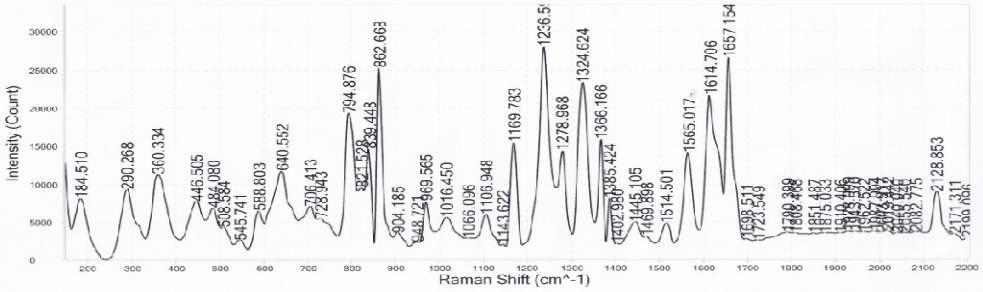
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 15:52:18
Method: Method_General_Purpose
Spectral ID: BS_20121107155218
Exposure Time: 16000 ms
Average Number: 4
Laser power = 300mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4 Acetamidophenol HQI = 71.6
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.41

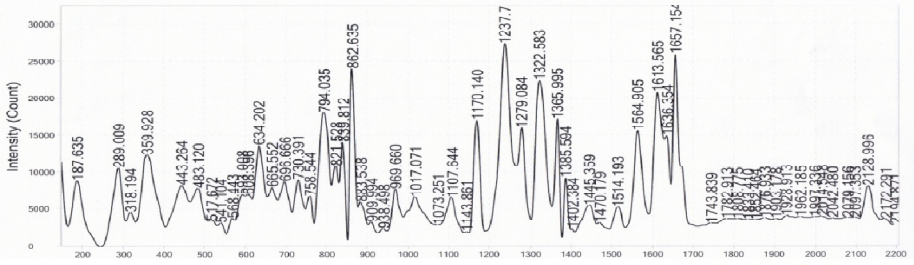
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 16:06:35
Method: Method_General_Purpose
Spectral ID: BS_20121107160635
Exposure Time: 16000 ms
Average Number: 4
Laser power = 350mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4 Acetamidophenol HQI = 68.7
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.42

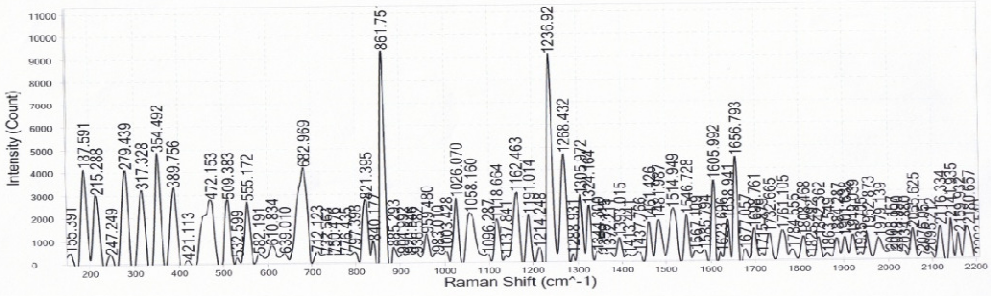
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 16:23:03
Method: Method_General_Purpose
Spectral ID: BS_20121107162303
Exposure Time: 16000 ms
Average Number: 4
Laser power = 400mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 64.7
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.5.43

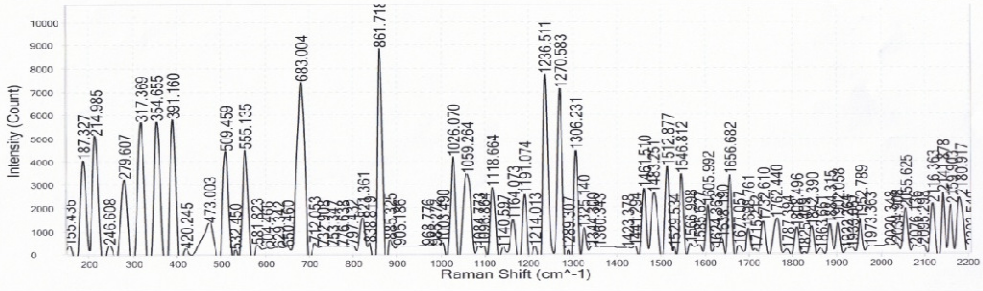
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 14:04:43
Method: Method_General_Purpose
Spectral ID: BS_20121107140443
Exposure Time: 32000 ms
Laser power = 50mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: FAIL

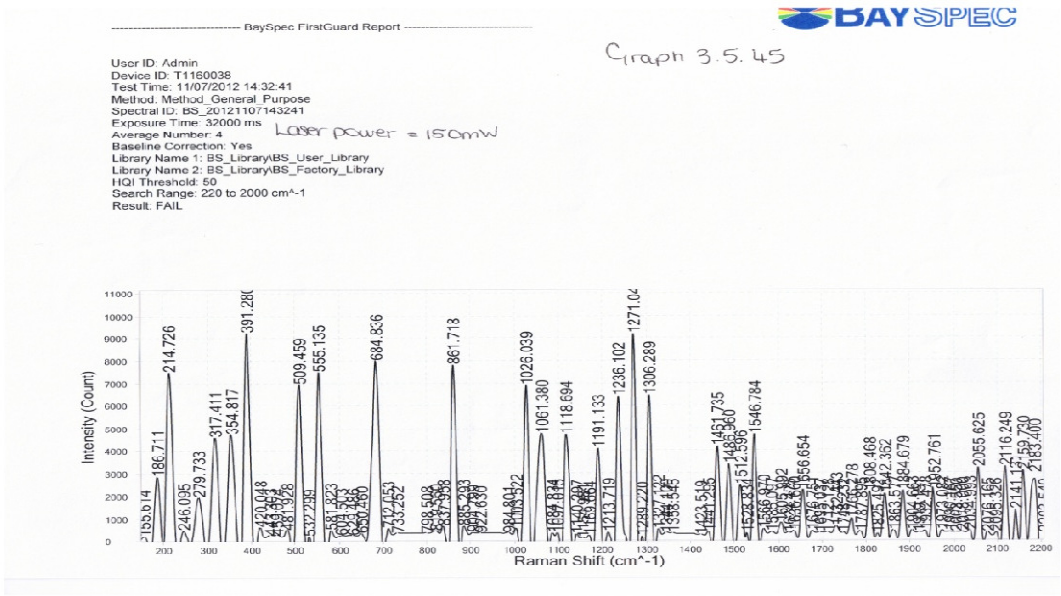


BaySpec FirstGuard Report

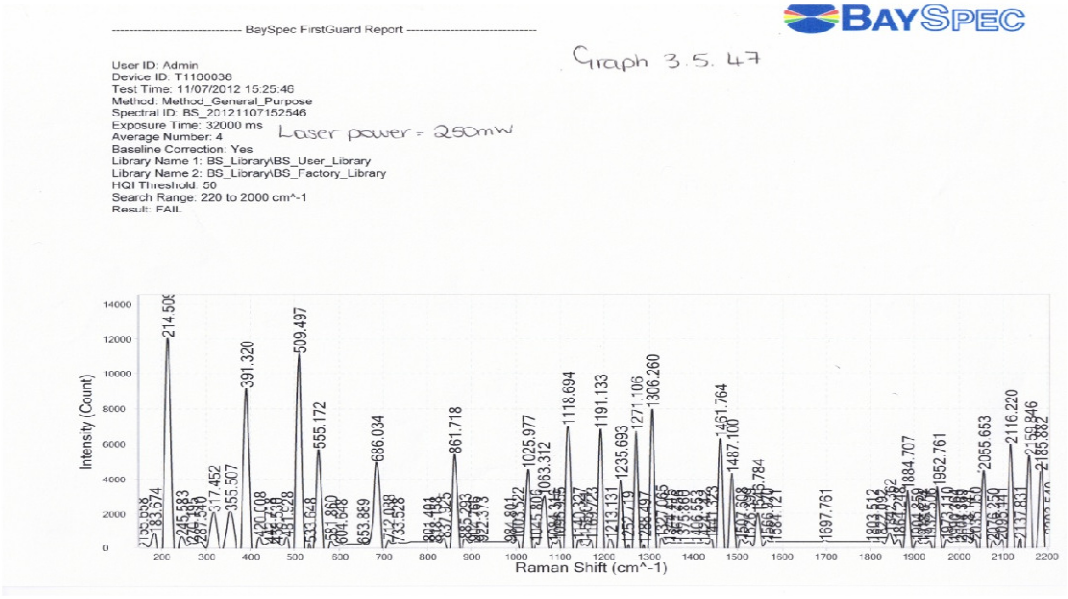
Graph 3.5.44

User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 14:18:09
Method: Method_General_Purpose
Spectral ID: BS_20121107141809
Exposure Time: 32000 ms
Laser power = 100mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: FAIL





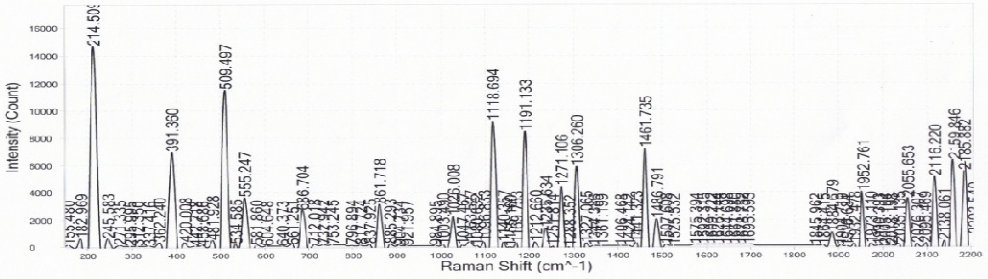
Human Haemoglobin:



BaySpec FirstGuard Report

Graph 3.5.49

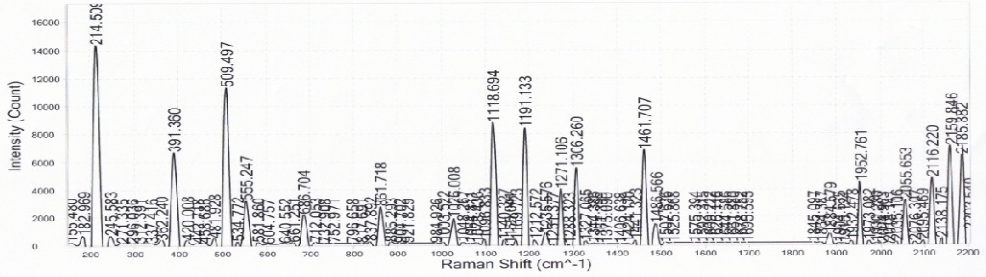
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 15:12:06
Method: Method_General_Purpose
Spectral ID: BS_20121107161206
Exposure Time: 32000 ms
Average Number: 4
Laserpower = 350mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.5.50

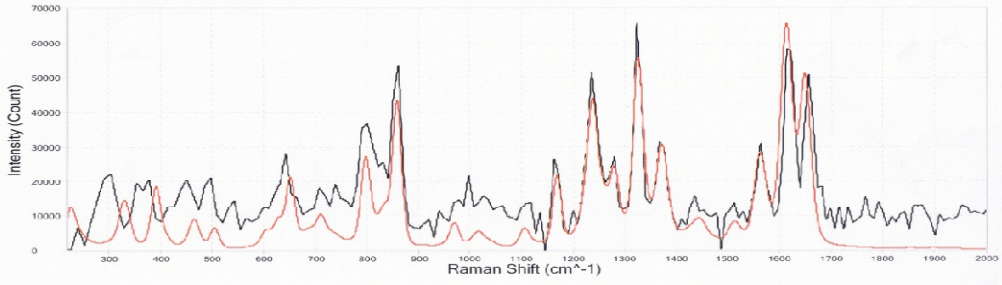
User ID: Admin
Device ID: T1160038
Test Time: 11/07/2012 16:28:11
Method: Method_General_Purpose
Spectral ID: BS_20121107162811
Exposure Time: 32000 ms
Average Number: 4
Laser power = 400mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.5.52

User ID: Admin
Device ID: T1160038
Test Time: 11/28/2012 12:00:06
Method: Method_General_Purpose
Spectral ID: BS_20121128120006
Exposure Time: 2000 ms
Average Number: 4
Laser power = 400mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 10
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 70.0
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

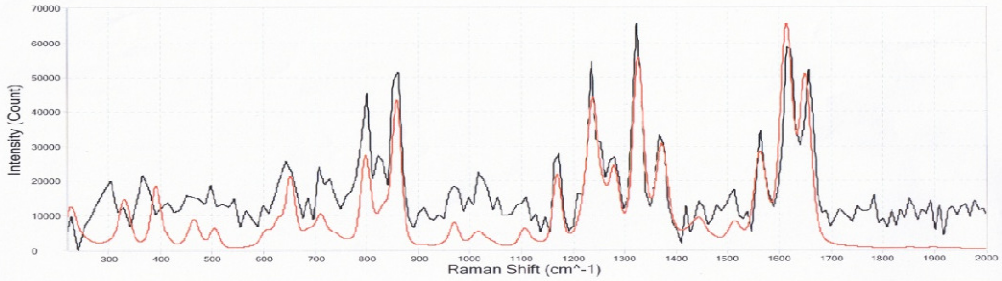


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.5.53

User ID: Admin
Device ID: T1160038
Test Time: 11/28/2012 12:04:26
Method: Method_General_Purpose
Spectral ID: BS_20121128120426
Exposure Time: 4000 ms
Average Number: 4 Laser power = 200mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 10
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 75.6
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

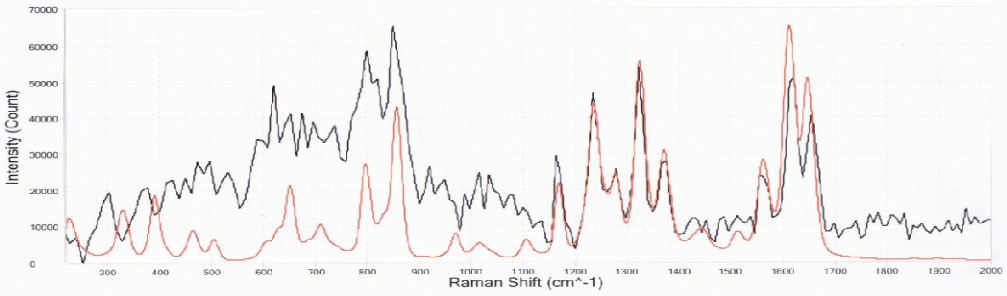


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.5.55

User ID: Admin
Device ID: T1160038
Test Time: 11/28/2012 12:15:10
Method: Method_General_Purpose
Spectral ID: BS_20121128121510
Exposure Time: 4000 ms
Average Number: 4 Laser power = 200mW, cone length = 0mm
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 10
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 29.9
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

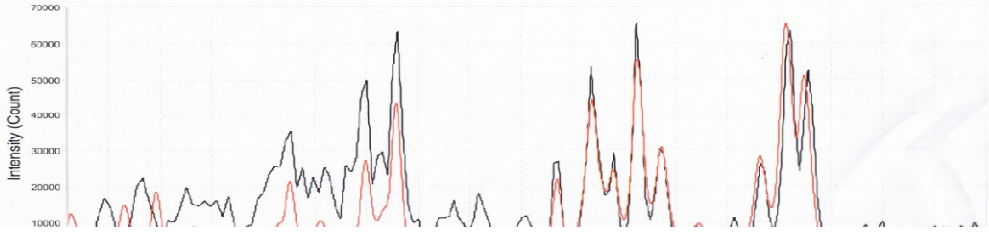


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.5.56

User ID: Admin
Device ID: T1160038
Test Time: 11/28/2012 12:13:16
Method: Method_General_Purpose
Spectral ID: BS_20121128121316
Exposure Time: 4000 ms
Average Number: 4 Laser power = 200mW, cone length = 4mm
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 10
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 64.7
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

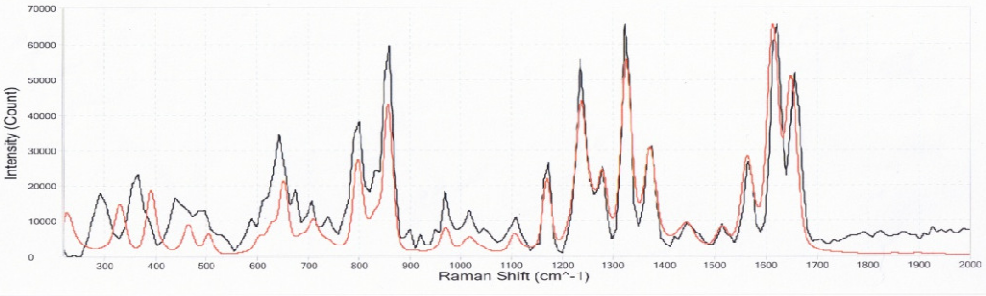


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.5.57

User ID: Admin
Device ID: T1160038
Test Time: 11/28/2012 12:18:13
Method: Method_General_Purpose
Spectral ID: BS_20121128121813
Exposure Time: 4000 ms
Average Number: 4
Laser power = 200mW, Cone length = 6mm
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 10
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 73.6
Unknown Substance: Black Spectrum
Library Substance: Red Spectrum

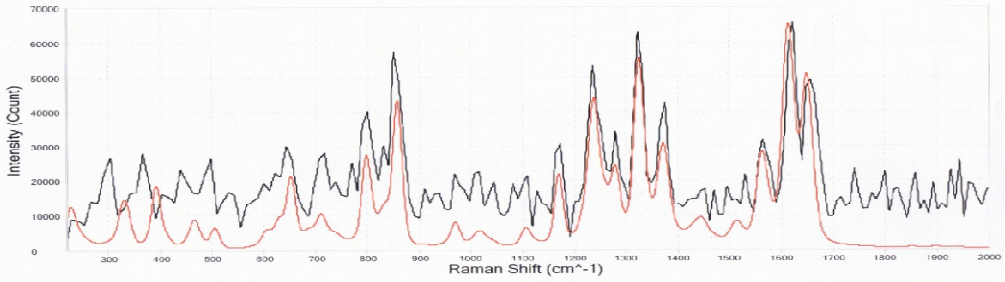


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.5.59

User ID: Admin
Device ID: T1160038
Test Time: 11/28/2012 12:08:19
Method: Method_General_Purpose
Spectral ID: BS_20121128120819
Exposure Time: 4000 ms
Average Number: 4
Laser power = 200mW, Cone length = 12mm
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 10
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 71.1
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

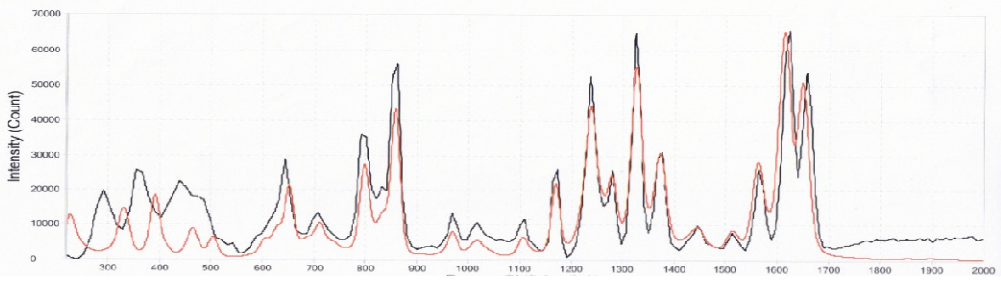


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.5.58

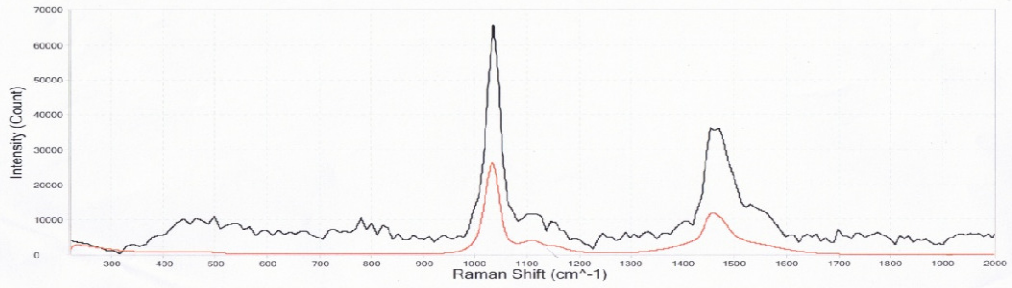
User ID: Admin
Device ID: T1160038
Test Time: 11/28/2012 12:10:21
Method: Method_General_Purpose
Spectral ID: BS_20121128121021
Exposure Time: 4000 ms
Average Number: 4
Laser power = 200mW, Cone length = 8mm
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 10
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 71.0
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.6.1

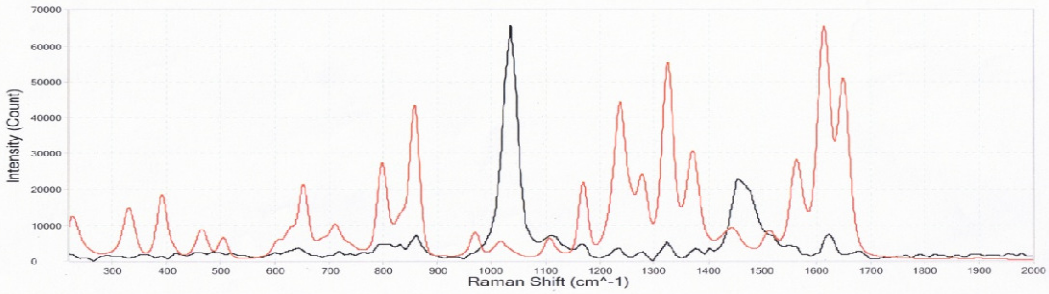
User ID: Admin
Device ID: T1160038
Test Time: 11/22/2012 14:18:24
Method: Method_General_Purpose
Spectral ID: BS_20121122141824
Exposure Time: 4000 ms
Average Number: 4
Laser power = 200mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: Methanol HQI = 89.2
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.6.4

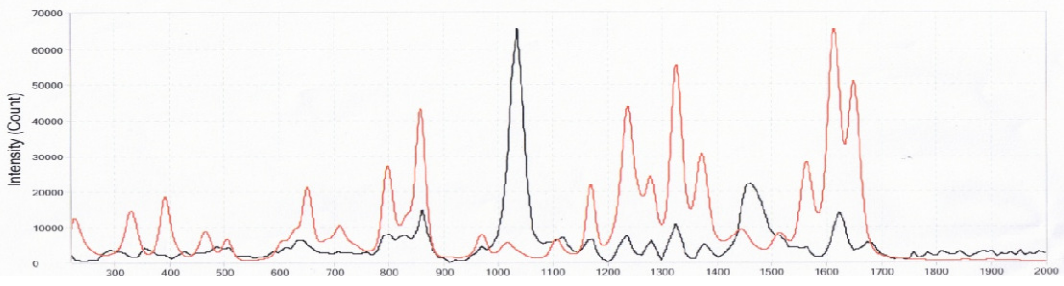
User ID: Admin
Device ID: T1160038
Test Time: 11/22/2012 15:28:16
Method: Method_General_Purpose
Spectral ID: BS_20121122152816
Exposure Time: 4000 ms
Laser power = 200mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.6.6

User ID: Admin
Device ID: T1160038
Test Time: 11/22/2012 15:33:53
Method: Method_General_Purpose
Spectral ID: BS_20121122153353
Exposure Time: 4000 ms
Laser power = 200mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: FAIL

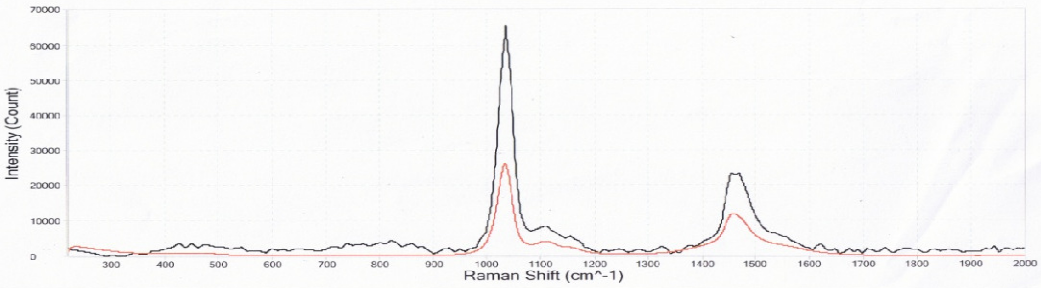


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.6.3

User ID: Admin
Device ID: T1160038
Test Time: 11/22/2012 15:22:06
Method: Method_General_Purpose
Spectral ID: BS_20121122152208
Exposure Time: 4000 ms
Average Number: 4
Laser power = 200mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: Methanol HQI = 93.9
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

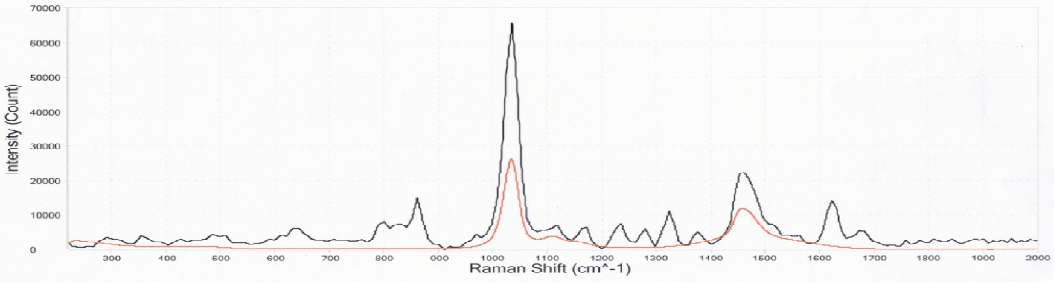


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.6.7

User ID: Admin
Device ID: T1160038
Test Time: 11/22/2012 15:33:41
Method: Method_General_Purpose
Spectral ID: BS_20121122153341
Exposure Time: 4000 ms
Laser power = 200mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: Methanol HQI = 82.8
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

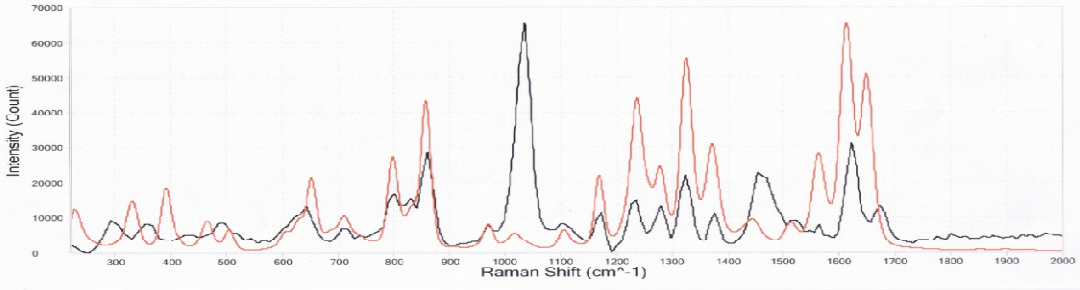


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.6.8

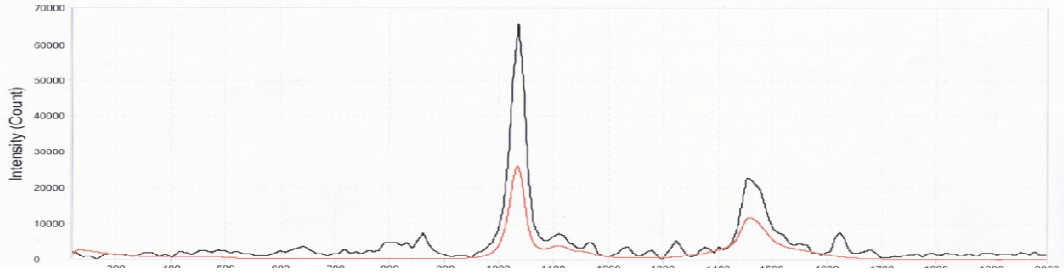
User ID: Admin
Device ID: T1160038
Test Time: 11/22/2012 15:39:13
Method: Method_General_Purpose
Spectral ID: BS_20121122153913
Exposure Time: 4000 ms
Laser power = 200mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.6.5

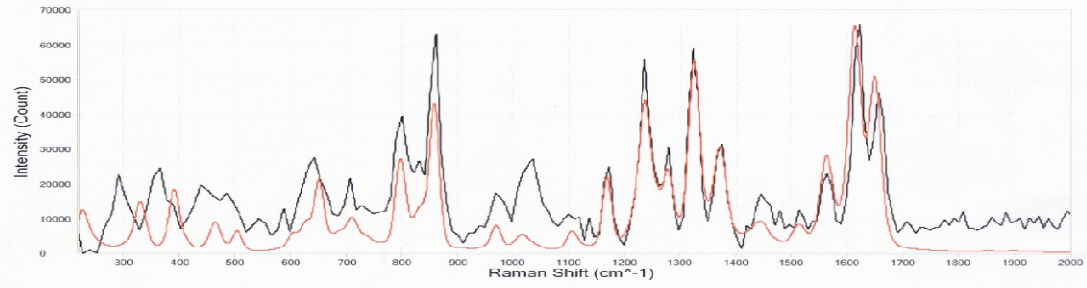
User ID: Admin
Device ID: T1180038
Test Time: 11/22/2012 15:27:58
Method: Method_General_Purpose
Spectral ID: BS_20121122152758
Exposure Time: 4000 ms
Average Number: 4
Laser power = 200mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: Methanol HQI = 91.4
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.6.10

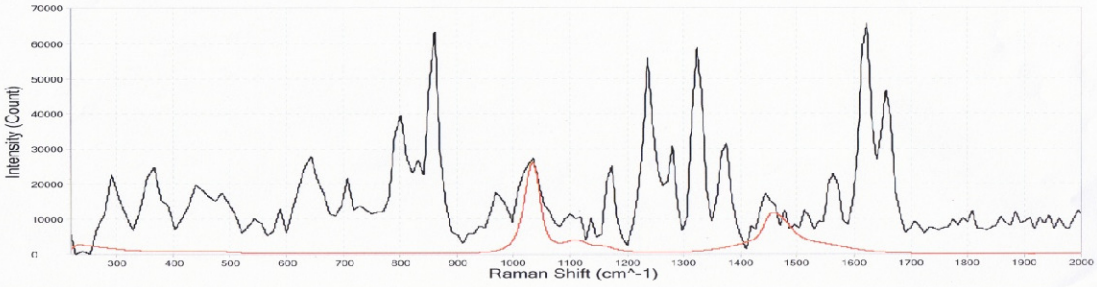
User ID: Admin
Device ID: T1180038
Test Time: 11/22/2012 15:45:49
Method: Method_General_Purpose
Spectral ID: BS_20121122154549
Exposure Time: 4000 ms
Average Number: 4
Laser power = 200mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: 4-Acetamidophenol HQI = 68.7
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

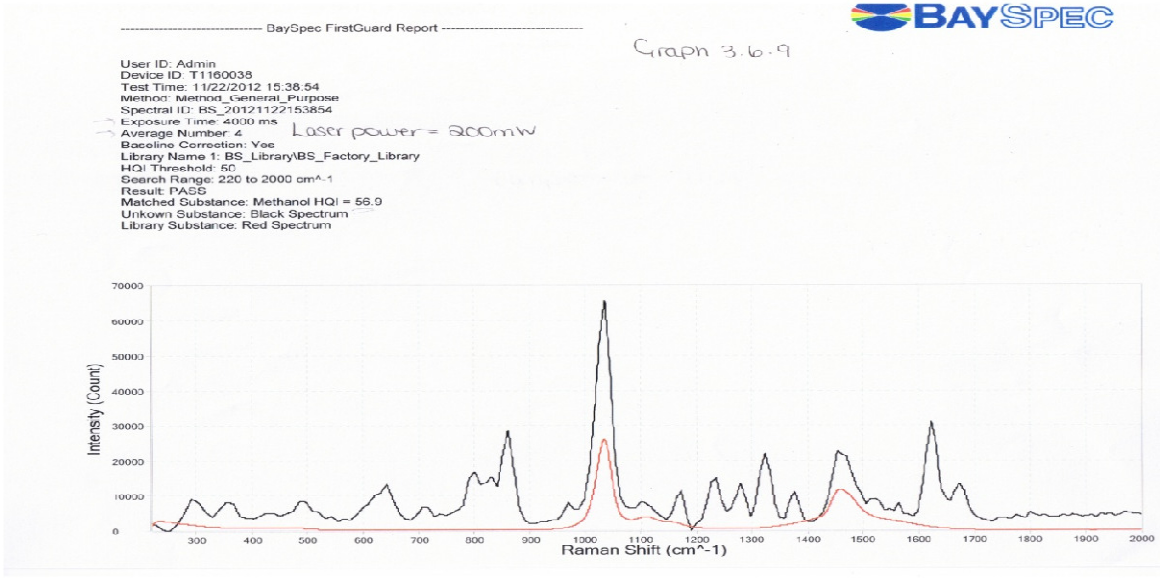


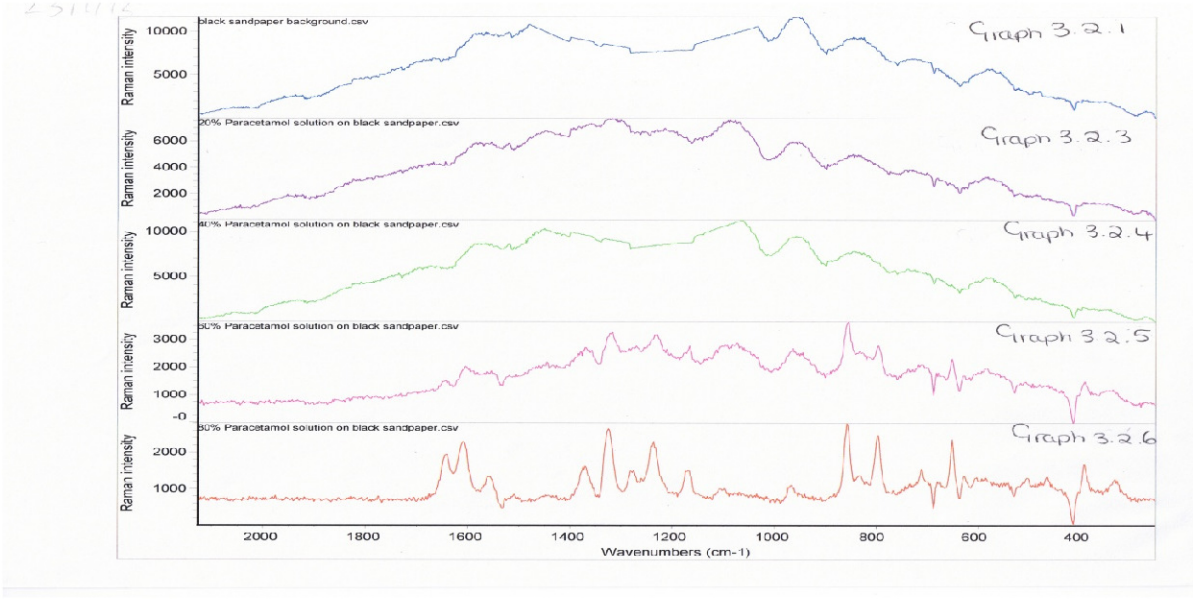
BaySpec FirstGuard Report

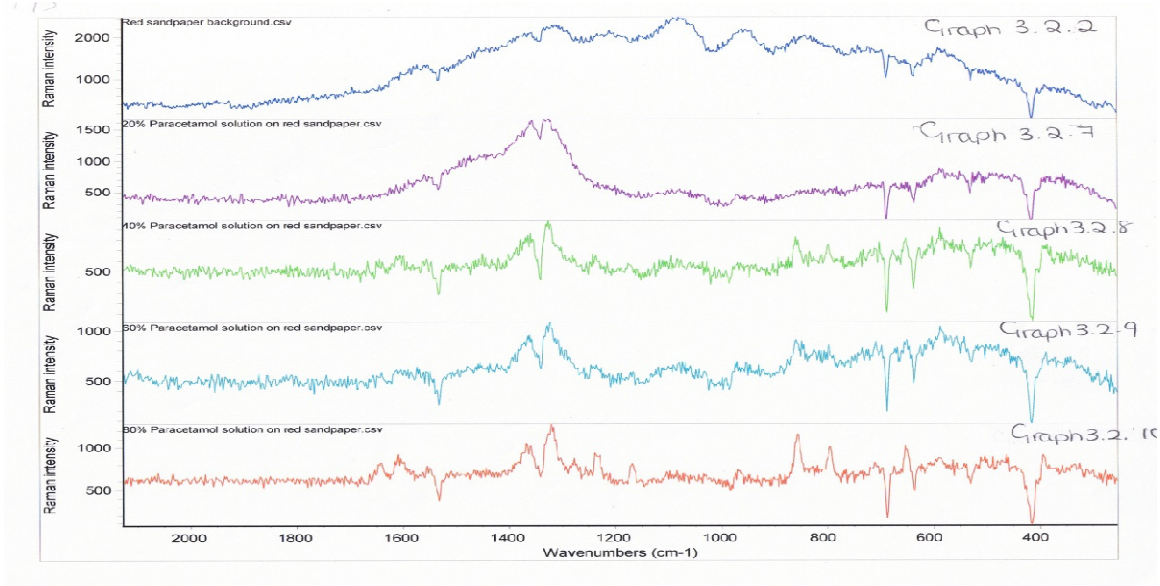
Graph 3.6.11

User ID: Admin
Device ID: T1180038
Test Time: 11/22/2012 15:45:33
Method: Method_General_Purpose
Spectral ID: BS_20121122154533
Exposure Time: 4000 ms
Laser power = 200mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: FAIL




BaySpec FirstGuard Report
Graph 3.6.9
User ID: Admin
Device ID: T1160038
Test Time: 11/22/2012 15:38:54
Method: Method_General_Purpose
Spectral ID: BS_20121122153854
Exposure Time: 4000 ms
Laser power = 300mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: Methanol HQI = 56.9
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum
Intensity (Count)
Raman Shift (cm^-1)

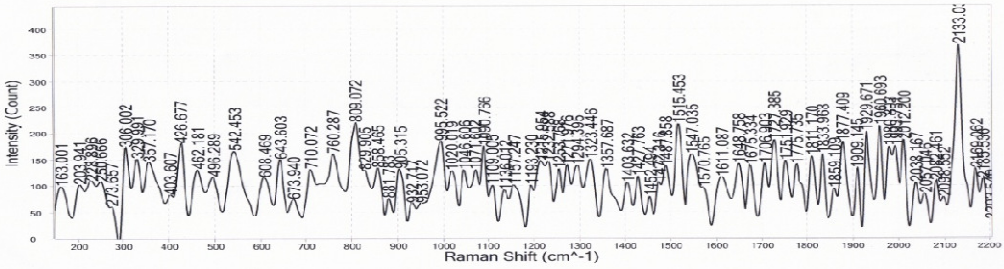

black sandpaper background.csv
Graph 3.2.1
20% Paracetamol solution on black sandpaper.csv
Graph 3.2.3
40% Paracetamol solution on black sandpaper.csv
Graph 3.2.4
60% Paracetamol solution on black sandpaper.csv
Graph 3.2.5
80% Paracetamol solution on black sandpaper.csv
Graph 3.2.6
Raman intensity
Wavenumbers (cm-1)


Red sandpaper background.csv
Graph 3.2.2
20% Paracetamol solution on red sandpaper.csv
Graph 3.2.7
40% Paracetamol solution on red sandpaper.csv
Graph 3.2.8
60% Paracetamol solution on red sandpaper.csv
Graph 3.2.9
80% Paracetamol solution on red sandpaper.csv
Graph 3.2.10
Raman intensity
Wavenumbers (cm-1)

BaySpec FirstGuard Report

Graph 3.3.1

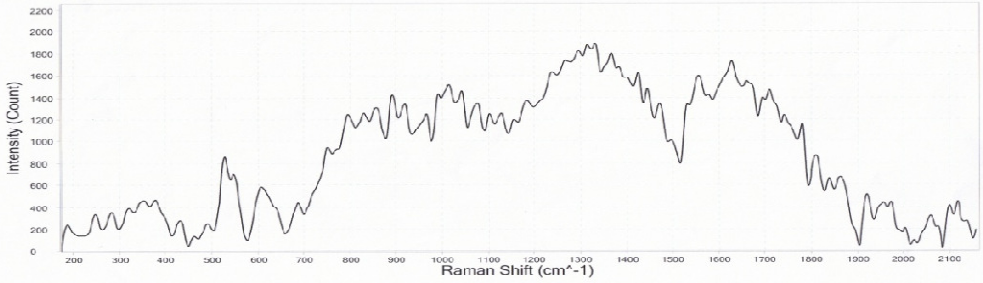
User ID: Admin
Device ID: T1160038
Test Time: 02/13/2013 10:06:18
Method: Method_General_Purpose
Spectral ID: BS_20130213100016
Exposure Time: 2000 ms
Average Number: 1
Laser power = 30mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.4

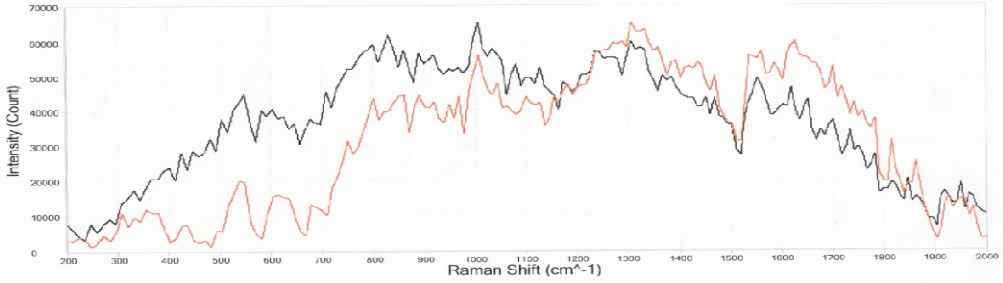
User ID: Admin
Device ID: T1160038
Test Time: 10/17/2012 10:46:51
Method: Method_General_Purpose
Spectral ID: BS_20121017104651
Exposure Time: 4000 ms
Average Number: 1
Laser power = 300mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.5

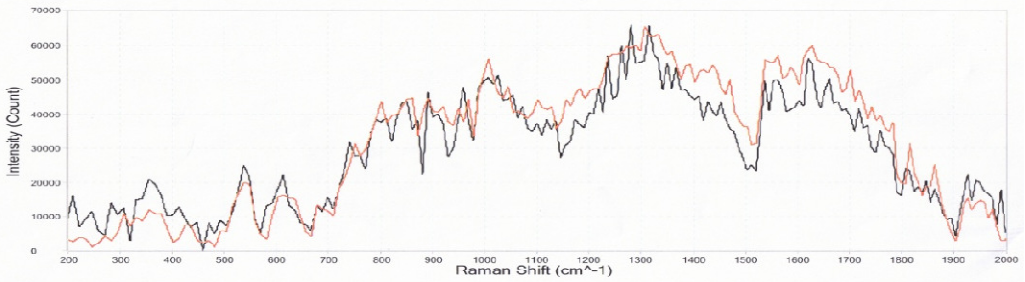
User ID: Admin
Device ID: T1160038
Test Time: 10/17/2012 11:00:26
Method: Method_General_Purpose
Spectral ID: BS_20121017110026
Exposure Time: 4000 ms
Average Number: 1
Laser power = 300mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Fresh equine blood HQI = 54.2
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.3.7

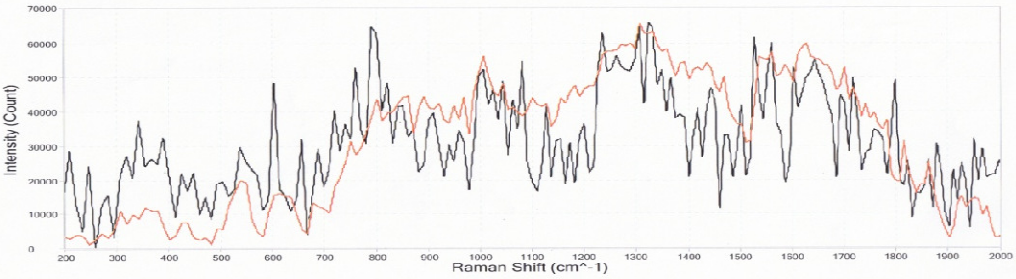
User ID: Admin
Device ID: T1160038
Test Time: 10/17/2012 11:07:46
Method: Method_General_Purpose
Spectral ID: BS_20121017110746
Exposure Time: 4000 ms Laser power = 260mW
Average Number: 1
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Fresh equine blood HQI = 93.6
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.3.8

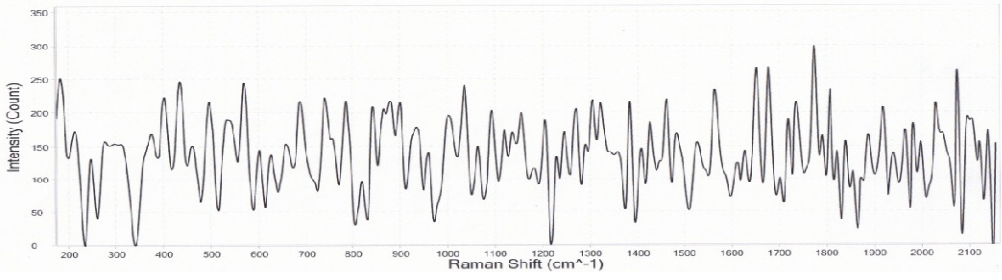
User ID: Admin
Device ID: T1160038
Test Time: 10/17/2012 11:19:33
Method: Method_General_Purpose
Spectral ID: BS_20121017111933
Exposure Time: 4000 ms Laser power = 150mW
Average Number: 1
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Fresh equine blood HQI = 57.7
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.3.9

User ID: Admin
Device ID: T1160038
Test Time: 10/17/2012 11:16:32
Method: Method_General_Purpose
Spectral ID: BS_20121017111632
Exposure Time: 4000 ms Laser power = 100mW
Average Number: 1
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Fresh equine blood HQI = 84.2
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

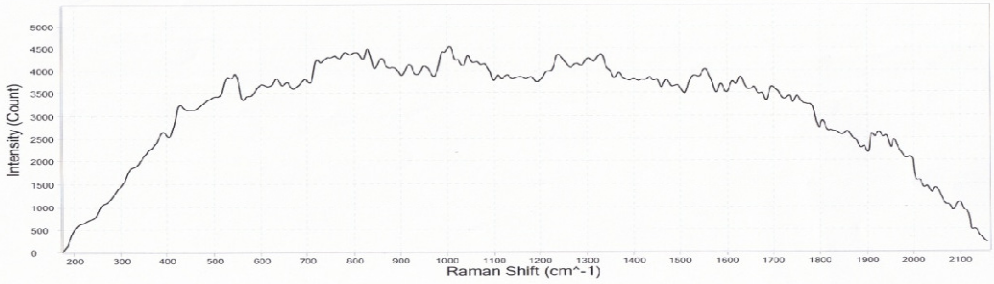


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.6

User ID: Admin
Device ID: T1160038
Test Time: 10/17/2012 11:05:18
Method: Method_General_Purpose
Spectral ID: BS_20121017110518
Exposure Time: 4000 ms Laser power = 300mW
Average Number: 1
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 220 to 2000 cm^-1
Result: PASS
Matched Substance: Antimony (III) Sulfide HQI = ~~51.9~~
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

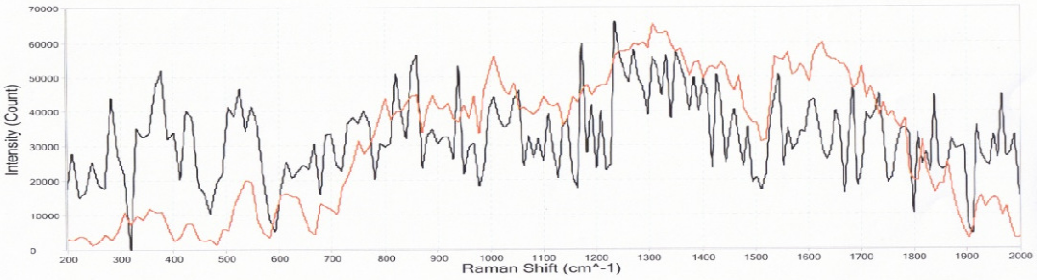


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.10

User ID: Admin
Device ID: T1160038
Test Time: 10/17/2012 11:38:09
Method: Method_General_Purpose
Spectral ID: BS_20121017113809
Exposure Time: 4000 ms Laser power = 200mW
Average Number: 1
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

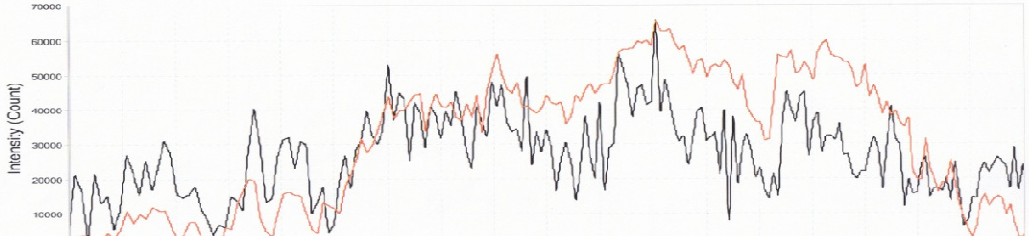


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.11

User ID: Admin
Device ID: T1160038
Test Time: 10/17/2012 11:35:10
Method: Method_General_Purpose
Spectral ID: BS_20121017113510
Exposure Time: 4000 ms Laser power = 200mW
Average Number: 1
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Fresh equine blood HQI = 52.5 (Air bubbles in sample)
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

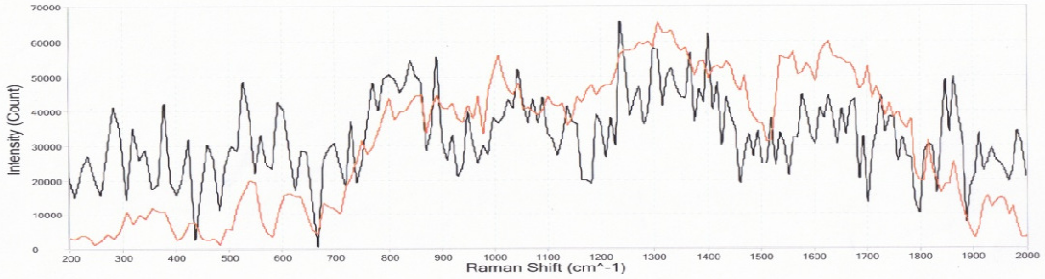


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.12

User ID: Admin
Device ID: T1160038
Test Time: 10/17/2012 11:42:14
Method: Method_General_Purpose
Spectral ID: BS_20121017114214
Exposure Time: 4000 ms
Average Number: 1
Laser power - 200mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
Library Name 2: BS_Library\BS_Factory_Library
HQI Threshold: 60
Search Range: 200 to 2000 cm^-1
Result: FAIL

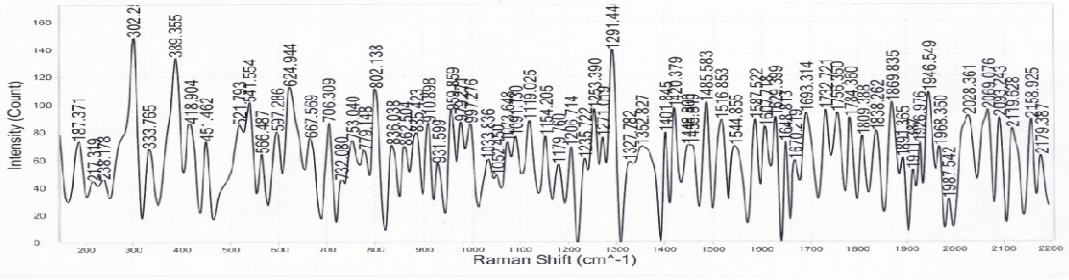


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.13

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 10:13:18
Method: Method_General_Purpose
Spectral ID: BS_20121115101318
Exposure Time: ~~8000 ms~~ 2000ms
Average Number: 4
Laser power = 30mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 0 to 0 cm^-1
Result: FAIL

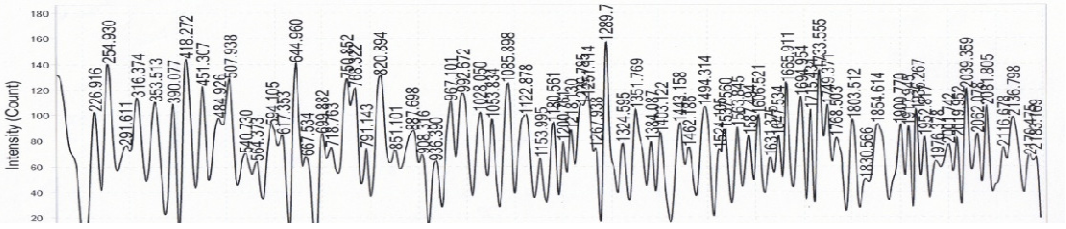


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.14

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 10:14:25
Method: Method_General_Purpose
Spectral ID: BS_20121115101425
Exposure Time: 8000 ms 2000ms
Average Number: 4
Laser power = 50mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

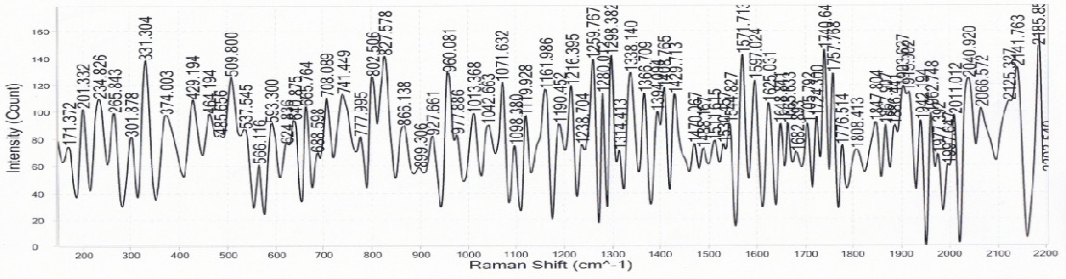


BaySpec FirstGuard Report

Graph 3.3.15

User ID: Admin
Device ID: T1160030
Test Time: 11/15/2012 10:16:07
Method: Method_General_Purpose
Spectral ID: BS_20121115101607
Exposure Time: 6000 ms 2000ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 100mW

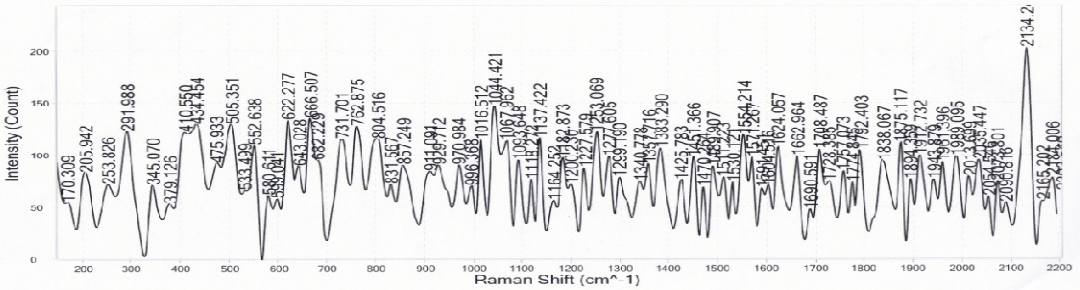


BaySpec FirstGuard Report

Graph 3.3.16

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 10:18:24
Method: Method_General_Purpose
Spectral ID: BS_20121115101824
Exposure Time: 6000 ms 2000ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

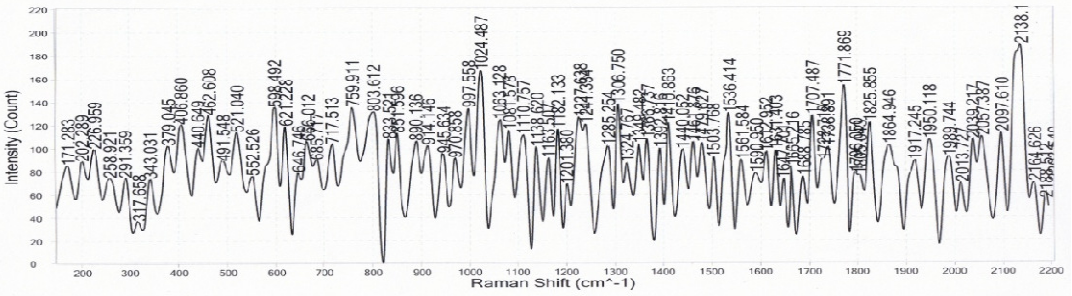
Laser power = 150mW



BaySpec FirstGuard Report

Graph 3.3.17

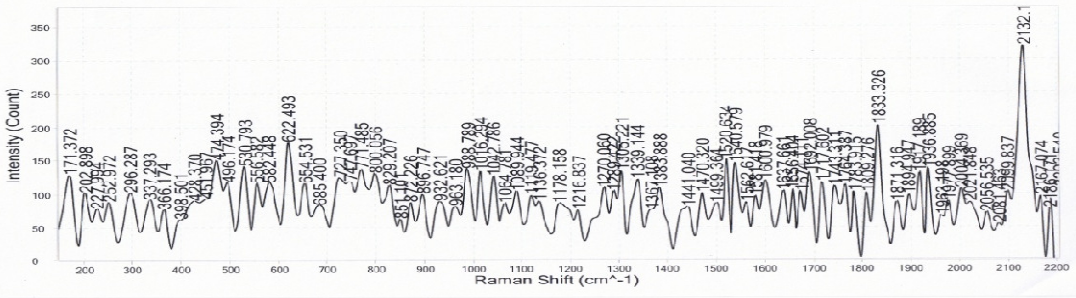
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 10:19:42
Method: Method_General_Purpose
Spectral ID: BS_20121115101942
Exposure Time: 8000 ms 2000ms Laser power = 200mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.18

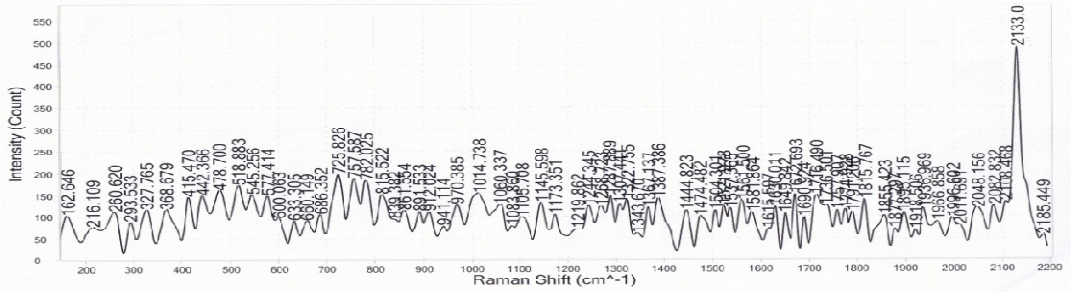
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 10:21:13
Method: Method_General_Purpose
Spectral ID: BS_20121115102113
Exposure Time: 8000 ms 2000ms Laser power = 250mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



Graph 3.3.20

BaySpec FirstGuard Report

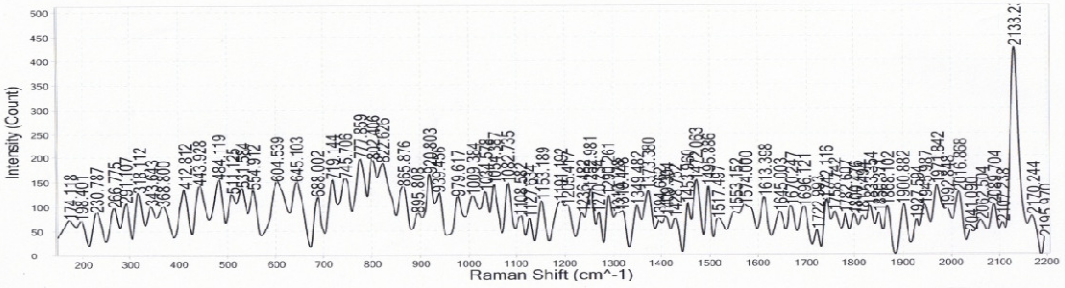
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 10:25:16
Method: Method_General_Purpose
Spectral ID: BS_20121115102516
Exposure Time: 8000 ms 2000ms Laser power = 350mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



Graph 3.3.21

BaySpec FirstGuard Report

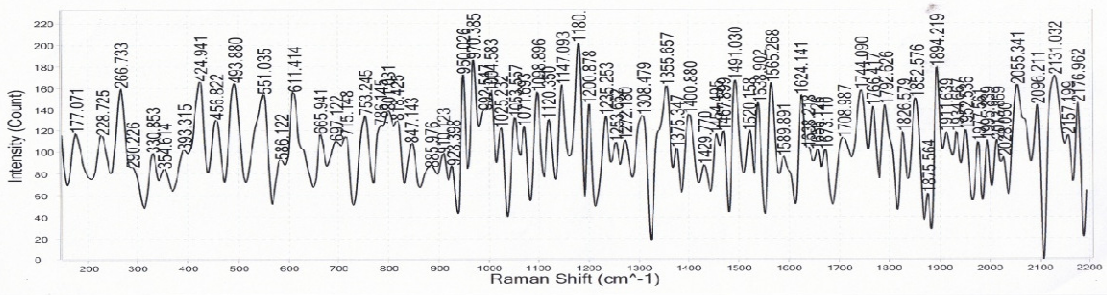
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 10:26:32
Method: Method_General_Purpose
Spectral ID: BS_20121115102632
Exposure Time: 8000 ms 2000ms Laser power =100mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



Graph 3.3.22

BaySpec FirstGuard Report

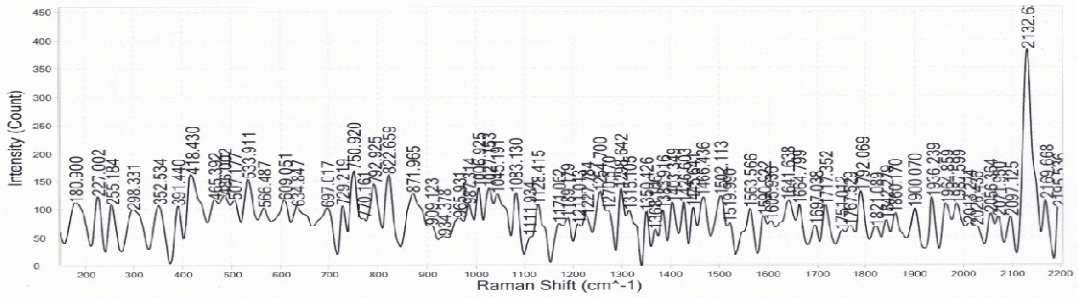
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 10:40:36
Method: Method_General_Purpose
Spectral ID: BS_20121115104036
Exposure Time: 4000 ms
Average Number: 4 Laser power = 30mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.19

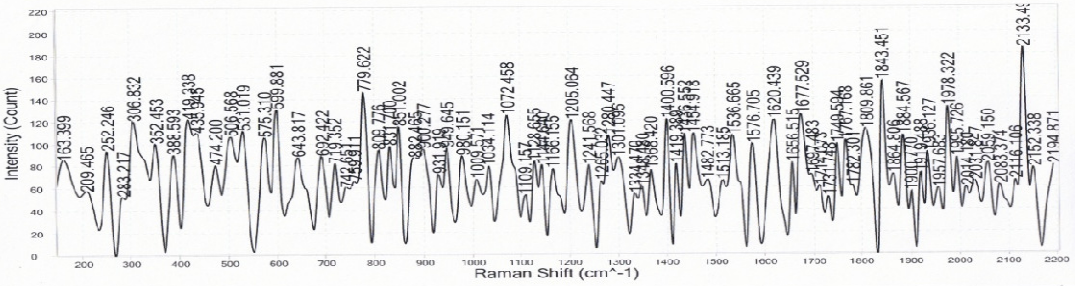
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 10:23:34
Method: Method_General_Purpose
Spectral ID: BS_20121115102334
Exposure Time: ~~8000~~ ms 2000ms Laser power = 300mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.23

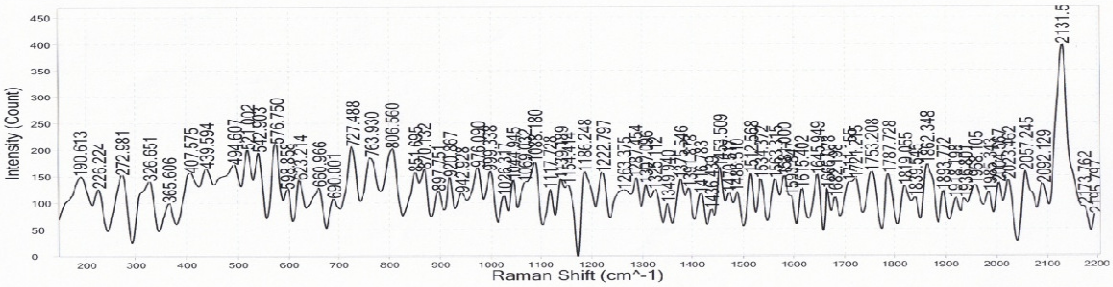
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 10:43:05
Method: Method_General_Purpose
Spectral ID: BS_20121115104305
Exposure Time: 4000 ms Laser power = 50mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.25

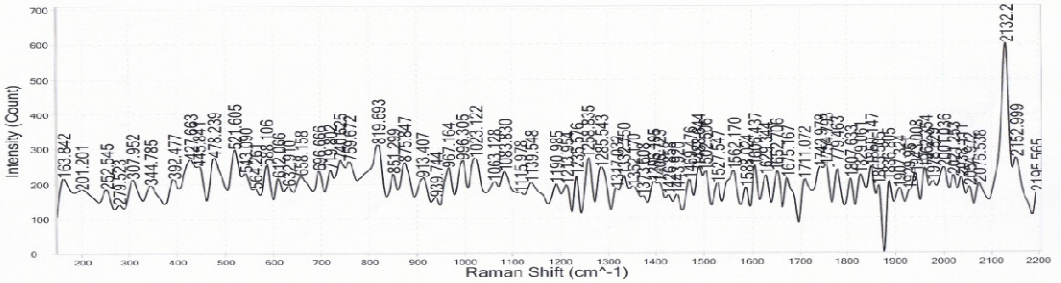
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 10:48:28
Method: Method_General_Purpose
Spectral ID: BS_20121115104828
Exposure Time: 4000 ms Laser power = 150mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.27

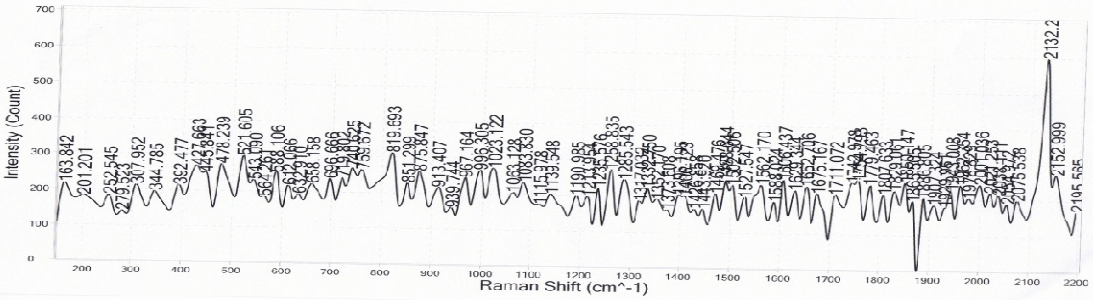
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 10:53:34
Method: Method_General_Purpose
Spectral ID: BS_20121115105334
Exposure Time: 4000 ms
Average Number: 4
Laser power = 250mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.26

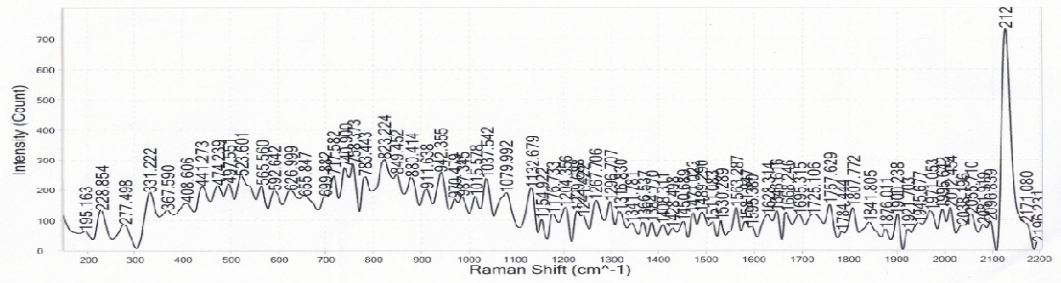
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 10:52:19
Method: Method_General_Purpose
Spectral ID: BS_20121115105219
Exposure Time: 4000 ms
Average Number: 4
Laser power = 200mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.28

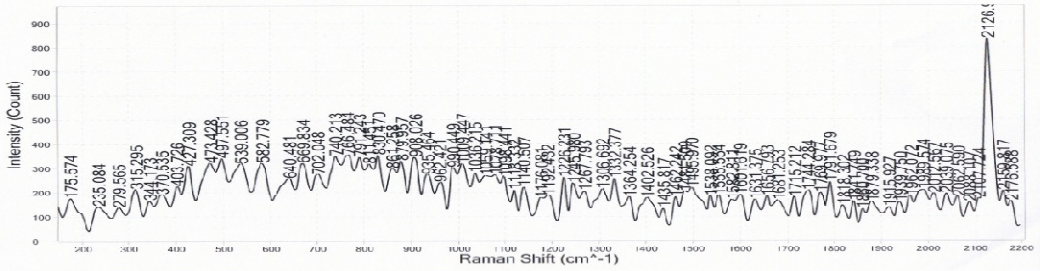
User ID: Admin
Device ID: T1160036
Test Time: 11/15/2012 10:55:18
Method: Method_General_Purpose
Spectral ID: BS_20121115105518
Exposure Time: 4000 ms
Average Number: 4
Laser power = 300mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.30

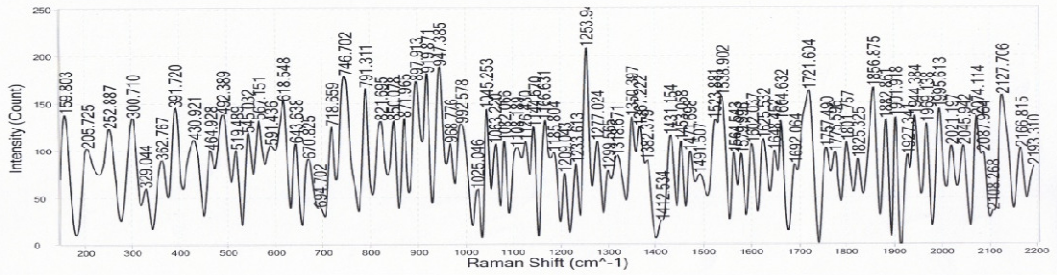
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 11:47:36
Method: Method_General_Purpose
Spectral ID: BS_20121115114736
Exposure Time: 4000 ms
Average Number: 4
Laser power 400mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial 400mW, 8000ms, 4 HQI = 92.0
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.3.31

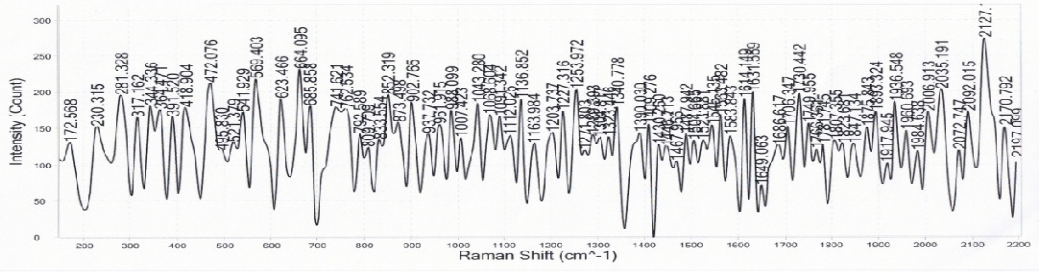
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 11:19:22
Method: Method_General_Purpose
Spectral ID: BS_20121115111922
Exposure Time: 8000 ms
Average Number: 4
Laser power = 30mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial 400mW, 8000ms, 4 HQI = 89.0
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.3.32

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 11:21:53
Method: Method_General_Purpose
Spectral ID: BS_20121115112153
Exposure Time: 8000 ms
Average Number: 4
Laser power - 50mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

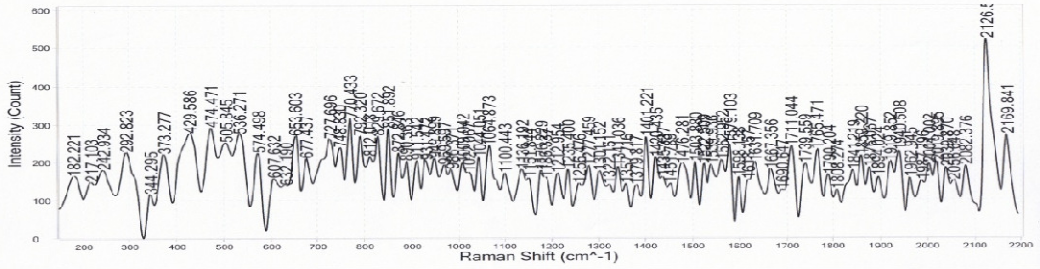


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.33

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 11:24:37
Method: Method_General_Purpose
Spectral ID: BS_20121115112437
Exposure Time: 3000 ms
Average Number: 4
Laser power = 100mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

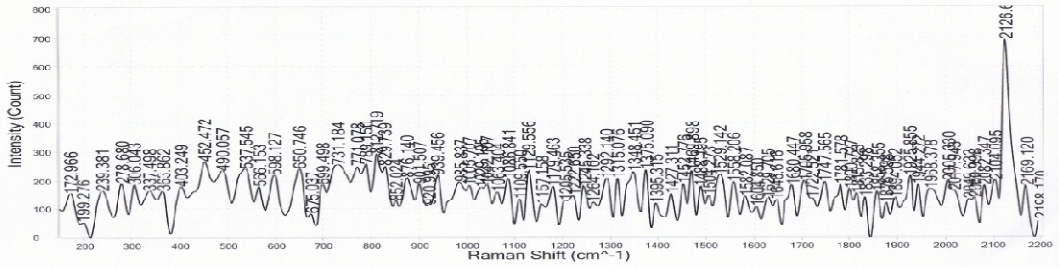


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.34

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 11:32:20
Method: Method_General_Purpose
Spectral ID: BS_20121115113220
Exposure Time: 8000 ms
Laser power = 150mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

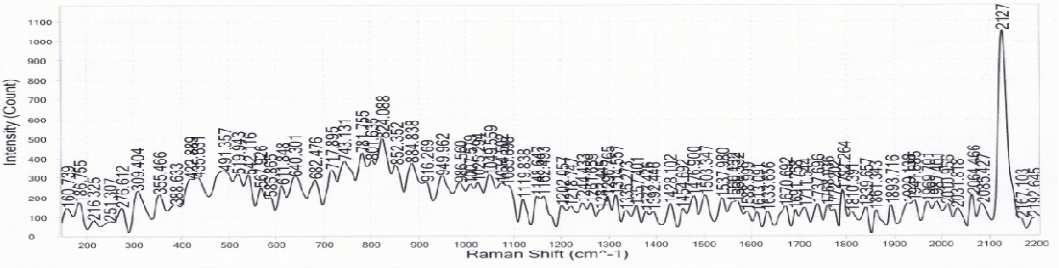


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.35

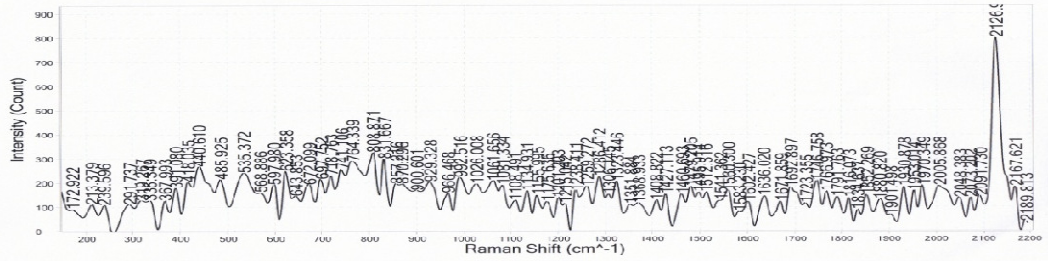
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 11:37:30
Method: Method_General_Purpose
Spectral ID: BS_20121115113730
Exposure Time: 8000 ms
Laser power = 200mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial 200mW, 8000ms, 4 HQI = 51.3
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.3.36

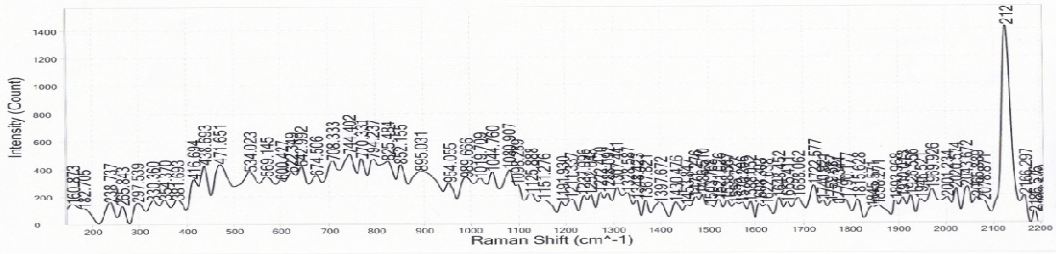
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 11:34:46
Method: Method_General_Purpose
Spectral ID: BS_20121115113448
Exposure Time: 8000 ms
Average Number: 4
Laser power = 250mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.37

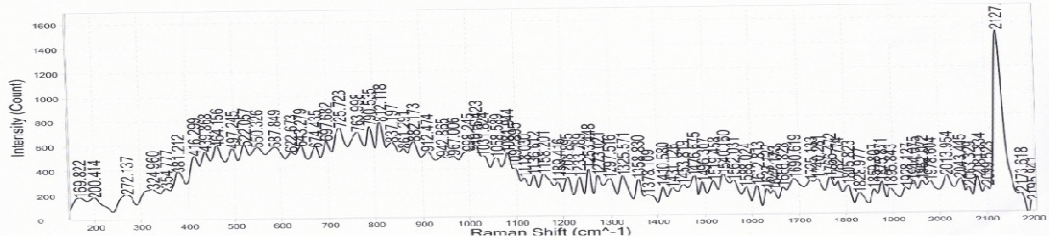
User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 11:40:59
Method: Method_General_Purpose
Spectral ID: BS_20121115114059
Exposure Time: 8000 ms
Average Number: 4
Laser power = 300mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial 250mW, 8000ms, 4 HQI = 79.7
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.3.38

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 11:45:21
Method: Method_General_Purpose
Spectral ID: BS_20121115114521
Exposure Time: 8000 ms
Average Number: 4
Laser power = 350mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial 350mW, 8000ms, 4 HQI = 87.3
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

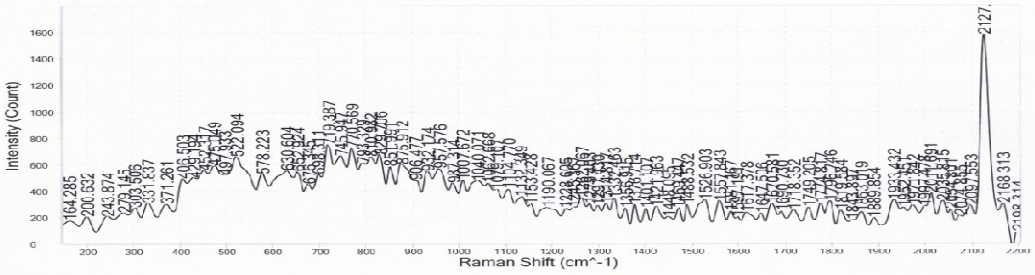


BAYSPEC

Graph 3.3.39

BaySpec FirstGuard Report

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 11:43:27
Method: Method_General_Purpose
Spectral ID: BS_20121115114327
Exposure Time: 8000 ms
Average Number: 4 Laser power=400mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial, 300mW, 8000ms, 4 HQI = 86.4
Unknown Substance: Black Spectrum
Library Substance: Red Spectrum

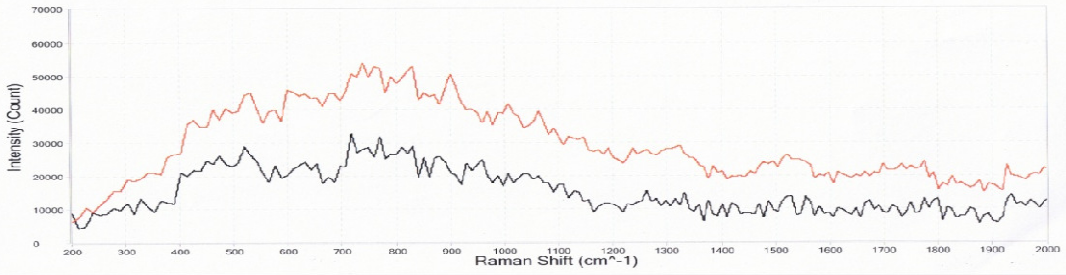


BAYSPEC

Graph 3.3.39A

BaySpec FirstGuard Report

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 11:43:53
Method: Method_General_Purpose
Spectral ID: BS_20121115114353
Exposure Time: 8000 ms
Average Number: 4 Laser power = 400mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial 350mW, 8000ms, 4 HQI = 87.3
Unknown Substance: Black Spectrum
Library Substance: Red Spectrum

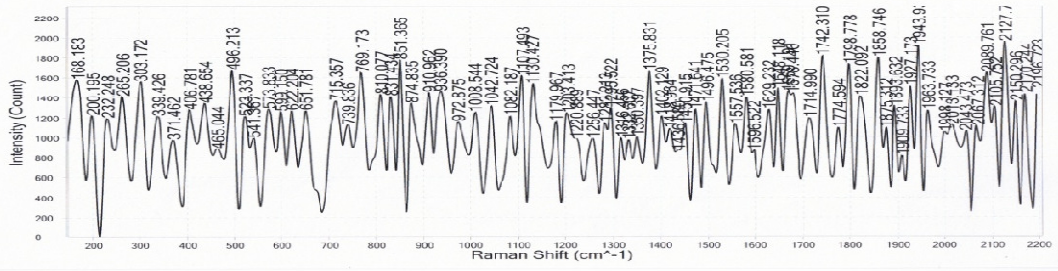


BAYSPEC

Graph 3.3.40

BaySpec FirstGuard Report

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 12:10:19
Method: Method_General_Purpose
Spectral ID: BS_20121115121019
Exposure Time: 16000 ms
Average Number: 4 Laser power = 30mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

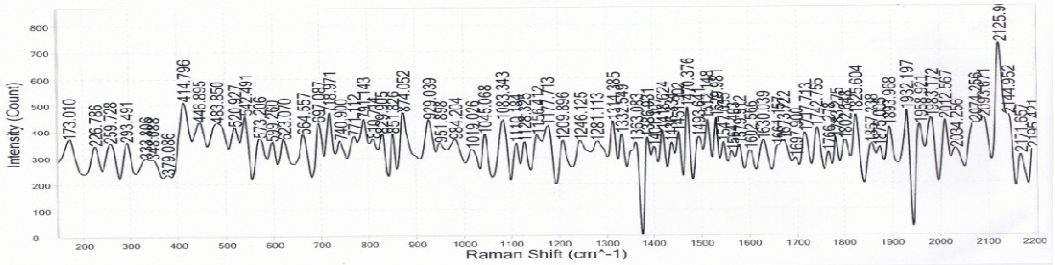


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.41

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 12:21:51
Method: Method_General_Purpose
Spectral ID: BS_20121115122151
Exposure Time: 16000 ms Laser power = 50mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

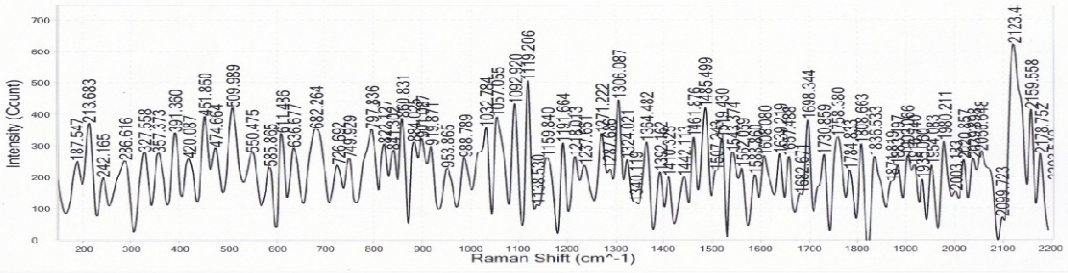


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.42

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 12:18:05
Method: Method_General_Purpose
Spectral ID: BS_20121115121805
Exposure Time: 16000 ms Laser power = 100mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

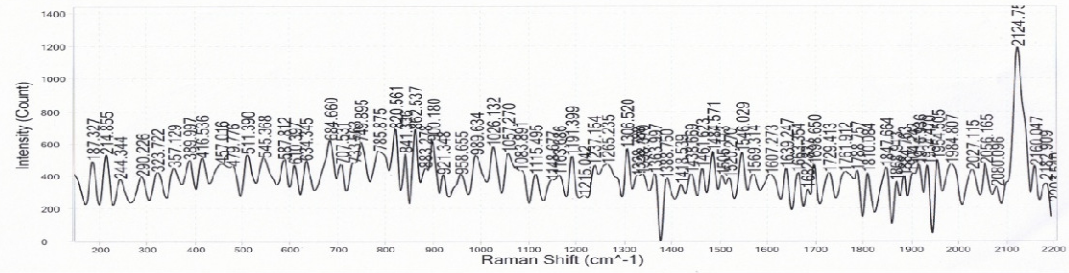


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.43

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 12:25:31
Method: Method_General_Purpose
Spectral ID: BS_20121115122531
Exposure Time: 16000 ms Laser power = 150mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

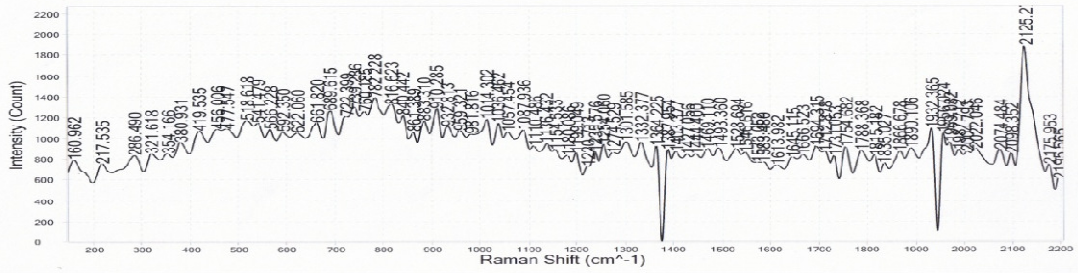


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.44

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 12:30:56
Method: Method_General_Purpose
Spectral ID: BS_20121115123056
Exposure Time: 16000 ms
Average Number: 4
Laser power-200mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial 400mW, 8000ms, 4 HQI = 76.4
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

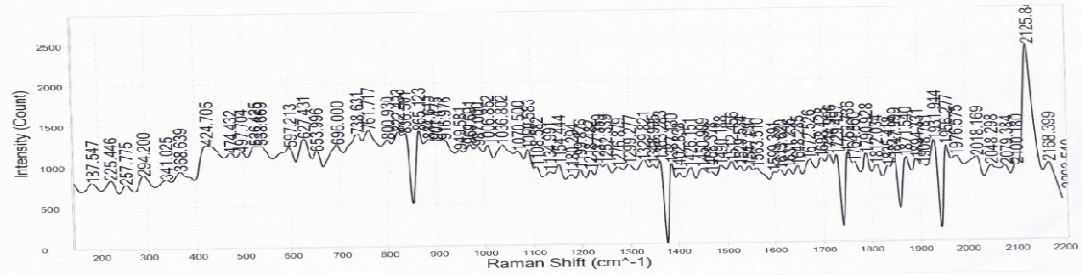


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.45

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 12:34:13
Method: Method_General_Purpose
Spectral ID: BS_20121115123413
Exposure Time: 16000 ms
Laser power = 250mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial 200mW, 8000ms, 4 HQI = 70.6
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

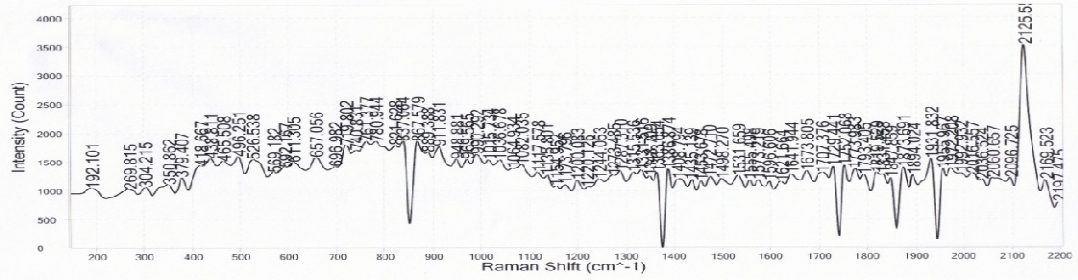


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.46

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 12:38:21
Method: Method_General_Purpose
Spectral ID: BS_20121115123821
Exposure Time: 16000 ms
Laser power = 300mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

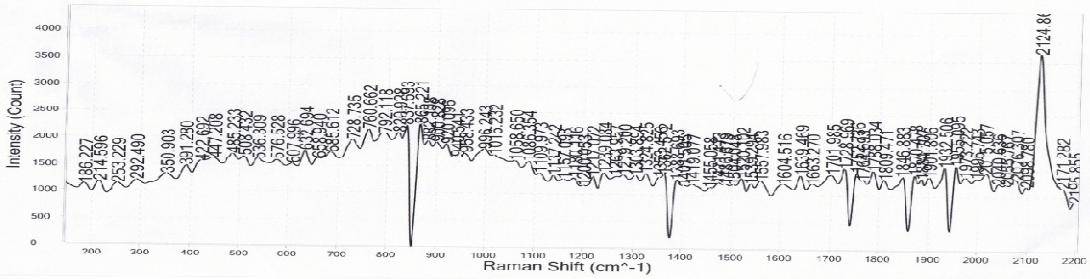


BaySpec FirstGuard Report

Graph 3.3.47

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 12:42:20
Method: Method_General_Purpose
Spectral ID: BS_201211151242?0
Exposure Time: 16000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial, 300mW, 8000ms, 4 HQI = 59.1
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

Laser power = 350mW

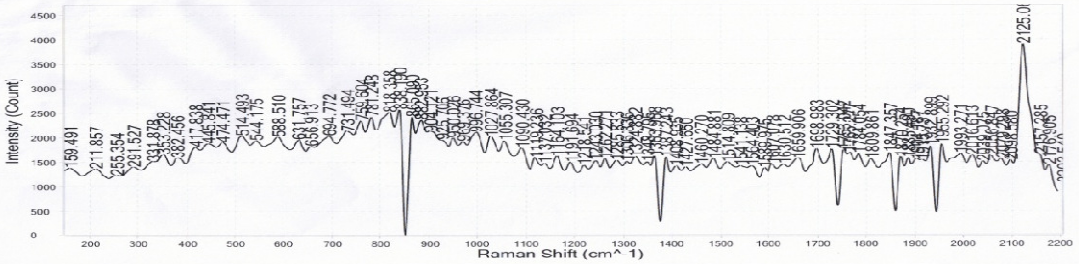


BaySpec FirstGuard Report

Graph 3.3.48

User ID: Admin
Device ID: T1160038
Test Time: 11/15/2012 12:46:04
Method: Method_General_Purpose
Spectral ID: BS_201211151246?4
Exposure Time: 16000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial 350mW, 8000ms, 4 HQI = 67.0
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

Laser power = 1160mW

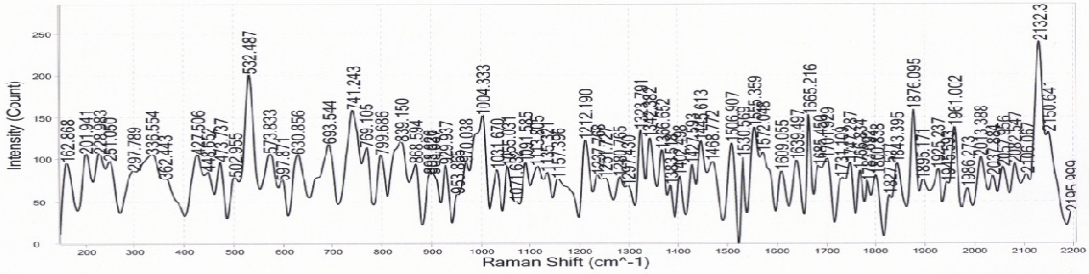


BaySpec FirstGuard Report

Graph 3.3.49

User ID: Admin
Device ID: T1160038
Test Time: 02/06/2013 14:44:55
Method: Method_General_Purpose
Spectral ID: BS_20130206144455
Exposure Time: 2000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 150mW, Cone distance = 0mm



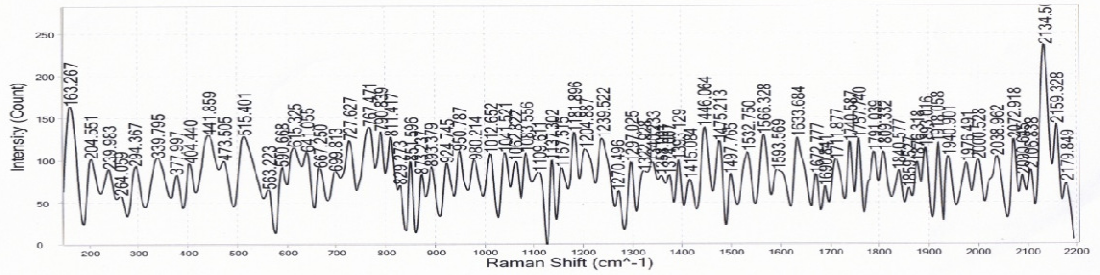
BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.50

User ID: Admin
Device ID: T1160038
Test Time: 02/06/2013 14:41:33
Method: Method_General_Purpose
Spectral ID: BS_20130206144133
Exposure Time: 2000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 150mW, Cone distance = 4mm



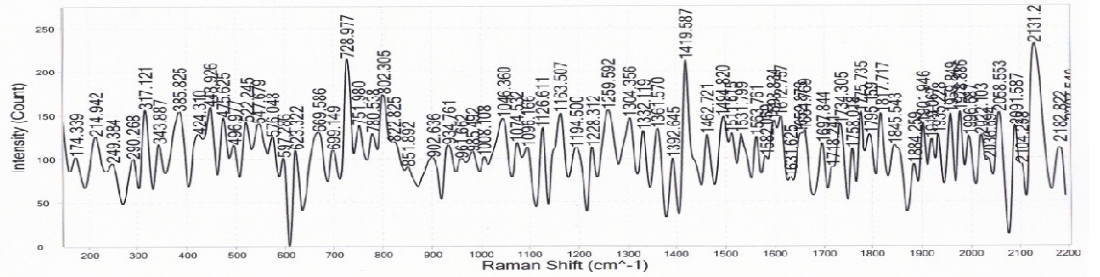
BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.51

User ID: Admin
Device ID: T1160038
Test Time: 02/13/2013 10:36:35
Method: Method_General_Purpose
Spectral ID: BS_20130213103635
Exposure Time: 2000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 150mW, Cone distance = 6mm



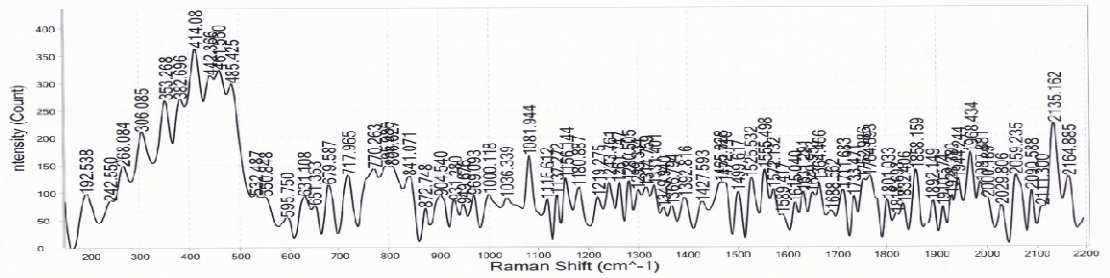
BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.52

User ID: Admin
Device ID: T1160038
Test Time: 02/06/2013 14:42:57
Method: Method_General_Purpose
Spectral ID: BS_20130206144257
Exposure Time: 2000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

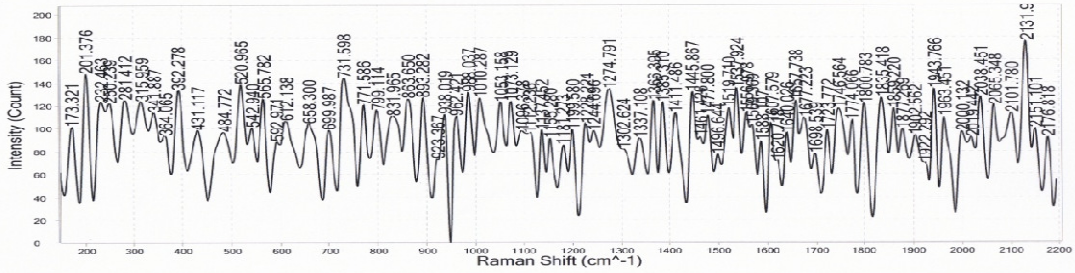
Laser power = 150mW, Cone distance = 8mm



BaySpec FirstGuard Report

Graph 3.3.53

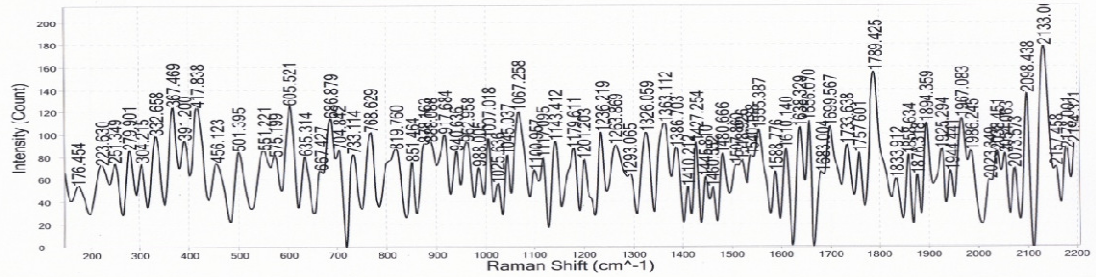
User ID: Admin
Device ID: T1160038
Test Time: 02/06/2013 14:47:07
Method: Method_General_Purpose
Spectral ID: BS_20130206144707
Exposure Time: 2000 ms
Average Number: 4
Laser power - 150mW, cone distance = 12mm
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.54

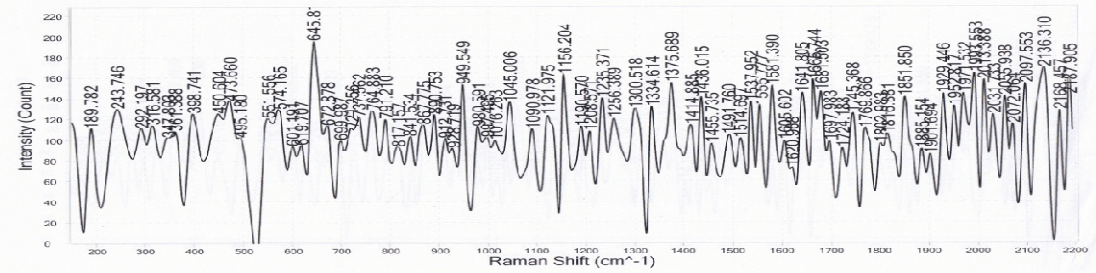
User ID: Admin
Device ID: T1160038
Test Time: 02/06/2013 14:48:05
Method: Method_General_Purpose
Spectral ID: BS_20130206144805
Exposure Time: 2000 ms
Average Number: 4
Laser power - 150mW, cone distance = 16mm
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.55

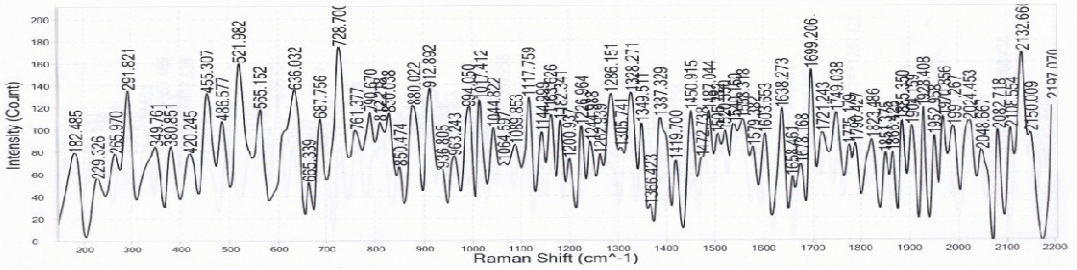
User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 11:04:39
Method: Method_General_Purpose
Spectral ID: BS_20121121110439
Exposure Time: 2000 ms
Average Number: 4
Laser power = 30mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.56

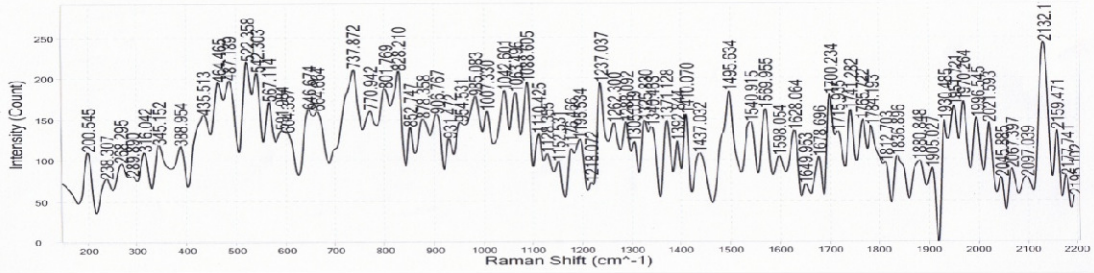
User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 11:06:12
Method: Method_General_Purpose
Spectral ID: BS_20121121110612
Exposure Time: 2000 ms
Average Number: 4
Laser power = 45mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.57

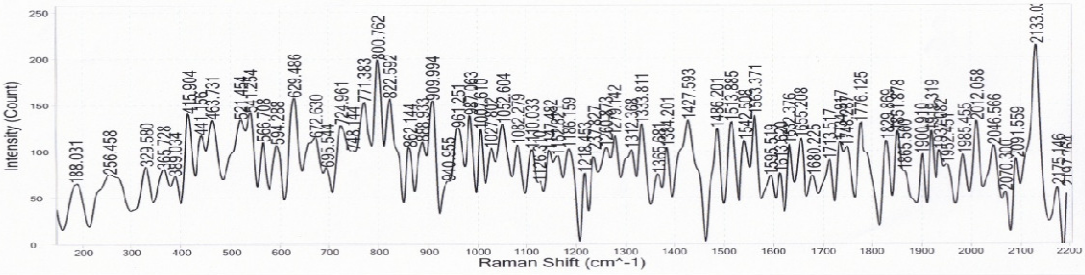
User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 11:07:59
Method: Method_General_Purpose
Spectral ID: BS_20121121110759
Exposure Time: 2000 ms
Average Number: 4
Laser power = 50mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.58

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 11:09:32
Method: Method_General_Purpose
Spectral ID: BS_20121121110932
Exposure Time: 2000 ms
Average Number: 4
Laser power = 100mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

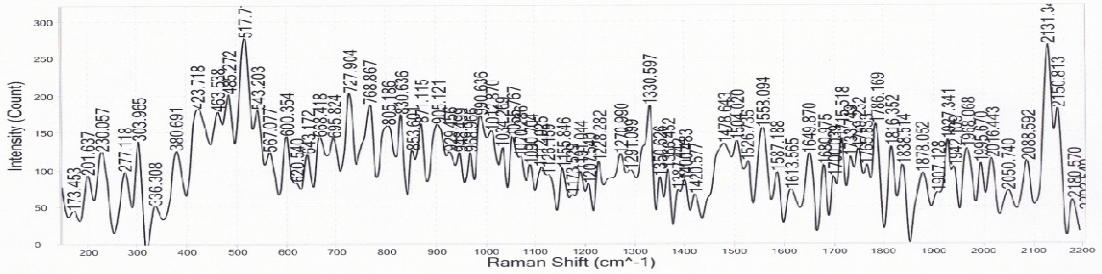


BaySpec FirstGuard Report

Graph 3.3.59

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 11:11:21
Method: Method_General_Purpose
Spectral ID: BS_20121121111121
Exposure Time: 2000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 60
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 150mW

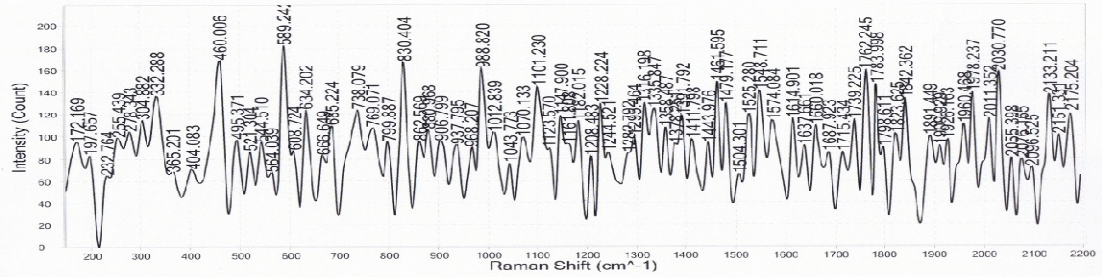


BaySpec FirstGuard Report

Graph 3.3.60

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 11:14:29
Method: Method_General_Purpose
Spectral ID: BS_20121121111429
Exposure Time: ~~4000 ms~~ 2000ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 60
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 200mW

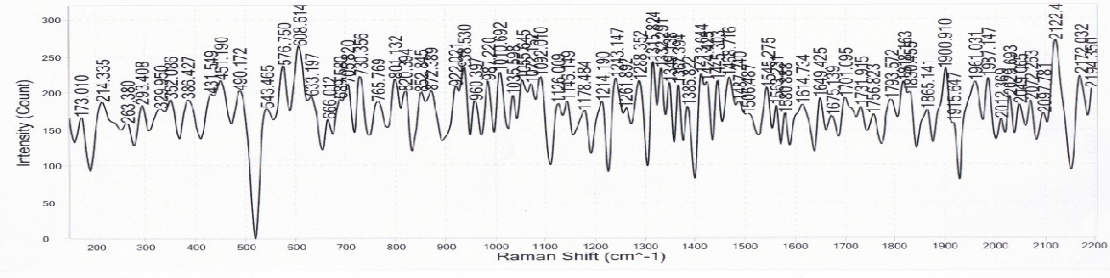


BaySpec FirstGuard Report

Graph 3.3.61

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 11:16:42
Method: Method_General_Purpose
Spectral ID: BS_20121121111642
Exposure Time: ~~4000 ms~~ 2000ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 60
Search Range: 200 to 2000 cm^-1
Result: FAIL

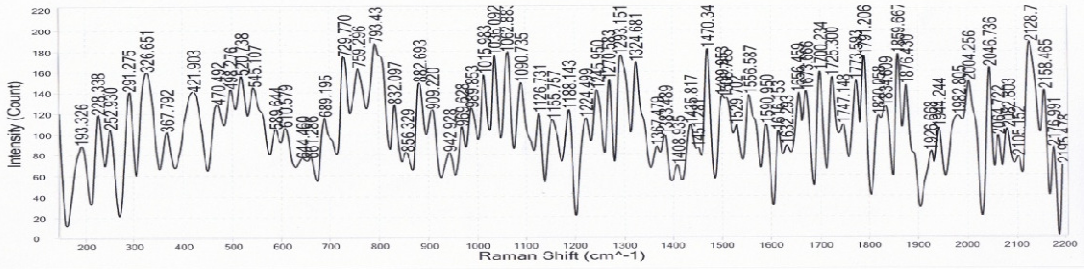
Laser power = 250mW



BaySpec FirstGuard Report

Graph 3.3.62

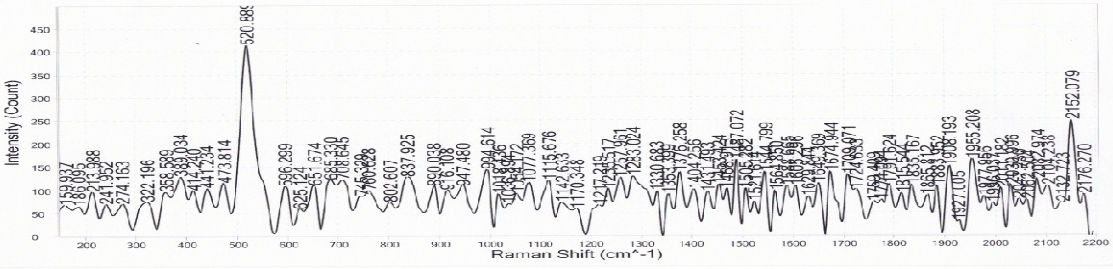
User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 11:22:58
Method: Method_General_Purpose
Spectral ID: BS_20121121112256
Exposure Time: 4000 ms
Laser power = 30mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 60
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.63

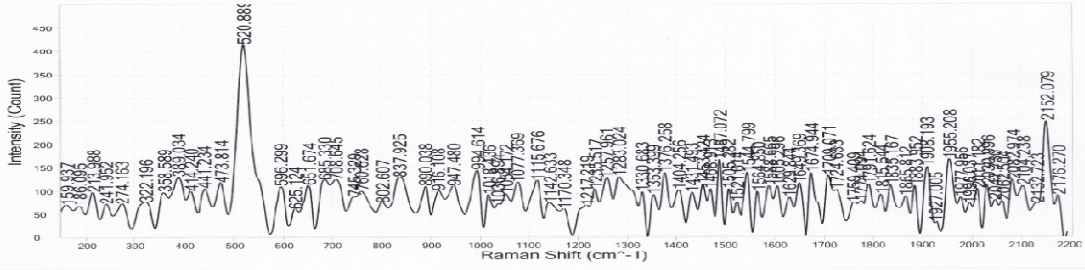
User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 11:19:57
Method: Method_General_Purpose
Spectral ID: BS_20121121111957
Exposure Time: 4000 ms
Laser power = 40mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 60
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.64

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 11:20:54
Method: Method_General_Purpose
Spectral ID: BS_20121121112054
Exposure Time: 4000 ms
Laser power = 50mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 60
Search Range: 200 to 2000 cm^-1
Result: FAIL

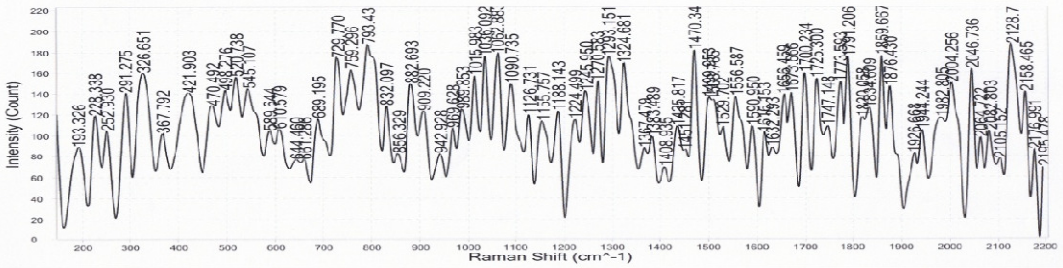


BAYSPEC

Graph 3.3.65

---------- BaySpec FirstGuard Report ----------

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 11:21:57
Method: Method_General_Purpose
Spectral ID: BS_20121121112157
Exposure Time: 4000 ms
Average Number: 4
Laser power = 100mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

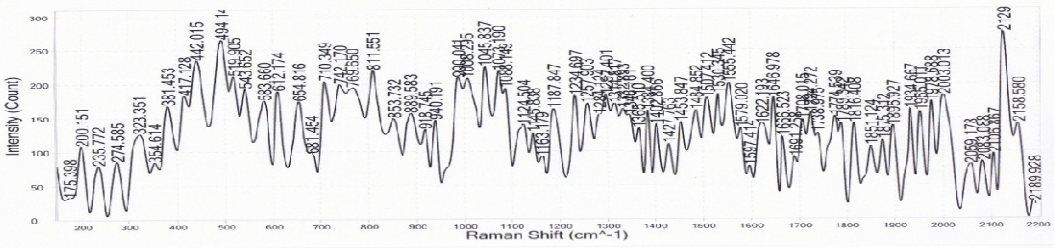


BAYSPEC

Graph 3.3.66

---------- BaySpec FirstGuard Report ----------

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 11:24:27
Method: Method_General_Purpose
Spectral ID: BS_20121121112427
Exposure Time: 4000 ms
Average Number: 4
Laser power = 150mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

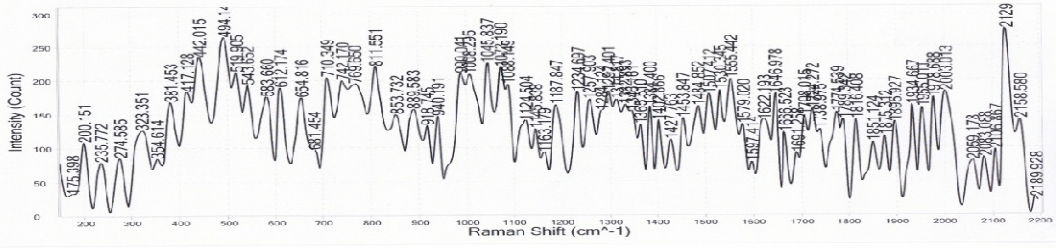


BAYSPEC

Graph 3.3.66

---------- BaySpec FirstGuard Report ----------

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 11:24:27
Method: Method_General_Purpose
Spectral ID: BS_20121121112427
Exposure Time: 4000 ms
Average Number: 4
Laser power = 150mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



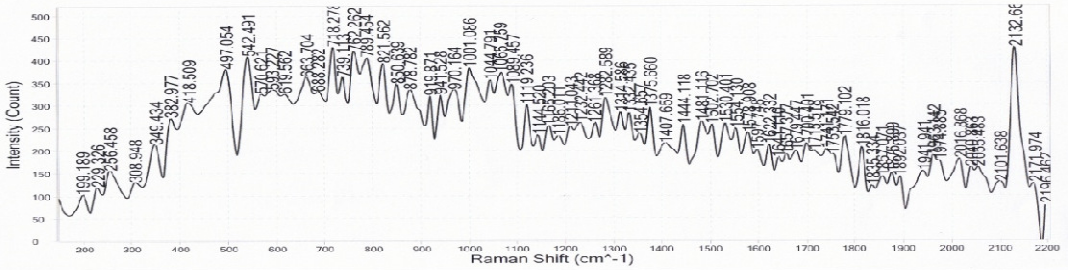
BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.68

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 10:34:26
Method: Method_General_Purpose
Spectral ID: BS_20121121103426
Exposure Time: 4000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 60
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 250mW



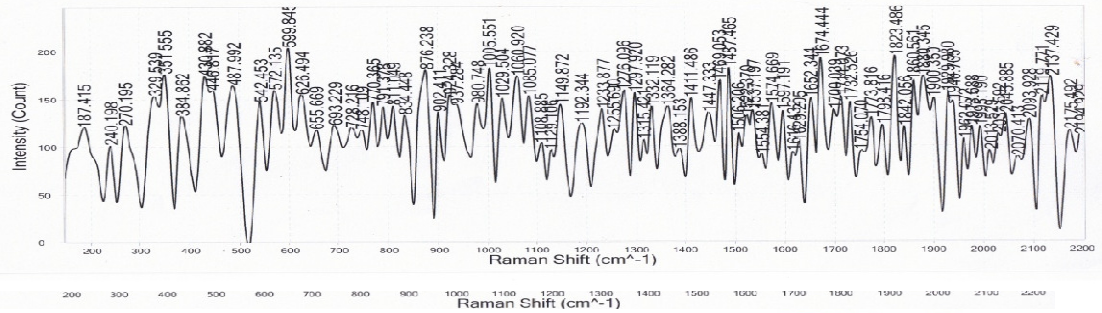
BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.69

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 10:38:40
Method: Method_General_Purpose
Spectral ID: BS_20121121103840
Exposure Time: 8000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 30mW



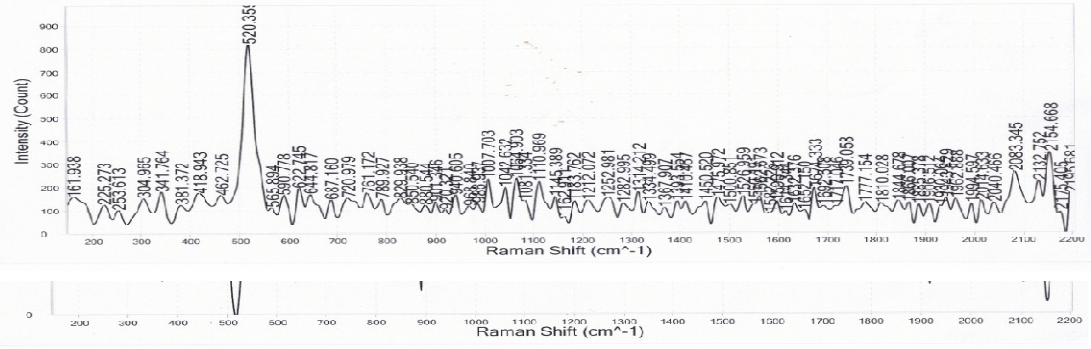
BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.70

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 10:40:36
Method: Method_General_Purpose
Spectral ID: BS_20121121104036
Exposure Time: 8000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 40mW

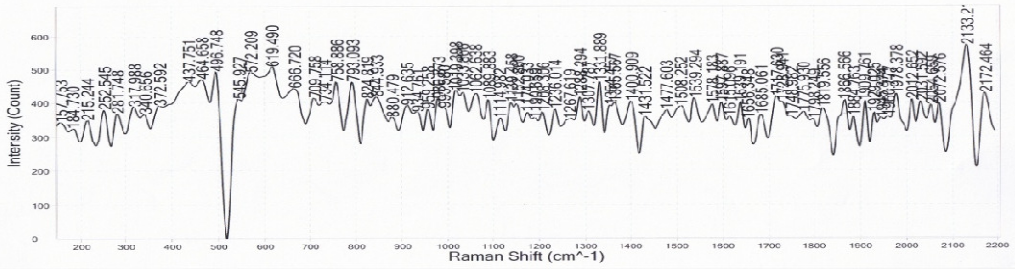


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.71

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 10:43:01
Method: Method_General_Purpose
Spectral ID: BS_20121121104301
Exposure Time: 8000 ms
Average Number: 4
Laser power = 50mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

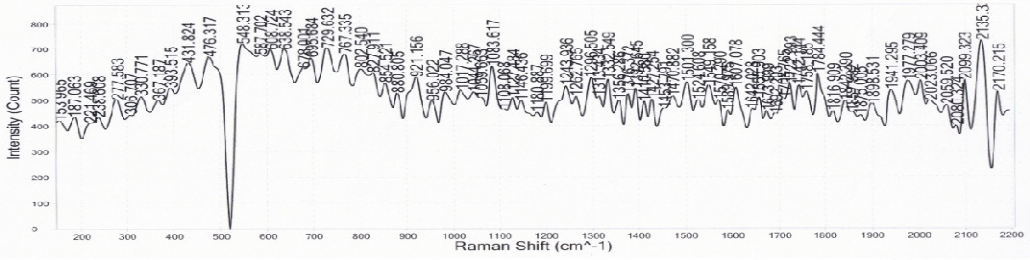


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.72

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 10:45:29
Method: Method_General_Purpose
Spectral ID: BS_20121121104529
Exposure Time: 8000 ms
Average Number: 4
Laser power = 100mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

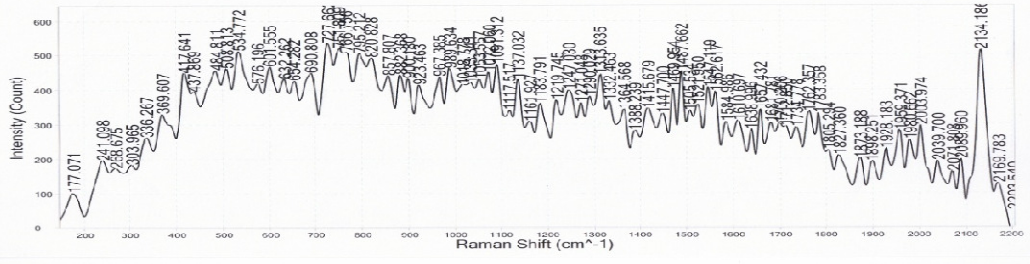


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.73

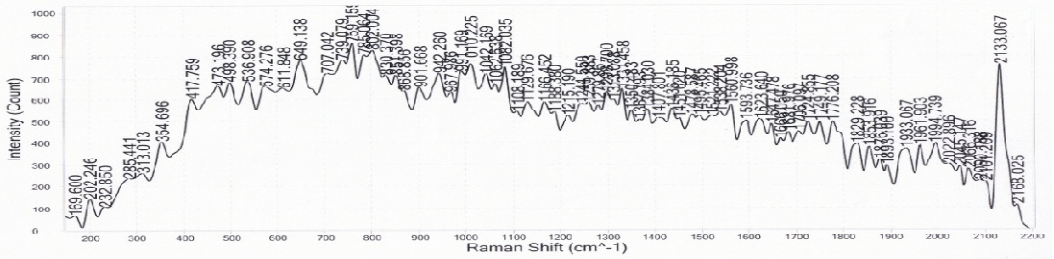
User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 10:47:34
Method: Method_General_Purpose
Spectral ID: BS_20121121104734
Exposure Time: 8000 ms
Average Number: 4
Laser power = 150mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.74

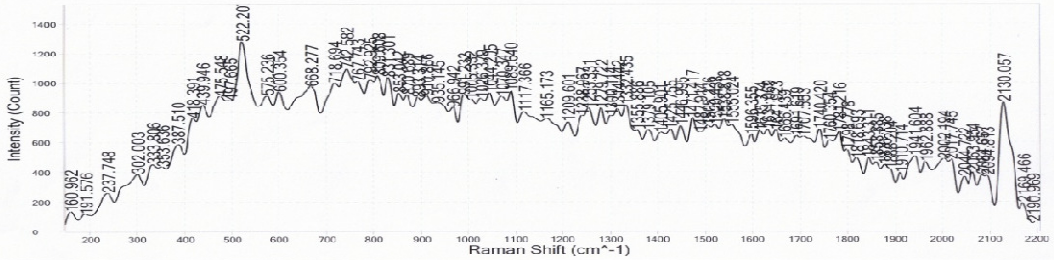
User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 10:52:23
Method: Method_General_Purpose
Spectral ID: BS_20121121105223
Exposure Time: 8000 ms
Laser power = 200mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.75

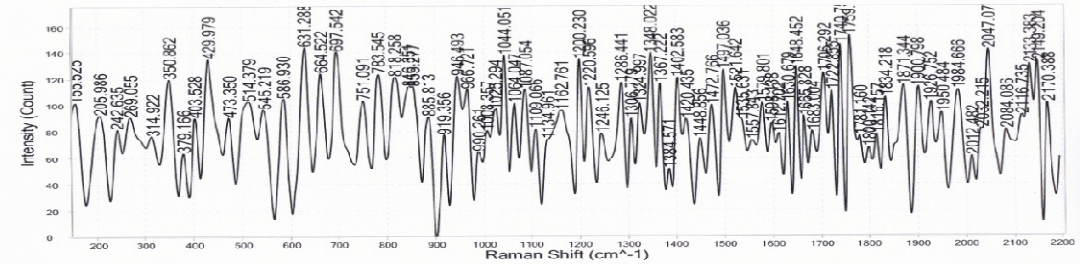
User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 10:55:21
Method: Method_General_Purpose
Spectral ID: BS_20121121105521
Exposure Time: 8000 ms
Laser power = 250mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.76

User ID: Admin
Device ID: T1160038
Test Time: 02/13/2013 11:45:00
Method: Method_General_Purpose
Spectral ID: BS_20130213114500
Exposure Time: 2000 ms
Laser power = 30mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

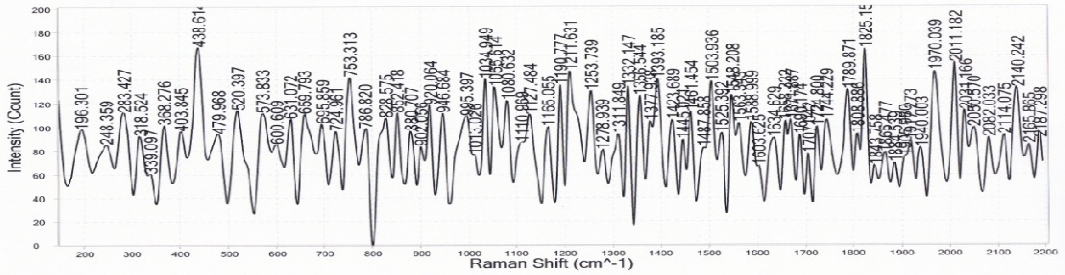


BaySpec FirstGuard Report

Graph 3.3.77

User ID: Admin
Device ID: T1160038
Test Time: 02/13/2013 11:45:57
Method: Method_General_Purpose
Spectral ID: BS_20130213114557
Exposure Time: 2000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 40mW

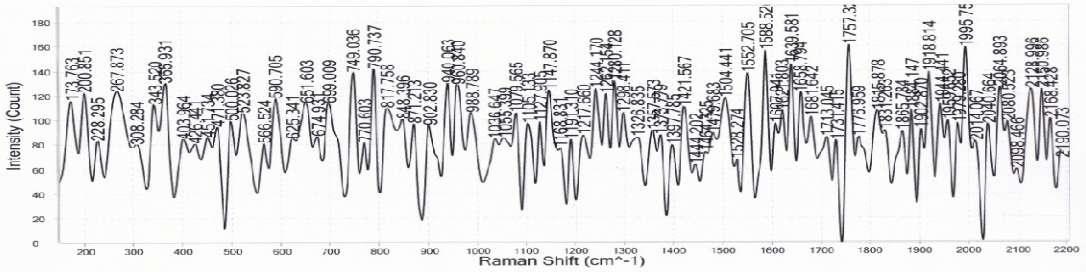


BaySpec FirstGuard Report

Graph 3.3.78

User ID: Admin
Device ID: T1160038
Test Time: 02/13/2013 11:51:50
Method: Method_General_Purpose
Spectral ID: BS_20130213115150
Exposure Time: 2000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 50mW

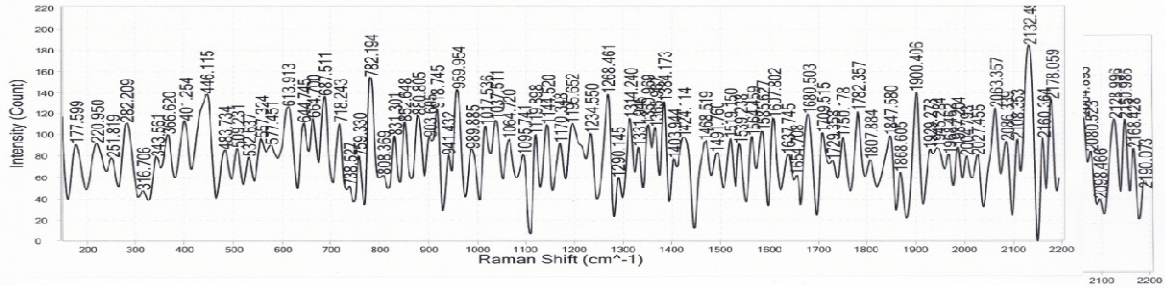


BaySpec FirstGuard Report

Graph 3.3.79

User ID: Admin
Device ID: T1160038
Test Time: 02/13/2013 13:55:29
Method: Method_General_Purpose
Spectral ID: BS_20130213135529
Exposure Time: 2000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

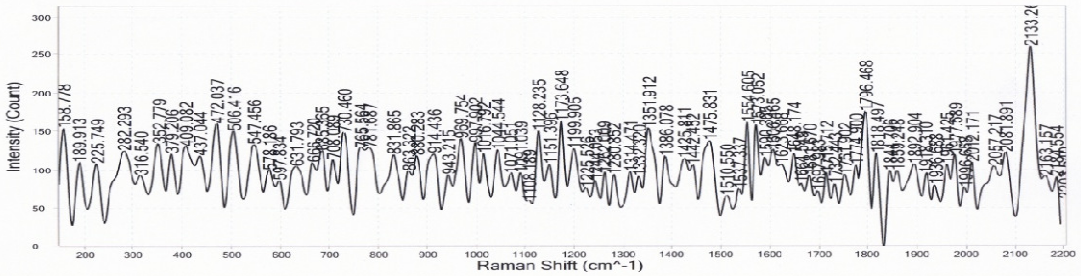
Laser power = 100mW



BaySpec FirstGuard Report

Graph 3.3.80

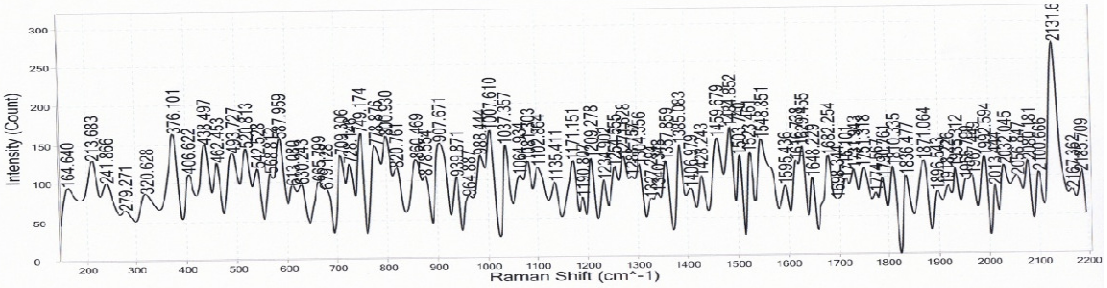
User ID: Admin
Device ID: T1160038
Test Time: 02/13/2013 14:01:54
Method: Method_General_Purpose
Spectral ID: BS_20130213140154
Exposure Time: 2000 ms
Average Number: 4
Laser power = 150mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.81

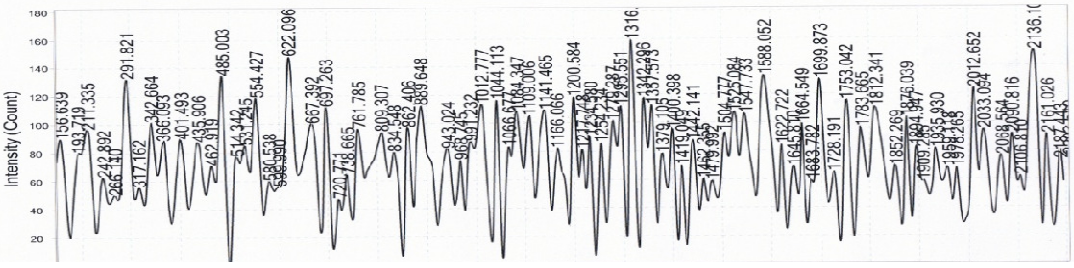
User ID: Admin
Device ID: T1160038
Test Time: 02/13/2013 14:09:44
Method: Method_General_Purpose
Spectral ID: BS_20130213140944
Exposure Time: 2000 ms
Average Number: 4
Laser power = 200mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.3.83

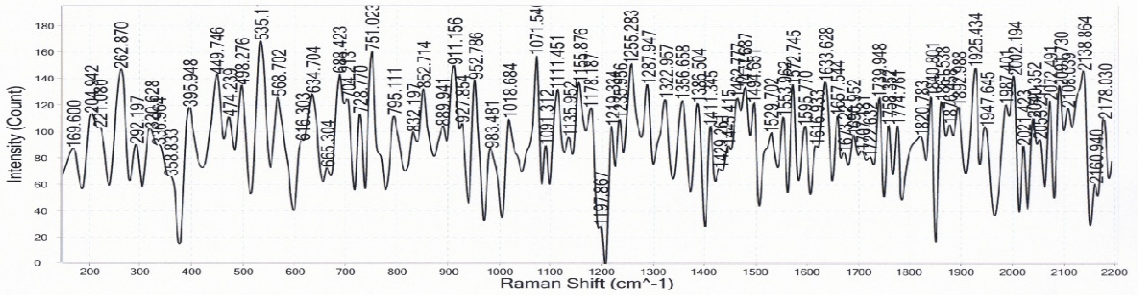
User ID: Admin
Device ID: T1160038
Test Time: 02/13/2013 14:13:52
Method: Method_General_Purpose
Spectral ID: BS_20130213141352
Exposure Time: 4000 ms
Average Number: 4
Laser power = 30mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



---------- BaySpec FirstGuard Report ----------

Graph 3.3.84

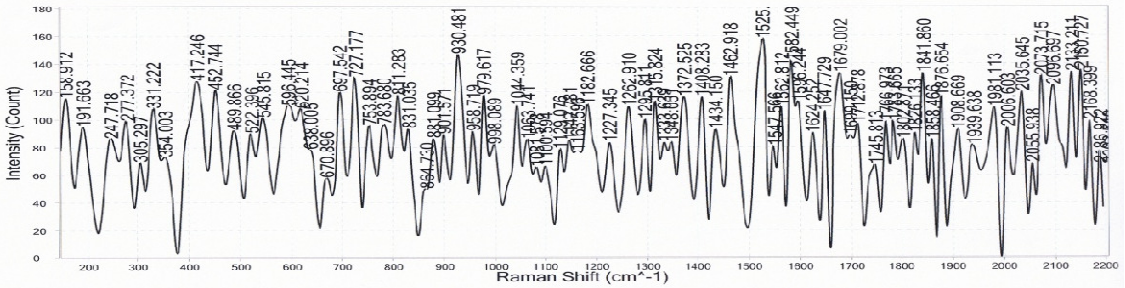
User ID: Admin
Device ID: T1160038
Test Time: 02/13/2013 14:15:57
Method: Method_General_Purpose
Spectral ID: BS_20130213141557
Exposure Time: 4000 ms
Average Number: 4
Laser power = 40mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL



---------- BaySpec FirstGuard Report ----------

Graph 3.3.85

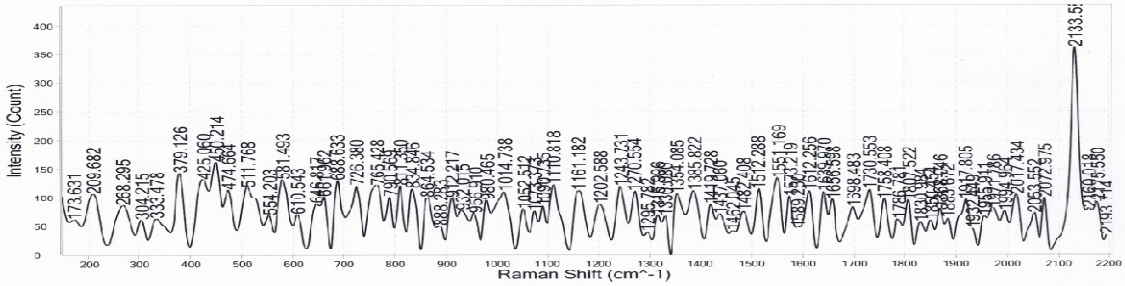
User ID: Admin
Device ID: T1160038
Test Time: 02/13/2013 14:19:09
Method: Method_General_Purpose
Spectral ID: BS_20130213141909
Exposure Time: 4000 ms
Average Number: 4
Laser power = 50mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

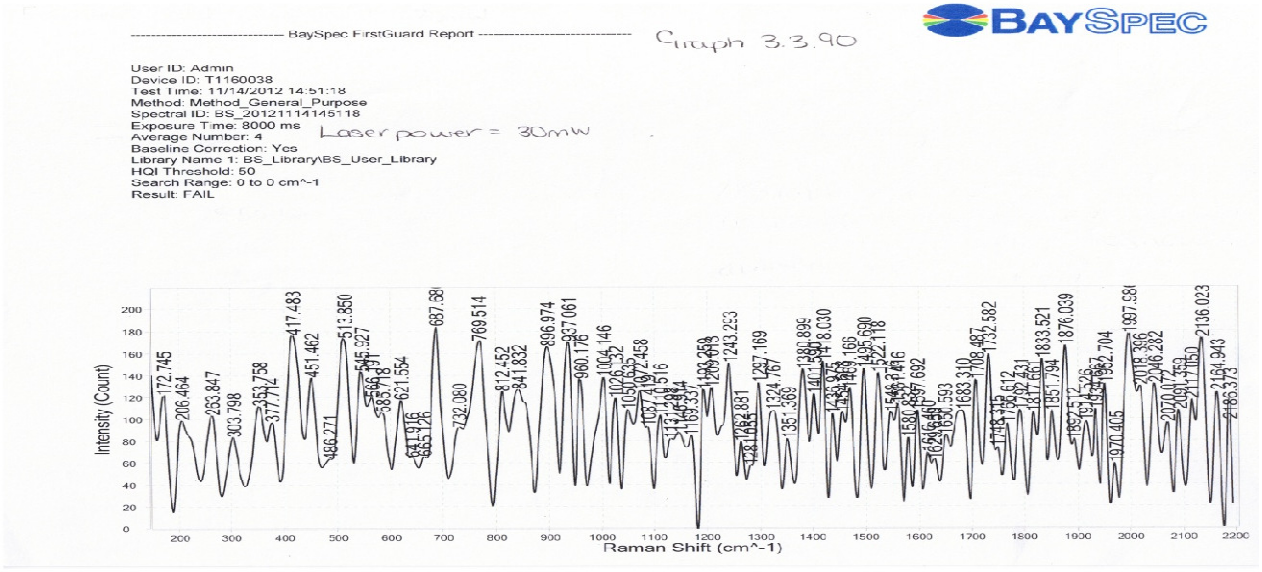


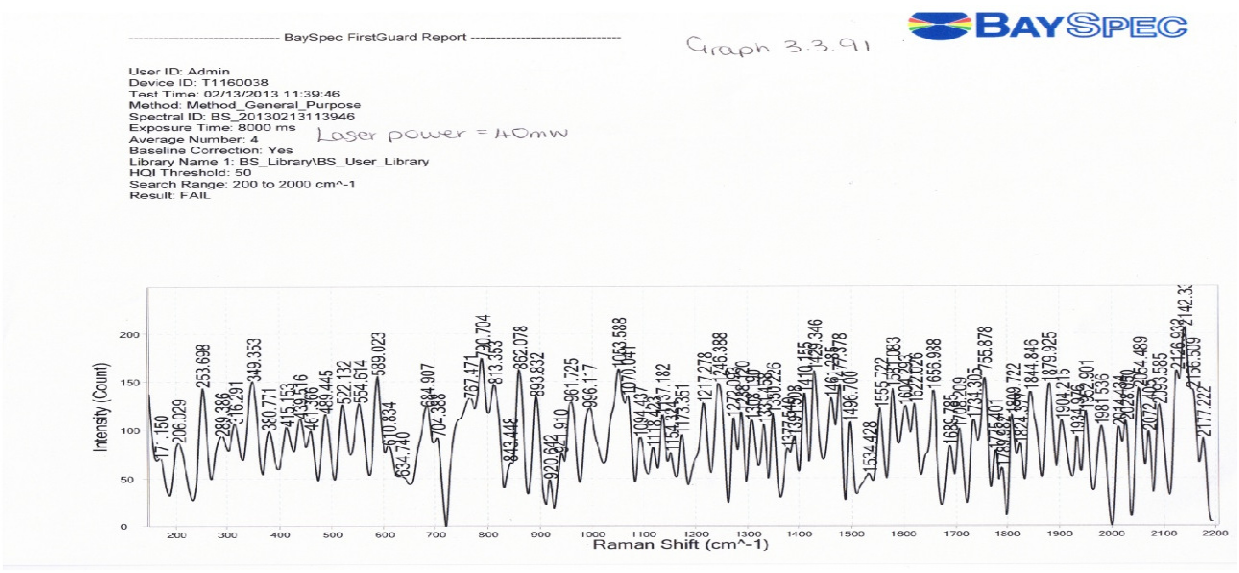
---------- BaySpec FirstGuard Report ----------

Graph 3.3.86

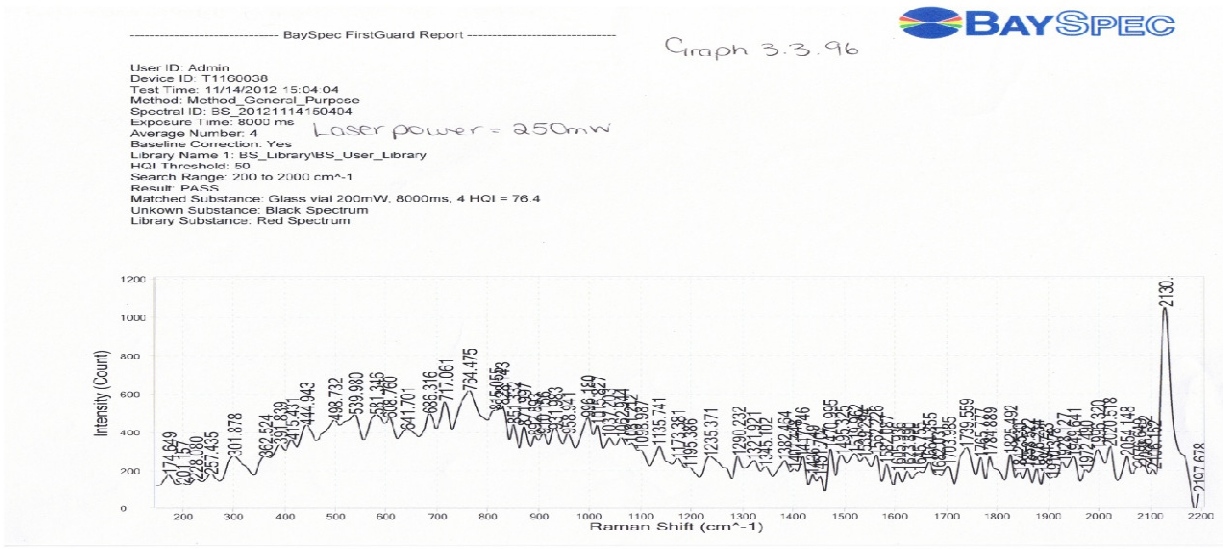
User ID: Admin
Device ID: T1160038
Test Time: 02/13/2013 14:23:48
Method: Method_General_Purpose
Spectral ID: BS_20130213142348
Exposure Time: 4000 ms
Average Number: 4
Laser power = 100mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL




BaySpec FirstGuard Report
Graph 3.3.90
User ID: Admin
Device ID: T1160038
Test Time: 11/14/2012 14:51:18
Method: Method_General_Purpose
Spectral ID: BS_20121114145118
Exposure Time: 8000 ms
Average Number: 4
Laser power = 30mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 0 to 0 cm^-1
Result: FAIL
Intensity (Count)
Raman Shift (cm^-1)


BaySpec FirstGuard Report
Graph 3.3.91
User ID: Admin
Device ID: T1160038
Test Time: 02/13/2013 11:39:46
Method: Method_General_Purpose
Spectral ID: BS_20130213113946
Exposure Time: 8000 ms
Average Number: 4
Laser power = 140mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL
Intensity (Count)
Raman Shift (cm^-1)

Human Blood:

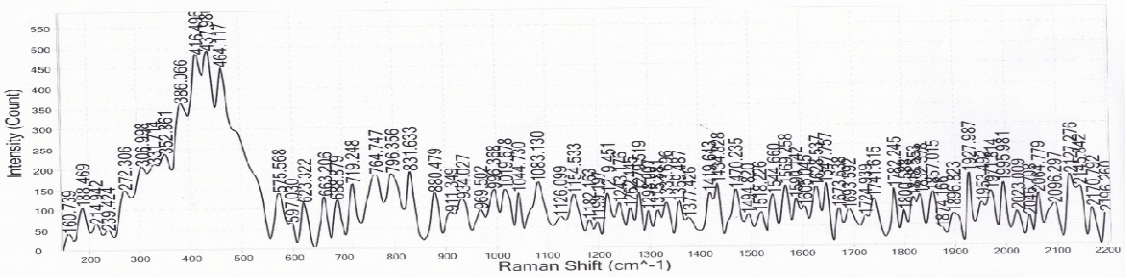

BaySpec FirstGuard Report
Graph 3.3.96
User ID: Admin
Device ID: T1160038
Test Time: 11/14/2012 15:04:04
Method: Method_General_Purpose
Spectral ID: BS_20121114150404
Exposure Time: 8000 ms
Average Number: 4
Laser power = 250mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial 200mW, 8000ms, 4 HQI = 76.4
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum
Intensity (Count)
Raman Shift (cm^-1)

BayGpec FirstGuard Report

Graph 3.3.100

User ID: Admin
Device ID: T1160038
Test Time: 11/28/2012 12:45:15
Method: Method_General_Purpose
Spectral ID: BS_20121128124515
Exposure Time: 8000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 50mW, cone distance = 8mm

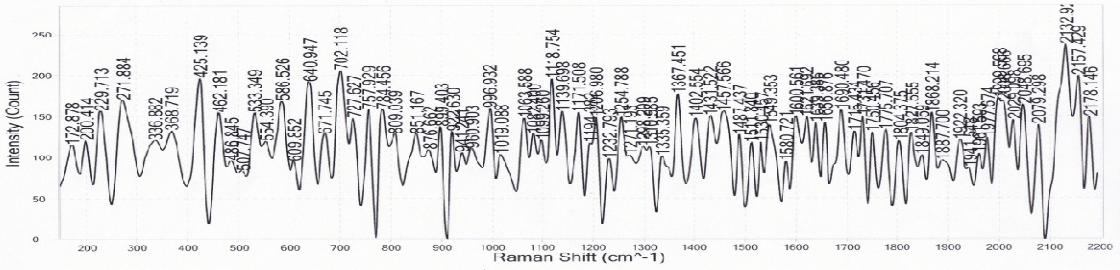


BayGpec FirstGuard Report

Graph 3.3.101

User ID: Admin
Device ID: T1160038
Test Time: 11/28/2012 12:42:49
Method: Method_General_Purpose
Spectral ID: BS_20121128124249
Exposure Time: 8000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 50mW, cone distance = 12mm

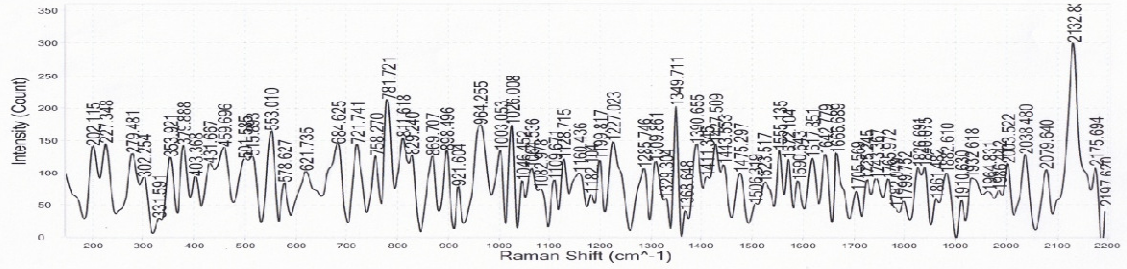


BayGpec FirstGuard Report

Graph 3.3.99

User ID: Admin
Device ID: T1160038
Test Time: 11/28/2012 12:35:06
Method: Method_General_Purpose
Spectral ID: BS_20121128123506
Exposure Time: 8000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 50mW, cone distance = 6mm

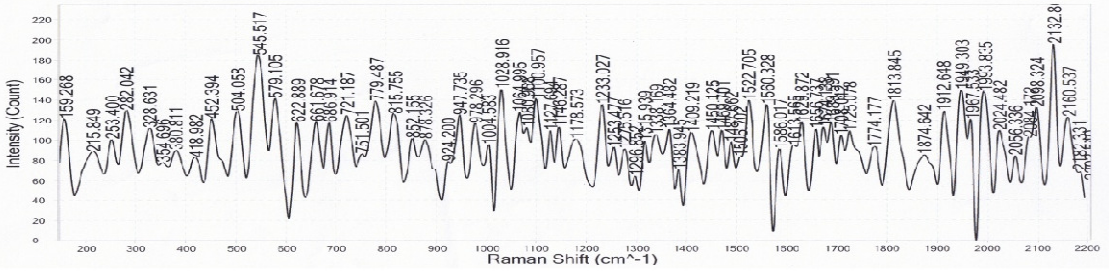


BaySpec FirstGuard Report

Graph 3.3.103

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 14:10:02
Method: Method_General_Purpose
Spectral ID: BS_20121121141002
Exposure Time: 2000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 30mW

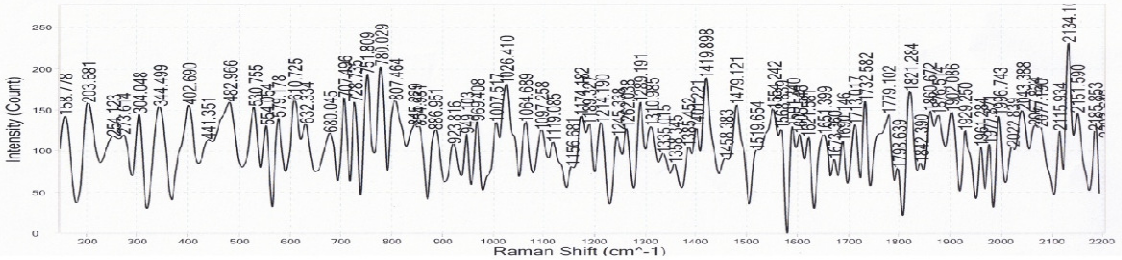


BaySpec FirstGuard Report

Graph 3.3.102

User ID: Admin
Device ID: T1160038
Test Time: 11/28/2012 12:38:42
Method: Method_General_Purpose
Spectral ID: BS_20121128123842
Exposure Time: 8000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 50mW, cone distance = 16mm

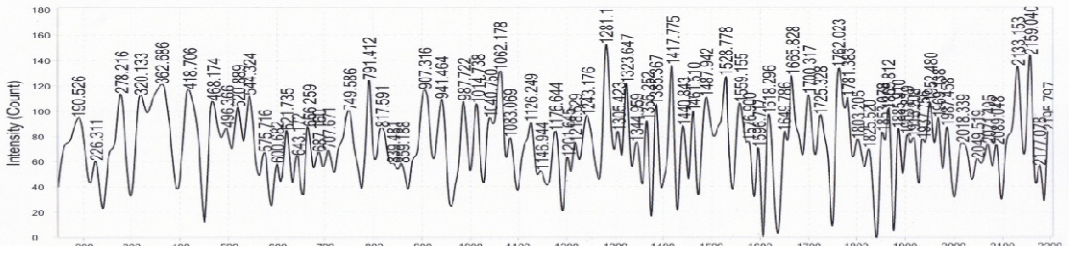


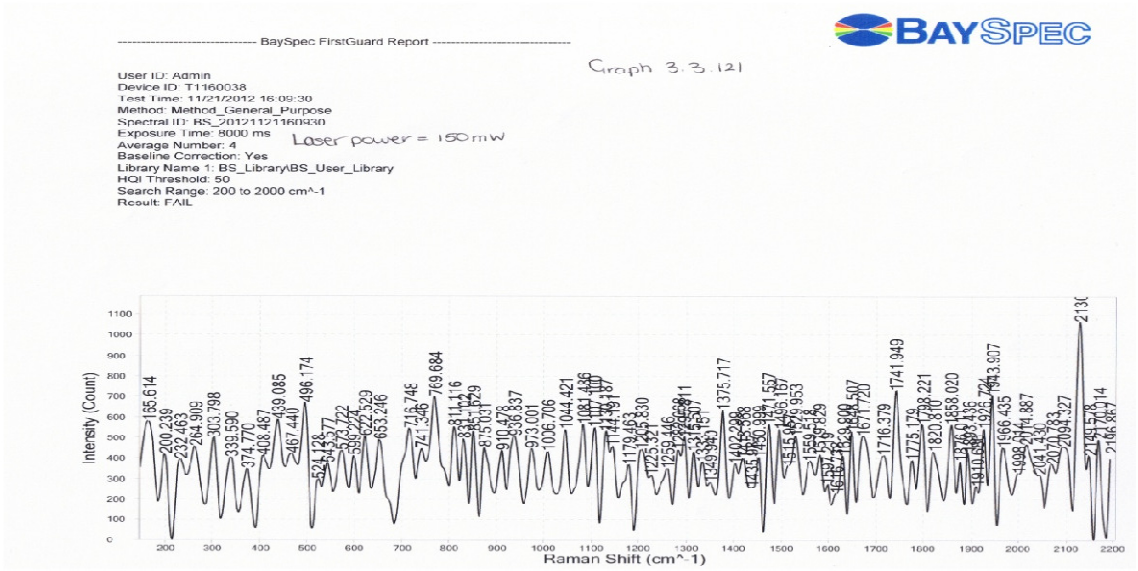
BaySpec FirstGuard Report

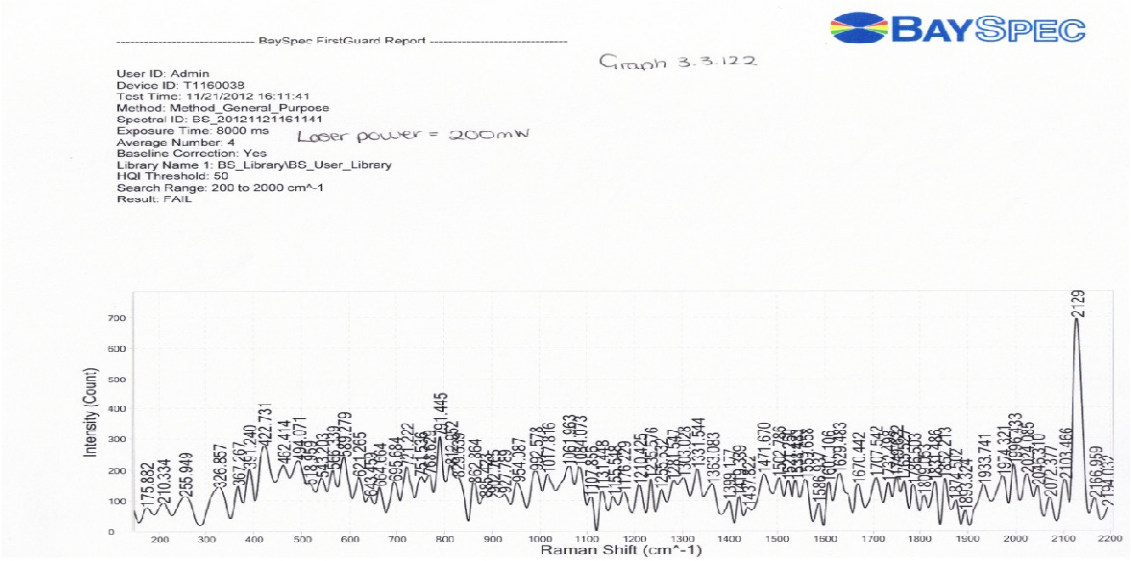
Graph 3.3.104

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 14:13:22
Method: Method_General_Purpose
Spectral ID: BS_20121121141322
Exposure Time: 2000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 60
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laserpower = 40mW




BaySpec FirstGuard Report
Graph 3.3.121
User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 16:09:30
Method: Method_General_Purpose
Spectral ID: BS_20121121160930
Exposure Time: 8000 ms
Laser power = 150mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL
Intensity (Count)
Raman Shift (cm^-1)


BaySpec FirstGuard Report
Graph 3.3.122
User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 16:11:41
Method: Method_General_Purpose
Spectral ID: BS_20121121161141
Exposure Time: 8000 ms
Laser power = 200mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL
Intensity (Count)
Raman Shift (cm^-1)

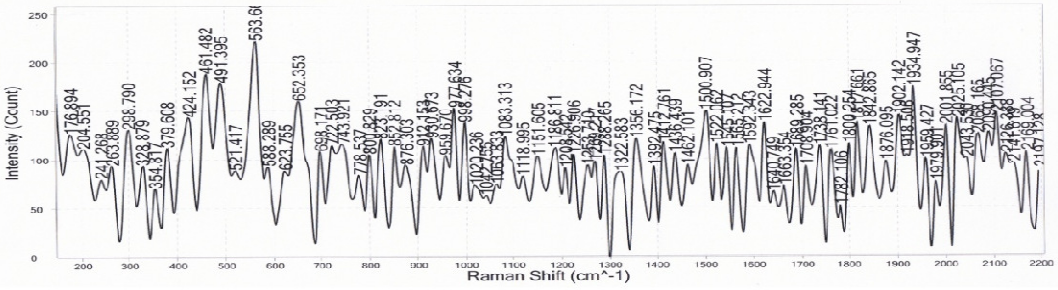
BAYSPEC

Graph 3.3.125

BaySpec FirstGuard Report

User ID: Admin
Device ID: T1160038
Test Time: 02/06/2013 11:49:05
Method: Method_General_Purpose
Spectral ID: BS_201302061149O5
Exposure Time: 4000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 40mW



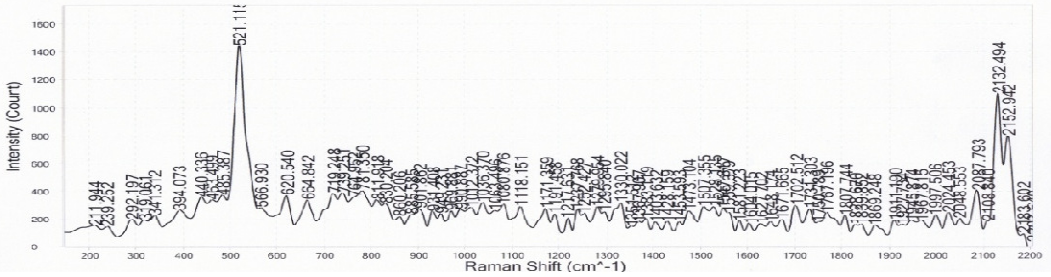
BAYSPEC

Graph 3.3.123

BaySpec FirstGuard Report

User ID: Admin
Device ID: T1160038
Test Time: 11/21/2012 16:14:39
Method: Method_General_Purpose
Spectral ID: BS_20121121161439
Exposure Time: 8000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 250mW



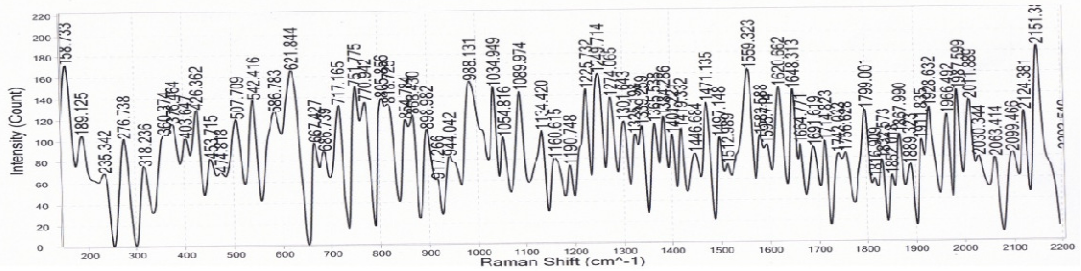
BAYSPEC

Graph 3.3.126

BaySpec FirstGuard Report

User ID: Admin
Device ID: T1160038
Test Time: 02/06/2013 11:51:25
Method: Method_General_Purpose
Spectral ID: BS_201302061151525
Exposure Time: 4000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 50mW



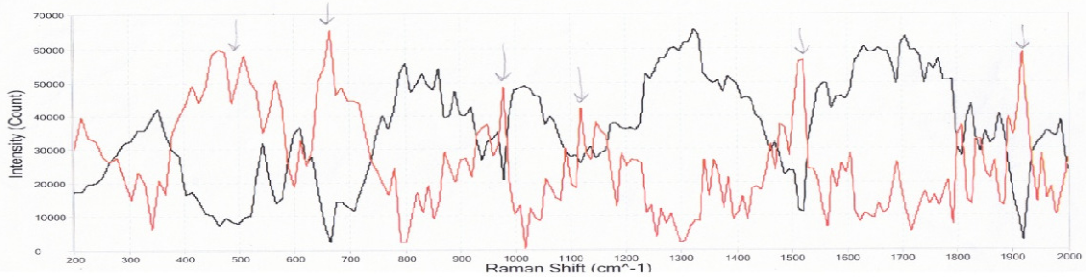
BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.127A

User ID: Admin
Device ID: T1160038
Test Time: 11/08/2012 14:25:24
Method: Method_General_Purpose
Spectral ID: BS_20121108142524
Exposure Time: 8000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 40
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Equine Haemoglobin HQI = 80.5
Unkown Substance: Black Spectrum Glass microscope slide (Substrate)
Library Substance: Red Spectrum Centrifuged equine blood (added to library)

Laser power = 30mW



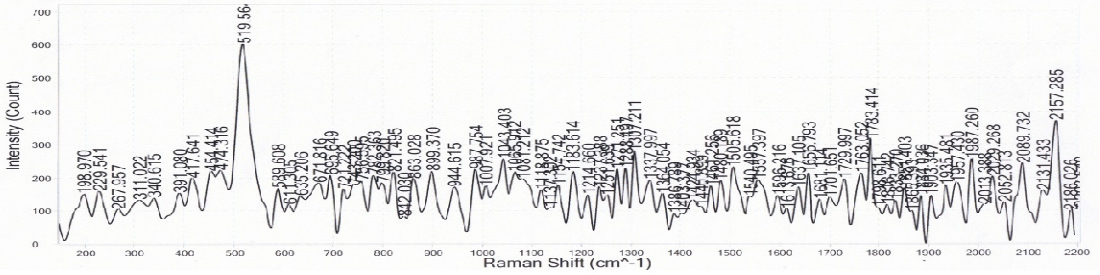
BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.3.128

User ID: Admin
Device ID: T1160038
Test Time: 11/14/2012 15:48:36
Method: Method_General_Purpose
Spectral ID: BS_20121114154836
Exposure Time: 8000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 50
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial, 300mW, 8000ms, 4 HQI = 79.4
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

Laser power = 30mW

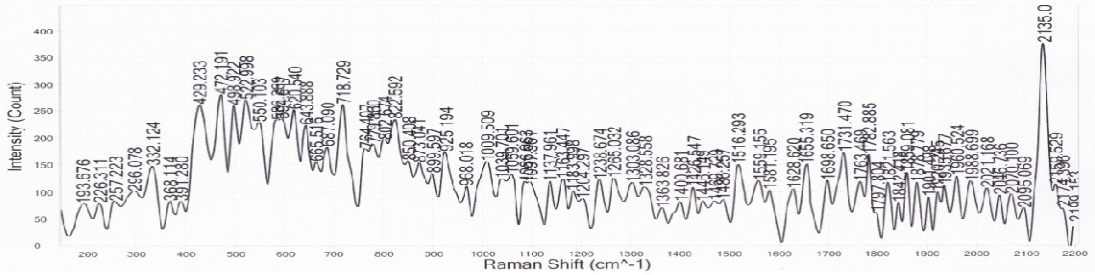


BaySpec

Graph 3.4.13

BaySpec FirstGuard Report

User ID: Admin
Device ID: T1160038
Test Time: 12/05/2012 12:47:29
Method: Method_General_Purpose
Spectral ID: BS_20121205124729
Exposure Time: 4000 ms
Average Number: 4
Laser power = 150mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Equine Haemoglobin HQI = 11.2
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

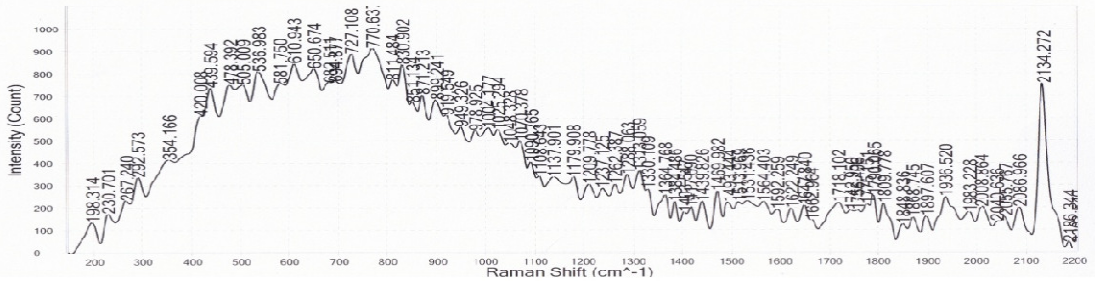


BaySpec

Graph 3.4.16

BaySpec FirstGuard Report

User ID: Admin
Device ID: T1160038
Test Time: 12/05/2012 12:51:04
Method: Method_General_Purpose
Spectral ID: BS_20121205125104
Exposure Time: 4000 ms
Laser power = 300mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial, 300mW, 8000ms, 4 HQI = 88.1
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

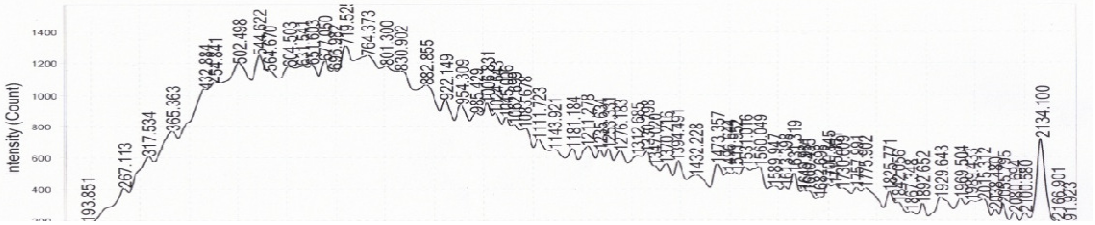


BaySpec

Graph 3.4.15

BaySpec FirstGuard Report

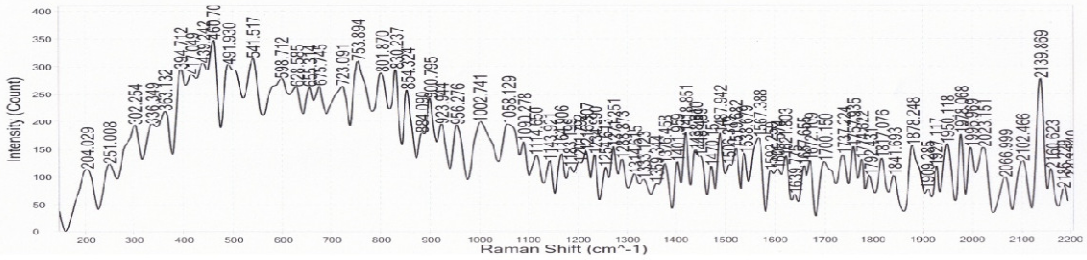
User ID: Admin
Device ID: T1160038
Test Time: 12/05/2012 12:52:51
Method: Method_General_Purpose
Spectral ID: BS_20121205125251
Exposure Time: 4000 ms
Laser power = 250mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial 250mW, 8000ms, 4 HQI = 89.1
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.4.18

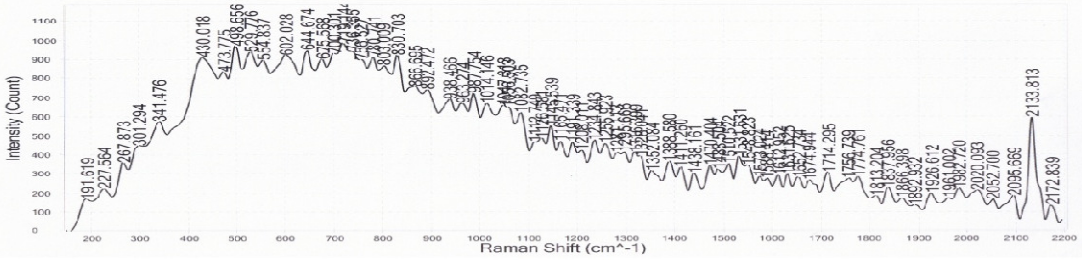
User ID: Admin
Device ID: T1160038
Test Time: 12/05/2012 12:58:11
Method: Method_General_Purpose
Spectral ID: BS_20121205125811
Exposure Time: 8000 ms
Average Number: 4
Laser power = 40mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Equine Haemoglobin HQI = 16.7
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.4.20

User ID: Admin
Device ID: T1160038
Test Time: 12/05/2012 13:02:44
Method: Method_General_Purpose
Spectral ID: BS_20121205130244
Exposure Time: 8000 ms
Laser power = 100mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Equine Haemoglobin HQI = 14.9
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum



BaySpec FirstGuard Report

Graph 3.4.21

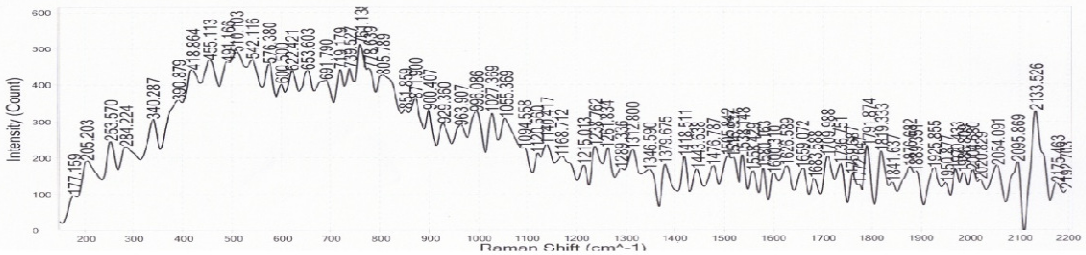
User ID: Admin
Device ID: T1160038
Test Time: 12/05/2012 13:04:31
Method: Method_General_Purpose
Spectral ID: BS_20121205130431
Exposure Time: 8000 ms
Laser power = 150mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial 150mW, 8000ms, 4 HQI = 78.7
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

BAYSPEC

Graph 3.4.19

BaySpec FirstGuard Report

User ID: Admin
Device ID: T1160038
Test Time: 12/05/2012 12:59:58
Method: Method_General_Purpose
Spectral ID: BS_20121205125058
Exposure Time: 8000 ms
Average Number: 4
Laser power = 50mw
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Equine Haemoglobin HQI = 20.9
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

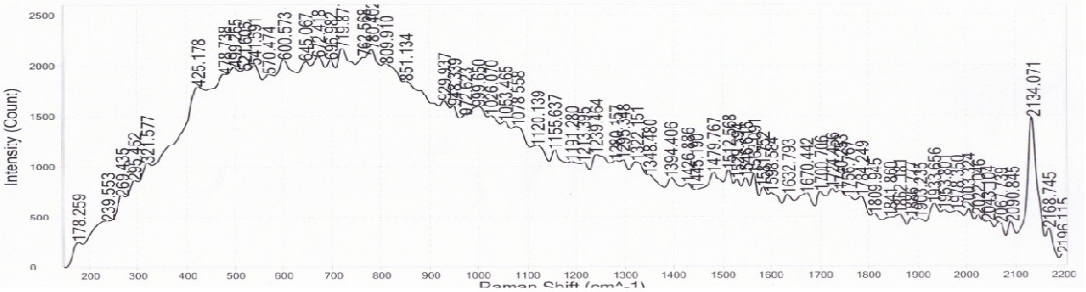


BAYSPEC

Graph 3.4.23

BaySpec FirstGuard Report

User ID: Admin
Device ID: T1160038
Test Time: 12/05/2012 13:07:56
Method: Method_General_Purpose
Spectral ID: BS_20121205130756
Exposure Time: 8000 ms
Laser power = 250mw
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial 250mW, 8000ms, 4 HQI = 90.6
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

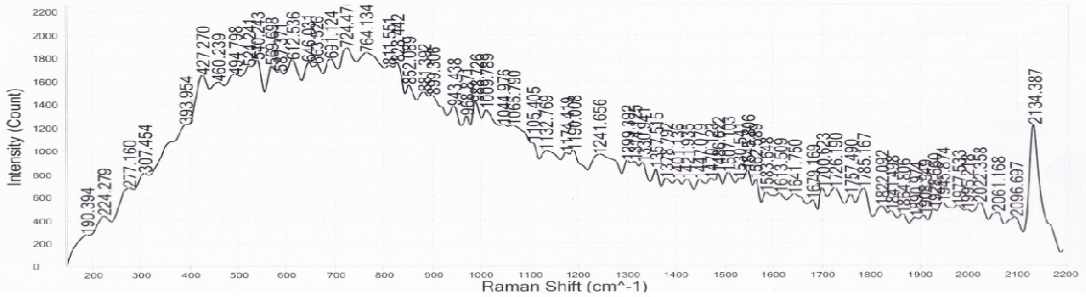


BAYSPEC

Graph 3.4.22

BaySpec FirstGuard Report

User ID: Admin
Device ID: T1160038
Test Time: 12/05/2012 13:06:15
Method: Method_General_Purpose
Spectral ID: BS_20121205130615
Exposure Time: 8000 ms
Laser power = 200mw
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial 200mW, 8000ms, 4 HQI = 90.1
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

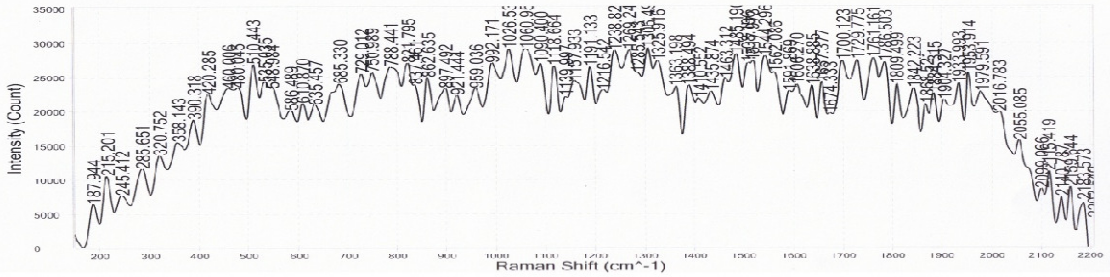


BaySpec

---------- BaySpec FirstGuard Report ----------

Graph 3.4.24

User ID: Admin
Device ID: T1160038
Test Time: 12/05/2012 13:09:53
Method: Method_General_Purpose
Spectral ID: BS_20121205130953
Exposure Time: 8000 ms
Average Number: 4
Laser power = 300mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Glass vial_300mW, 8000ms, 4 HQI = 13.3
Unknown Substance: Black Spectrum
Library Substance: Red Spectrum

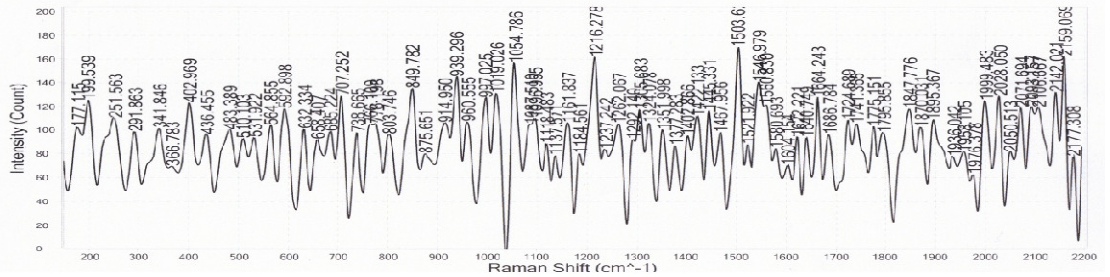


BaySpec

---------- BaySpec FirstGuard Report ----------

Graph 3.4.25

User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 09:57:04
Method: Method_General_Purpose
Spectral ID: BS_20121206095704
Exposure Time: ~~8000 ms~~ 2000ms
Laser power = 30mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

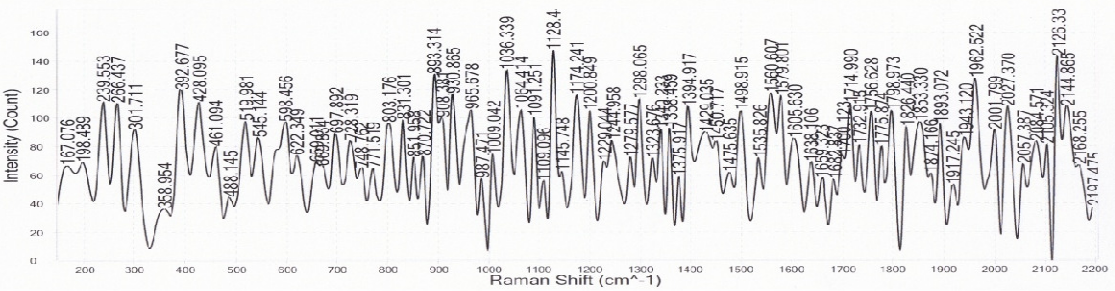


BaySpec

---------- BaySpec FirstGuard Report ----------

Graph 3.4.26

User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 09:59:41
Method: Method_General_Purpose
Spectral ID: BS_20121206095941
Exposure Time: 8000 ms
Laser power = 10mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

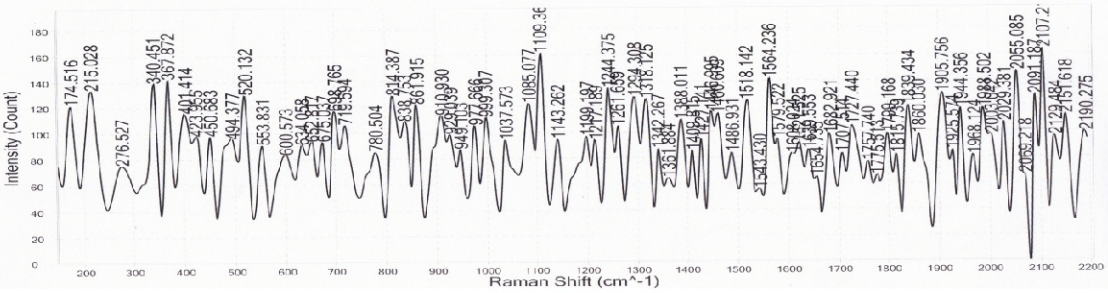


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.4.27

User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 10:01:00
Method: Method_General_Purpose
Spectral ID: BS_20121206100100
Exposure Time: ~~8000 ms~~ 2000ms Laser power = 50mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

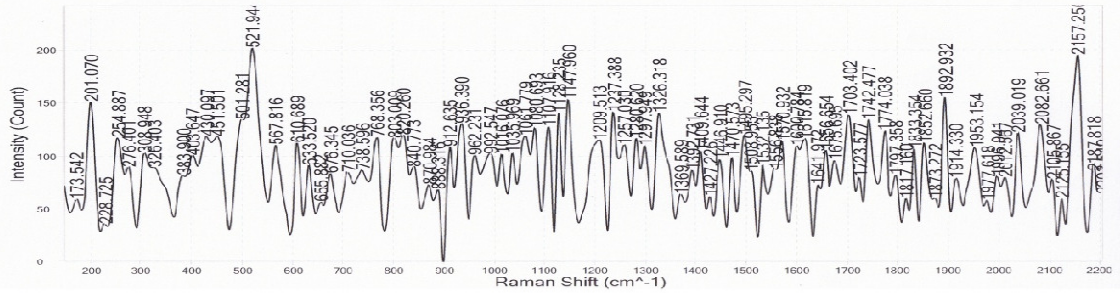


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.4.28

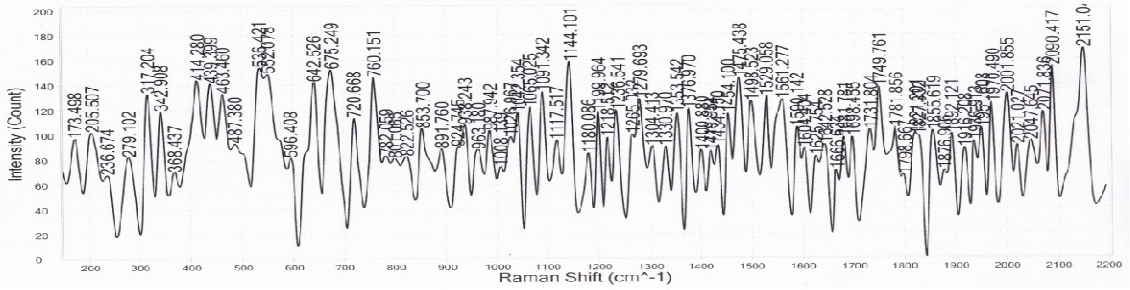
User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 10:03:07
Method: Method_General_Purpose
Spectral ID: BS_20121206100307
Exposure Time: ~~8000 ms~~ 2000ms Laser power = 100mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.4.29

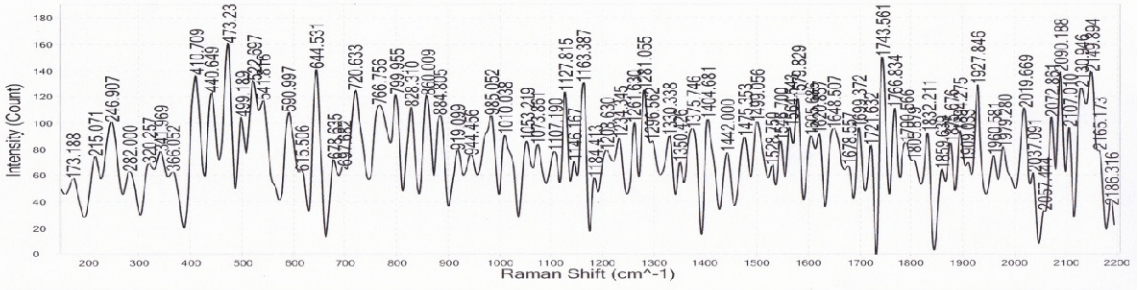
User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 10:04:26
Method: Method_General_Purpose
Spectral ID: BS_20121206100426
Exposure Time: ~~8000 ms~~ 2000ms Laser power = 150mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.4.30

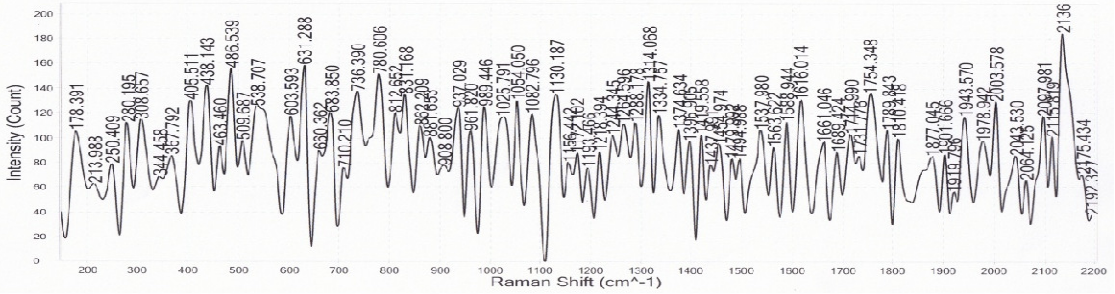
User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 10:06:31
Method: Method_General_Purpose
Spectral ID: BS_20121206100631
Exposure Time: ~~8000 ms~~ 2000ms Laser power = 200mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.4.31

User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 10:08:49
Method: Method_General_Purpose
Spectral ID: BS_20121206100849
Exposure Time: ~~8000 ms~~ 2000ms Laser power = 250mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

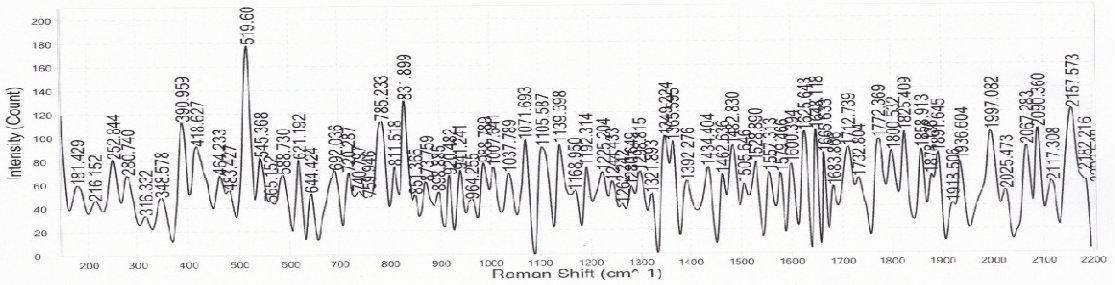


BaySpec FirstGuard Report

Graph 3.4.33

User ID: Admin
Device ID: T1100036
Test Time: 12/06/2012 10:12:38
Method: Method_General_Purpose
Spectral ID: BS_20121206101238
Exposure Time: 4000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 30mW

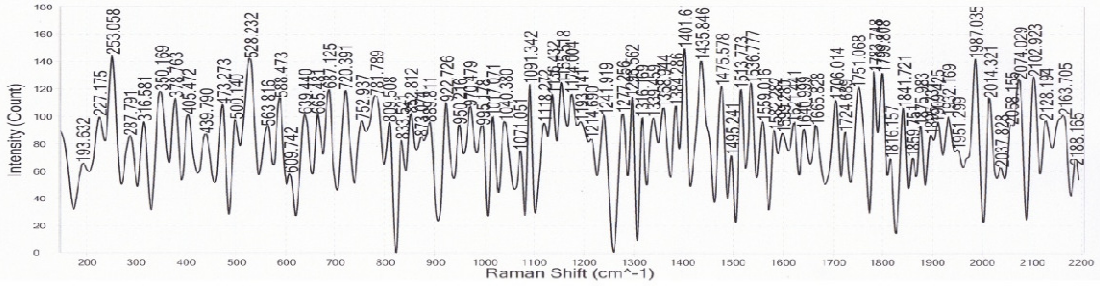


BaySpec FirstGuard Report

Graph 3.4.34

User ID: Admin
Device ID: T1100036
Test Time: 12/06/2012 10:14:48
Method: Method_General_Purpose
Spectral ID: BS_20121206101448
Exposure Time: 4000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 110mW

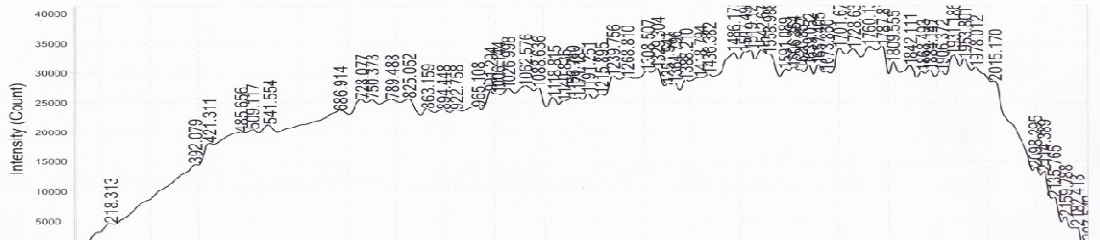


BaySpec FirstGuard Report

Graph 3.4.40

User ID: Admin
Device ID: T1100036
Test Time: 12/06/2012 10:27:28
Method: Method_General_Purpose
Spectral ID: BS_20121206102728
Exposure Time: 4000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 300mW

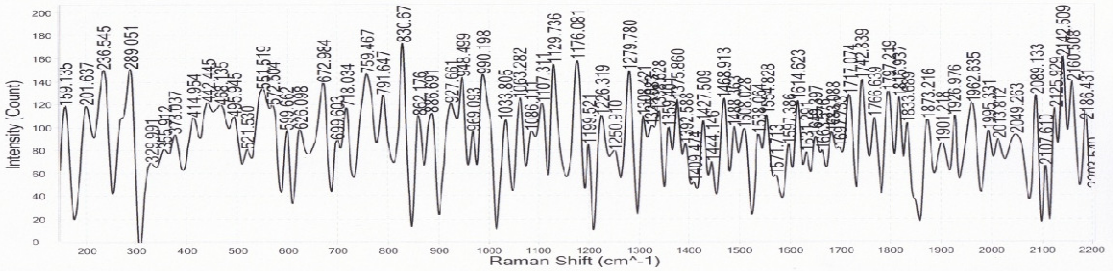


BaySpec FirstGuard Report

Graph 3.4.36

User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 10:20:50
Method: Method_General_Purpose
Spectral ID: BS_20121206102050
Exposure Time: 4000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 100mW

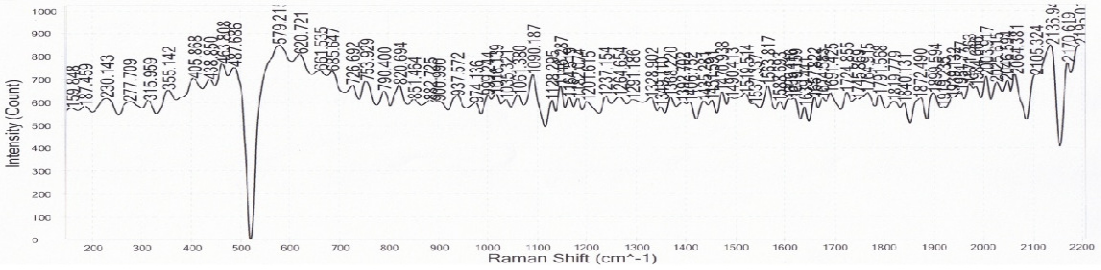


BaySpec FirstGuard Report

Graph 3.4.37

User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 10:22:55
Method: Method_General_Purpose
Spectral ID: BS_20121206102255
Exposure Time: 4000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

Laser power = 150mW

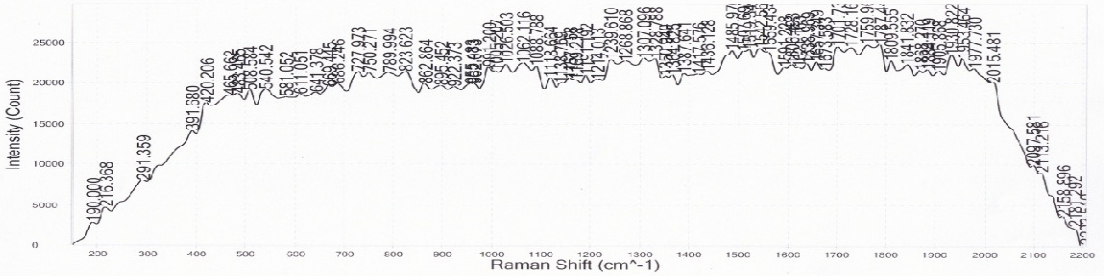


BaySpec FirstGuard Report

Graph 3.4.39

User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 10:25:00
Method: Method_General_Purpose
Spectral ID: BS_20121206102500
Exposure Time: 4000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

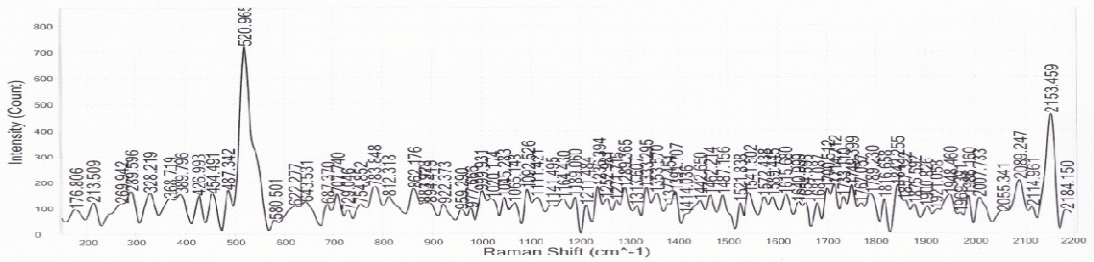
Laser power = 250mW



BaySpec FirstGuard Report

Graph 3.4.42

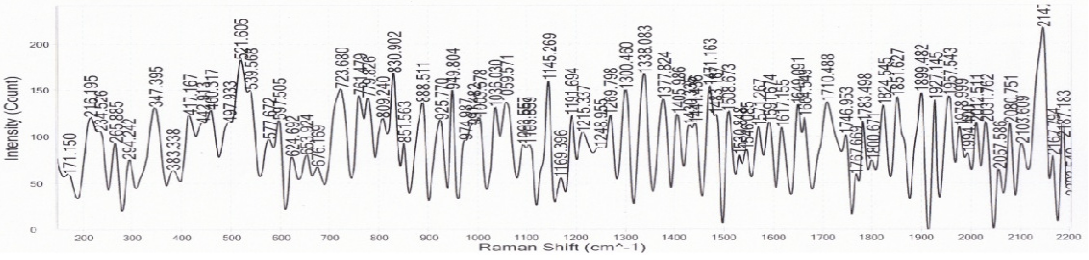
User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 10:46:36
Method: Method_General_Purpose
Spectral ID: BS_20121206104636
Exposure Time: 8000 ms
Laser power = 4.0mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.4.43

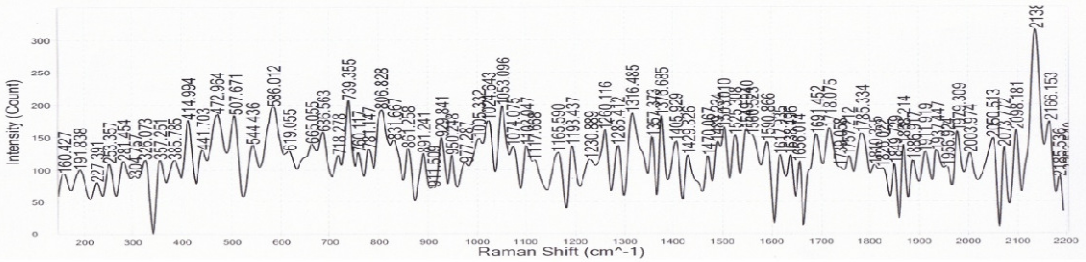
User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 10:50:15
Method: Method_General_Purpose
Spectral ID: BS_20121206105015
Exposure Time: 8000 ms
Laser power = 50mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL



BaySpec FirstGuard Report

Graph 3.4.44

User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 10:52:16
Method: Method_General_Purpose
Spectral ID: BS_20121206105218
Exposure Time: 8000 ms
Laser power = 100mW
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

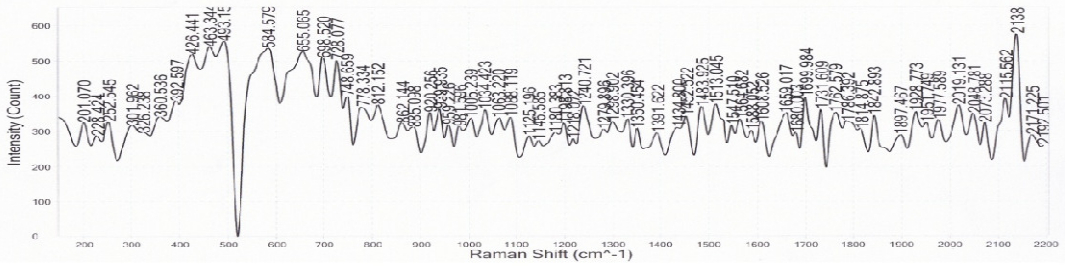


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.4.45

User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 10:56:49
Method: Method_General_Purpose
Spectral ID: BS_20121206105549
Exposure Time: 8000 ms
Average Number: 4
Laser power = 150mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Equine Haemoglobin HQI = 24.1
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

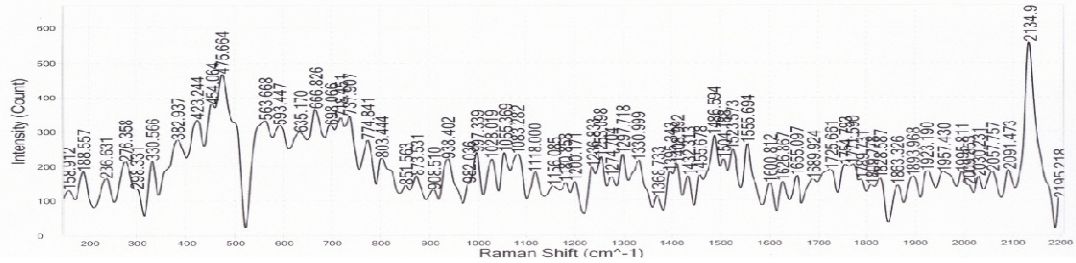


BAYSPEC

---------- BaySpec FirstGuard Report ----------

Graph 3.4.46

User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 11:01:04
Method: Method_General_Purpose
Spectral ID: BS_20121206110104
Exposure Time: 8000 ms
Average Number: 4
Laser power = 200mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: PASS
Matched Substance: Equine Haemoglobin HQI = 25.8
Unkown Substance: Black Spectrum
Library Substance: Red Spectrum

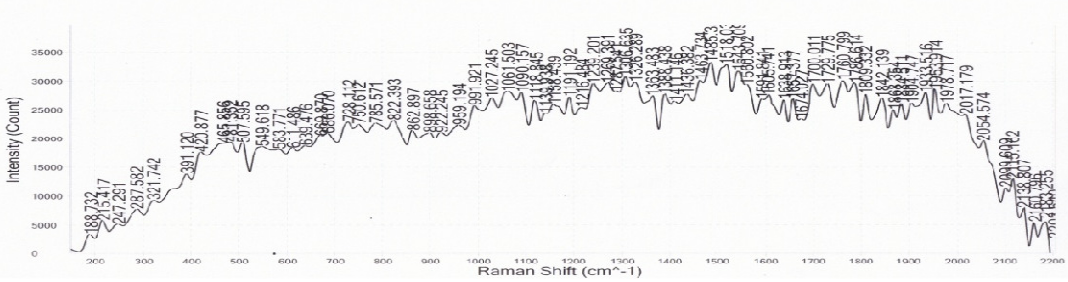


BAYSPEC

BaySpec FirstGuard Report

Graph 3.4.47

User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 11:03:50
Method: Method_General_Purpose
Spectral ID: BS_20121206110350
Exposure Time: 8000 ms
Average Number: 4
Laser power = 250mW
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

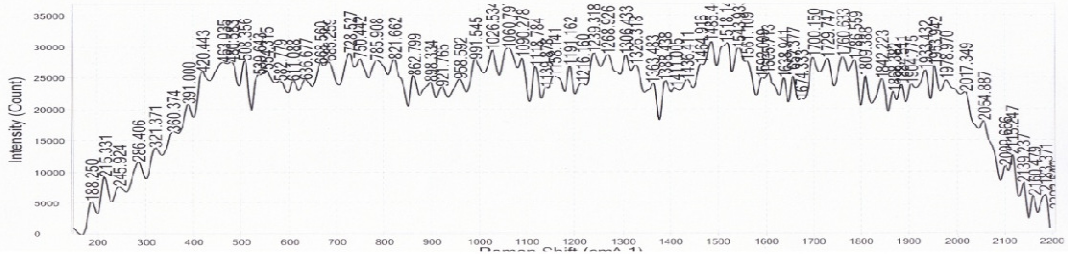


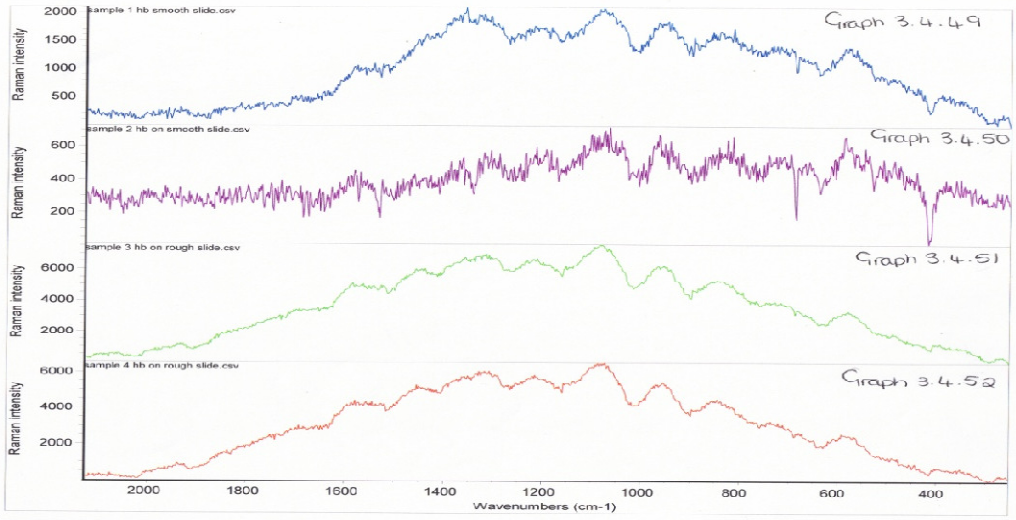
BaySpec FirstGuard Report

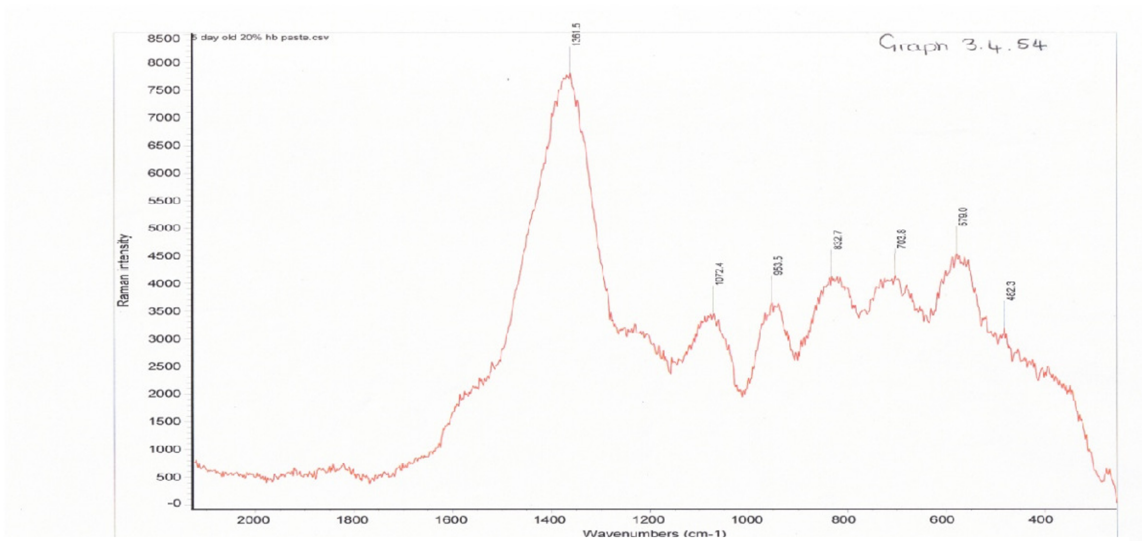
Graph 3.4.48

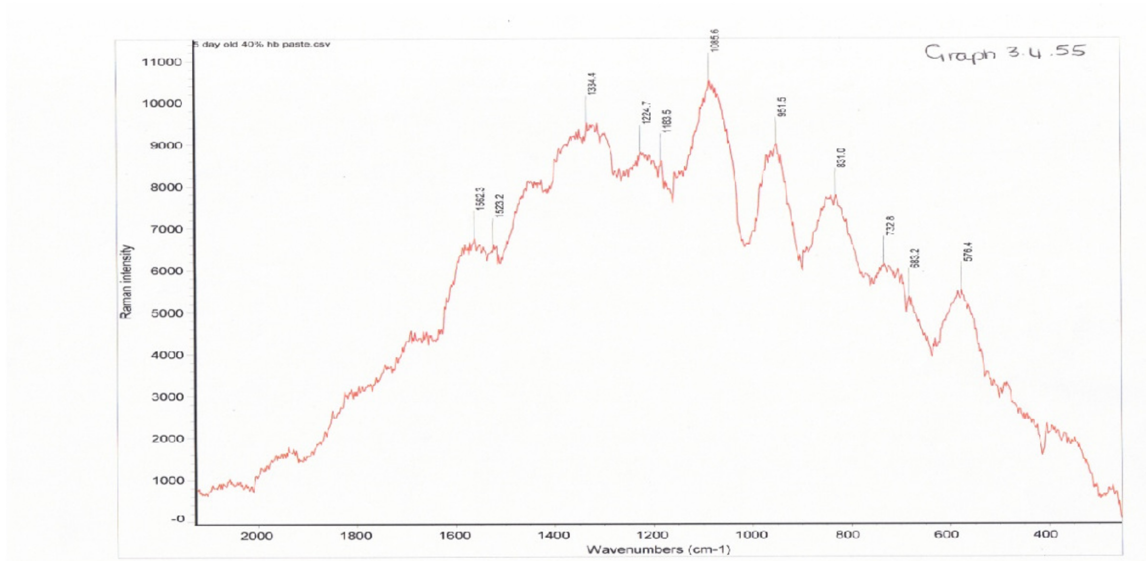
User ID: Admin
Device ID: T1160038
Test Time: 12/06/2012 11:06:40
Method: Method_General_Purpose
Spectral ID: BS_20121206110640
Exposure Time: 8000 ms
Average Number: 4
Baseline Correction: Yes
Library Name 1: BS_Library\BS_User_Library
HQI Threshold: 10
Search Range: 200 to 2000 cm^-1
Result: FAIL

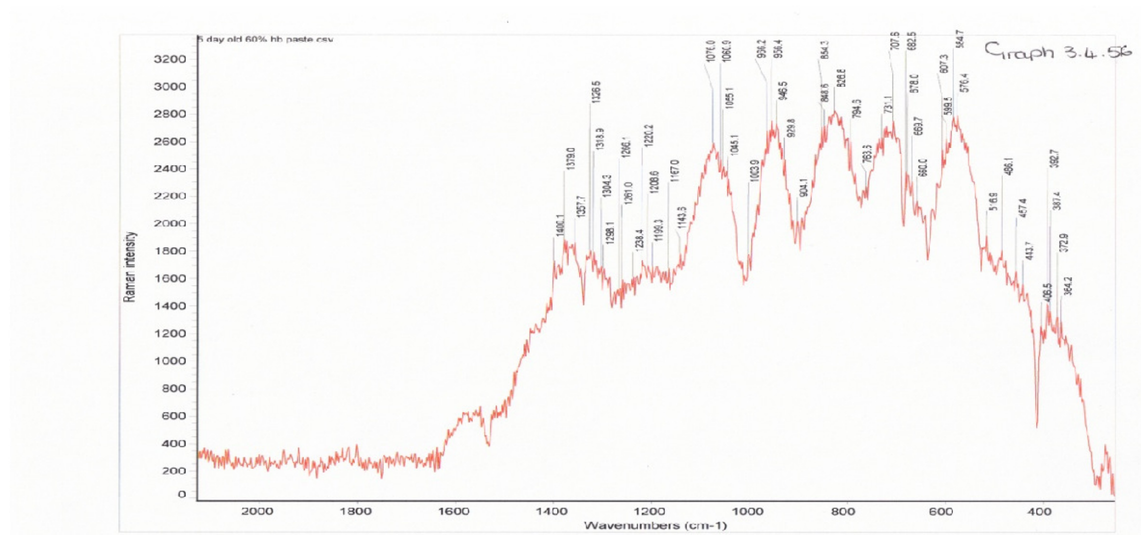
Laser power - 300mW

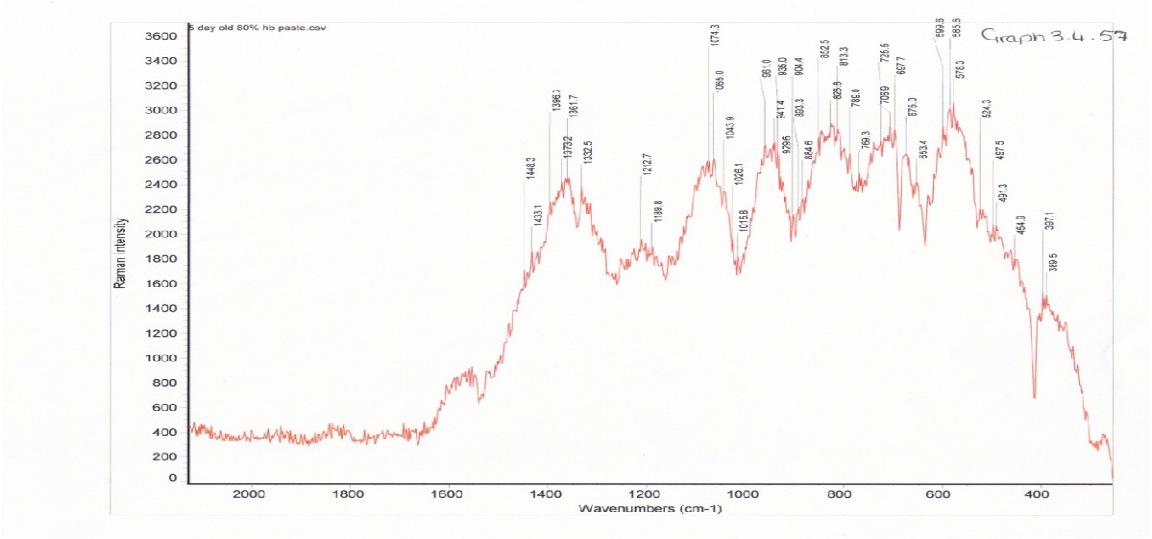


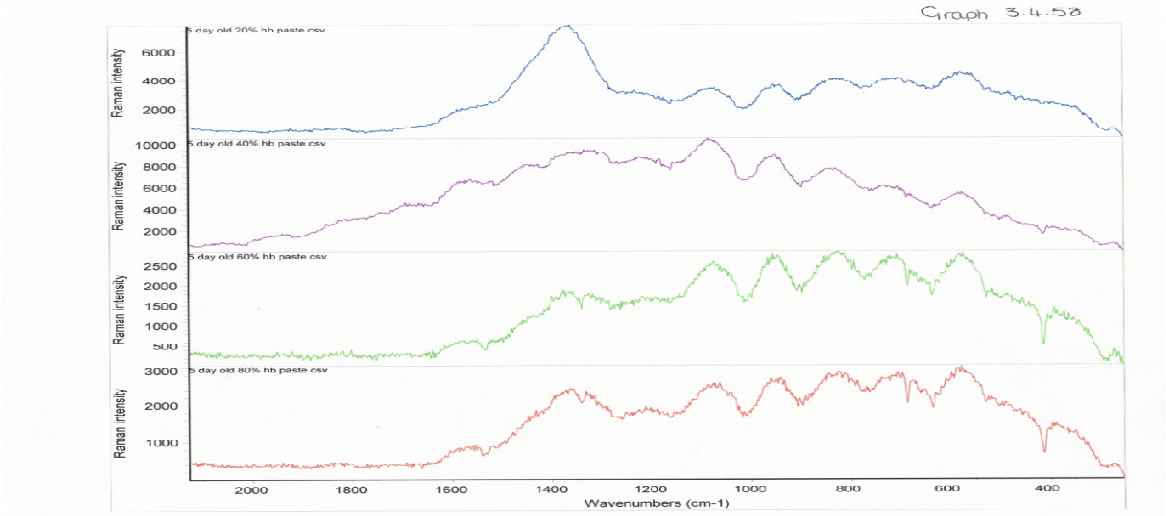


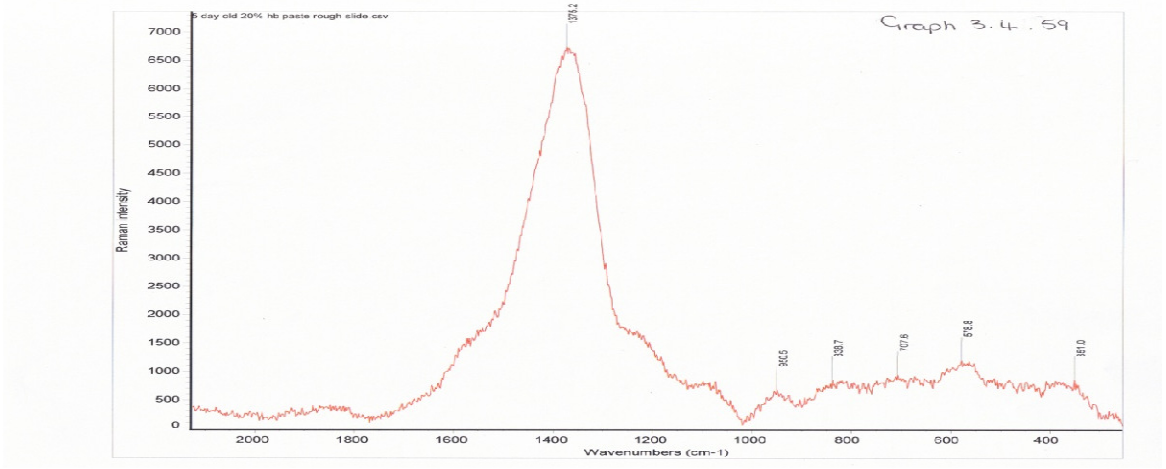

5 day old 20% hb paste.csv
Graph 3.4.54
Raman intensity
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
-0
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumbers (cm-1)

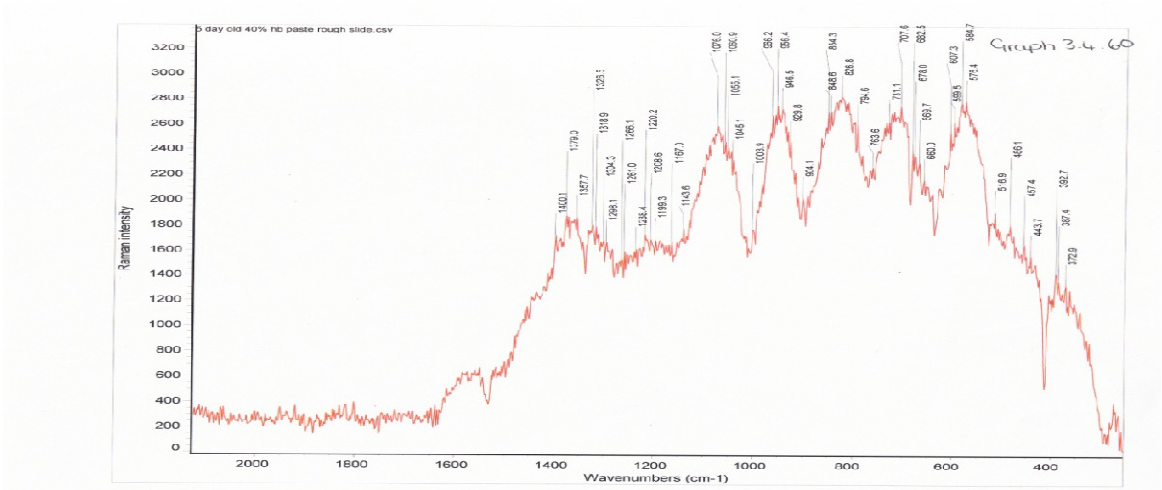

5 day old 40% hb paste.csv
Graph 3.4.55
Raman intensity
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
-0
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumbers (cm-1)

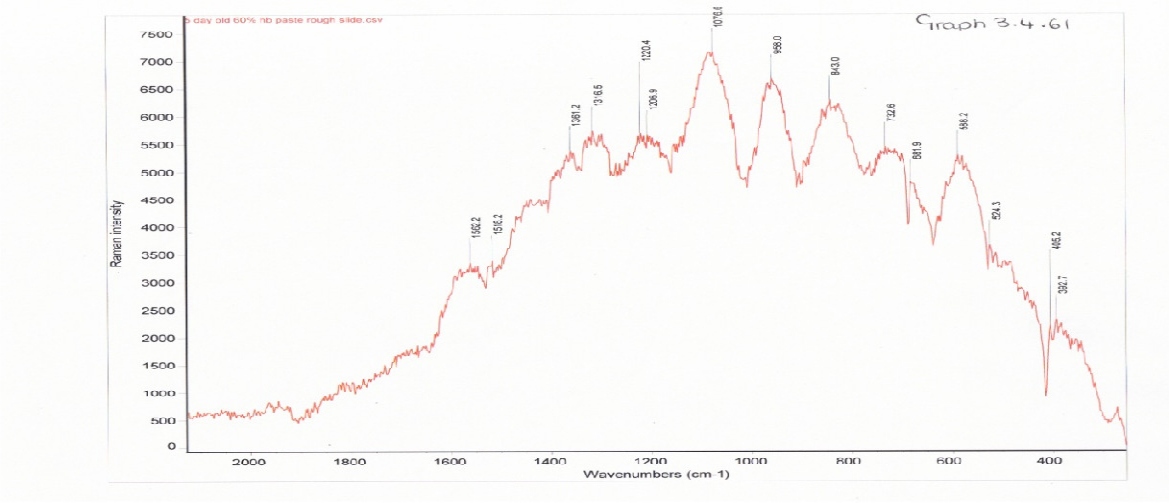

5 day old 60% hb paste.csv
Graph 3.4.56
Raman intensity
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumbers (cm-1)

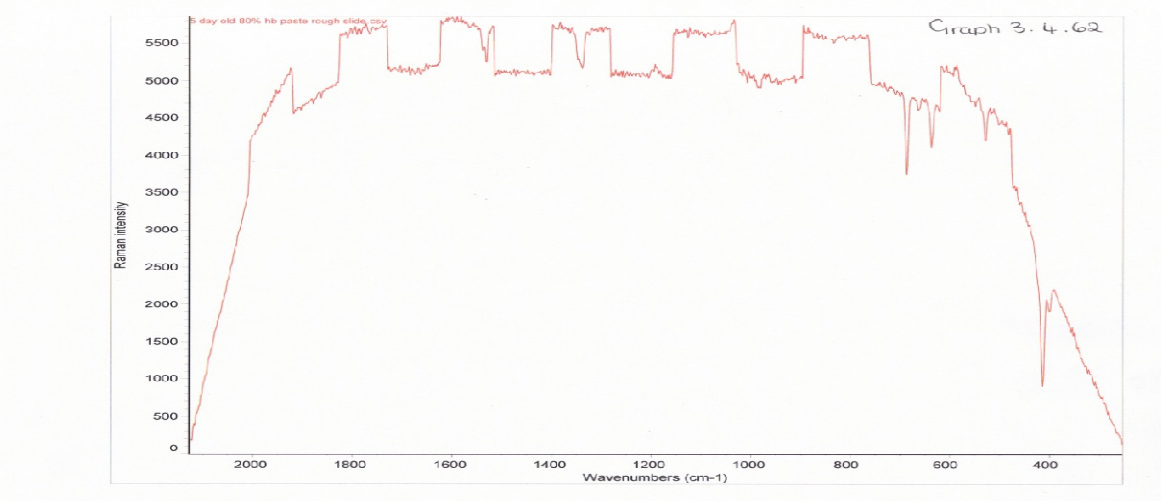

5 day old 80% hb paste.csv
Graph 3.4.57
Raman intensity
Wavenumbers (cm-1)


Graph 3.4.58
5 day old 20% hb paste.csv
5 day old 40% hb paste.csv
5 day old 60% hb paste.csv
5 day old 80% hb paste.csv
Raman intensity
Wavenumbers (cm-1)

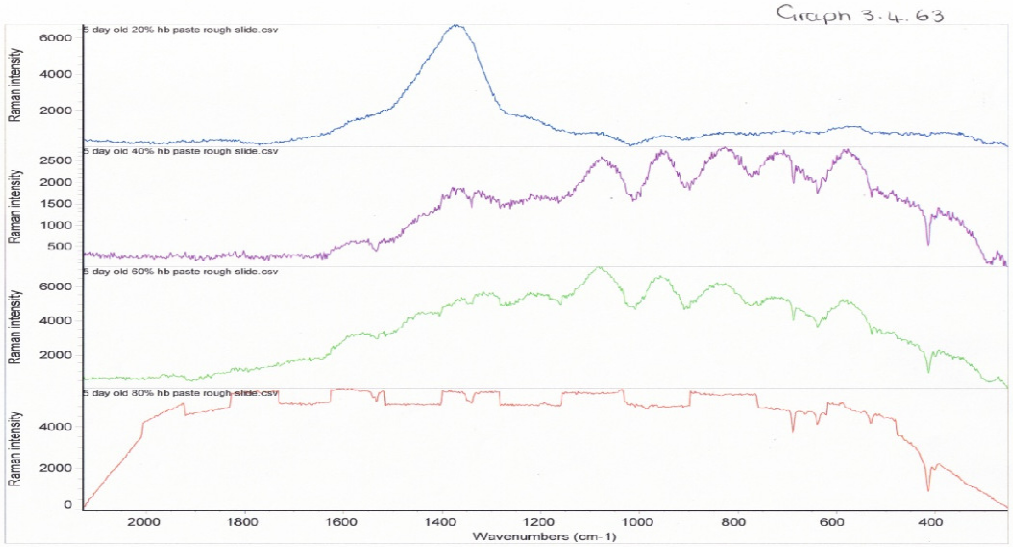

5 day old 20% hb paste rough slide.csv
Graph 3.4.59
Raman intensity
Wavenumbers (cm-1)


5 day old 40% hb paste rough slide.csv
Graph 3.4.60
Raman intensity
Wavenumbers (cm-1)

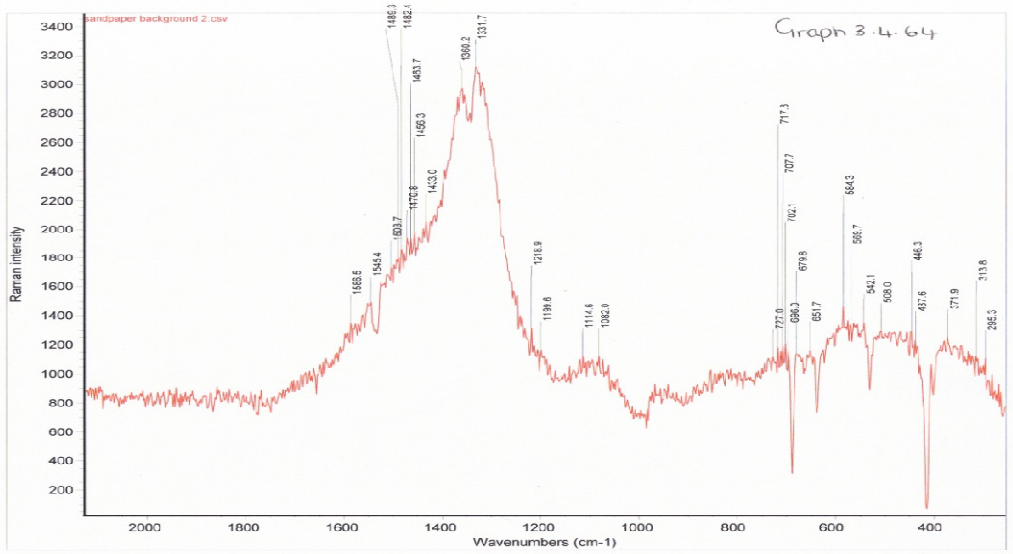

5 day old 60% hb paste rough slide.csv
Graph 3.4.61
Raman intensity
Wavenumbers (cm-1)


5 day old 80% hb paste rough slide.csv
Graph 3.4.62
Raman intensity
Wavenumbers (cm-1)

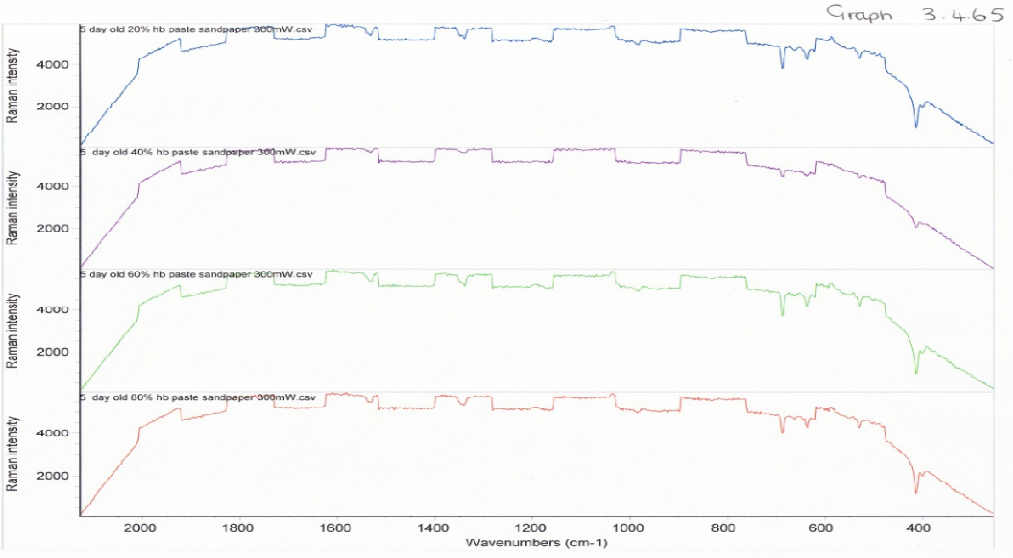
Graph 3.4.63



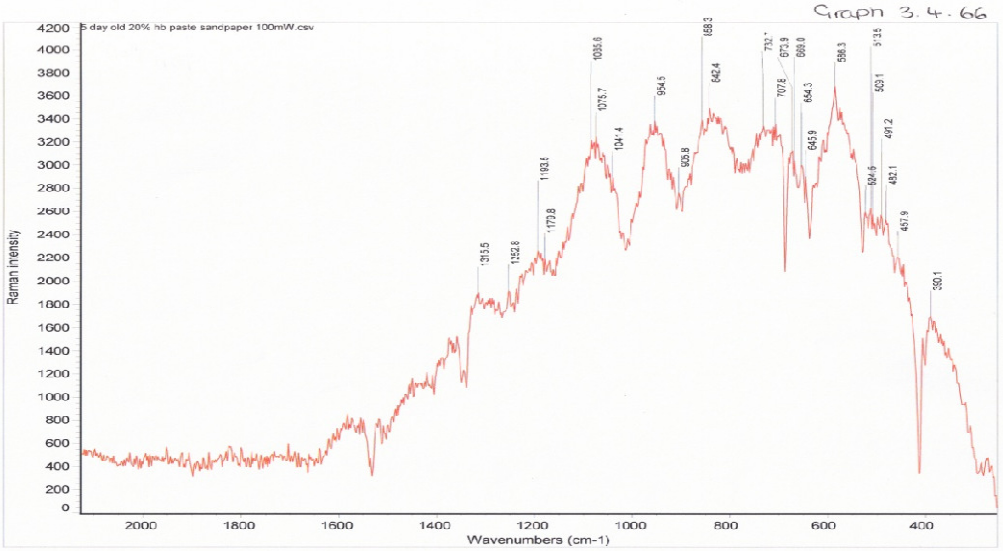
Graph 3.4.64



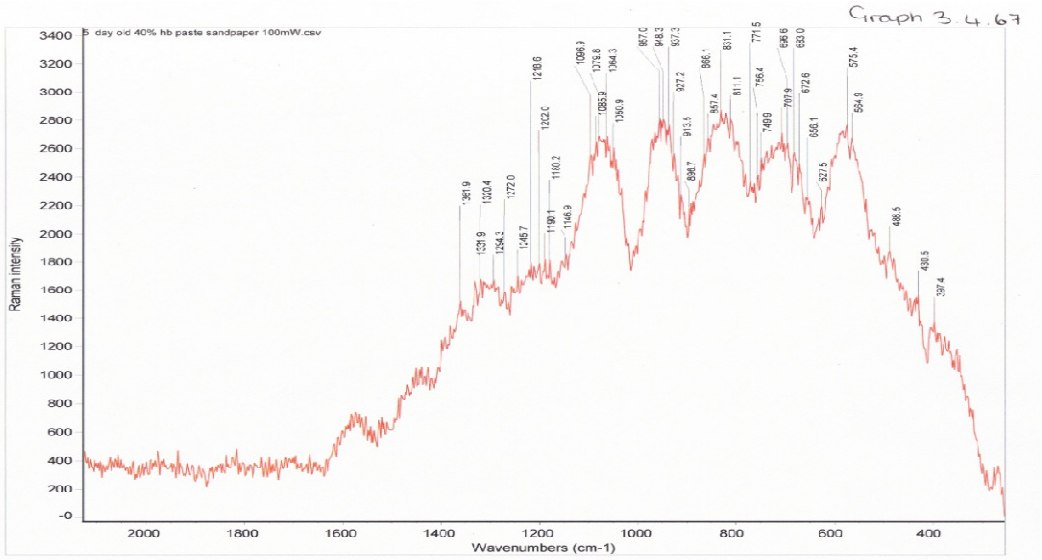
Graph 3.4.65



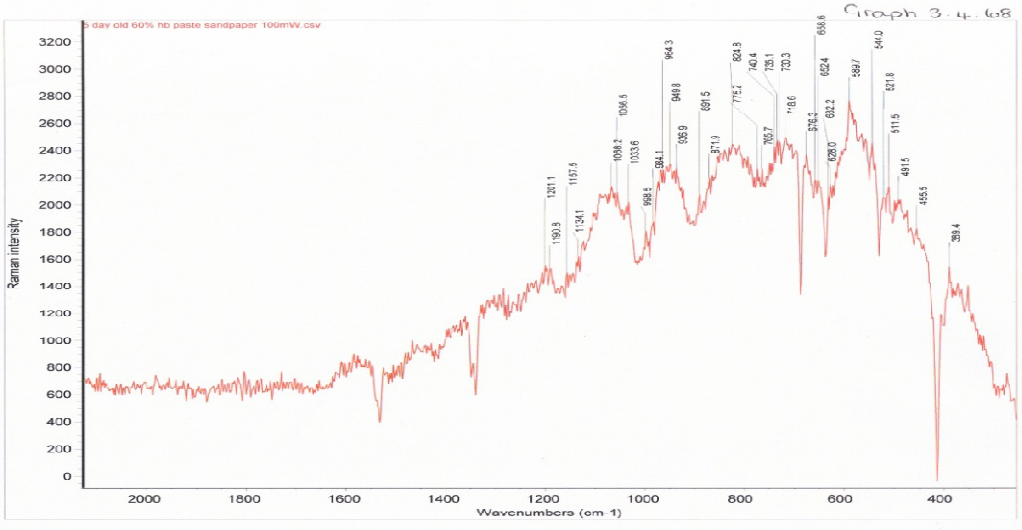
Graph 3.4.66



Graph 3.4.67

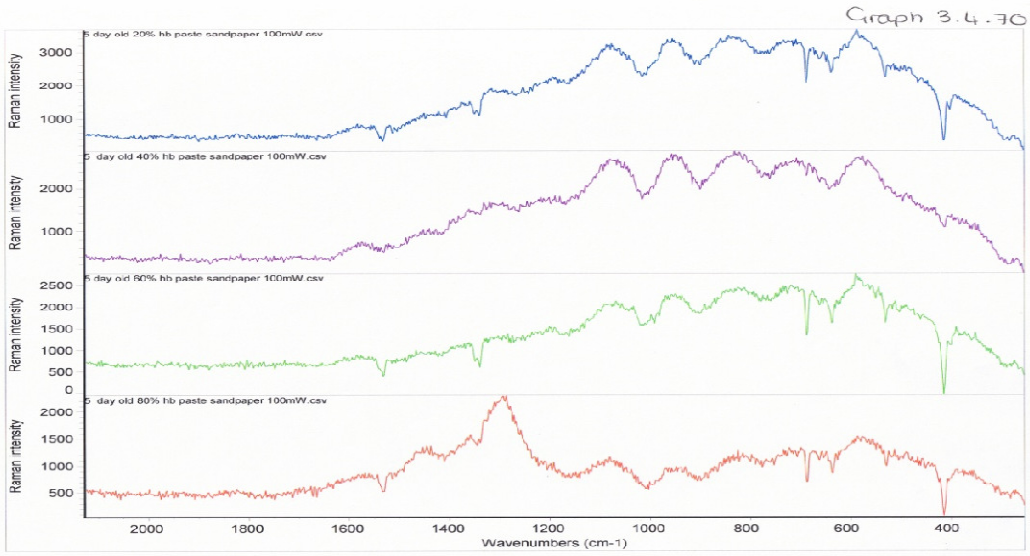


Graph 3.4.68

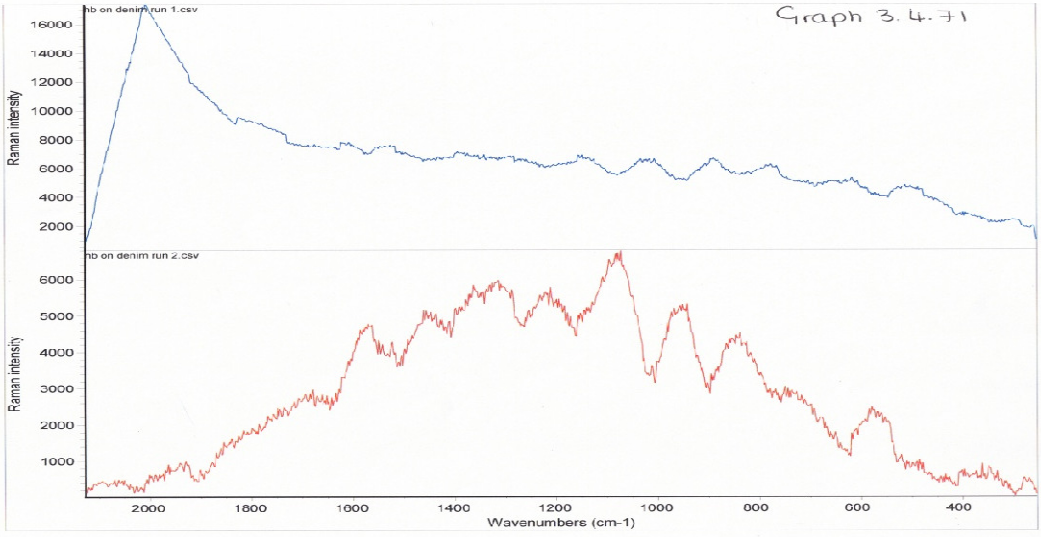


Graph 3.4.69

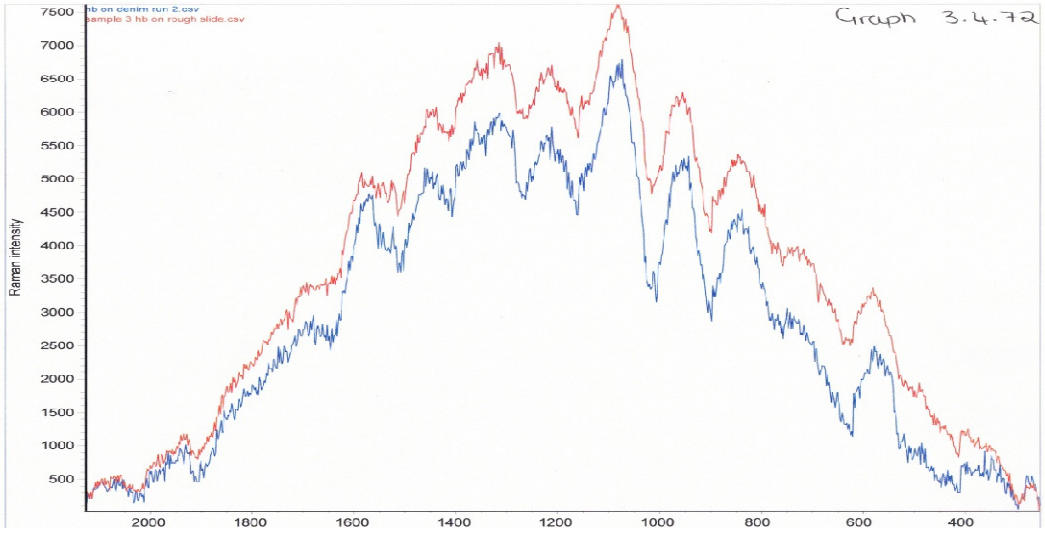
Graph 3.4.70



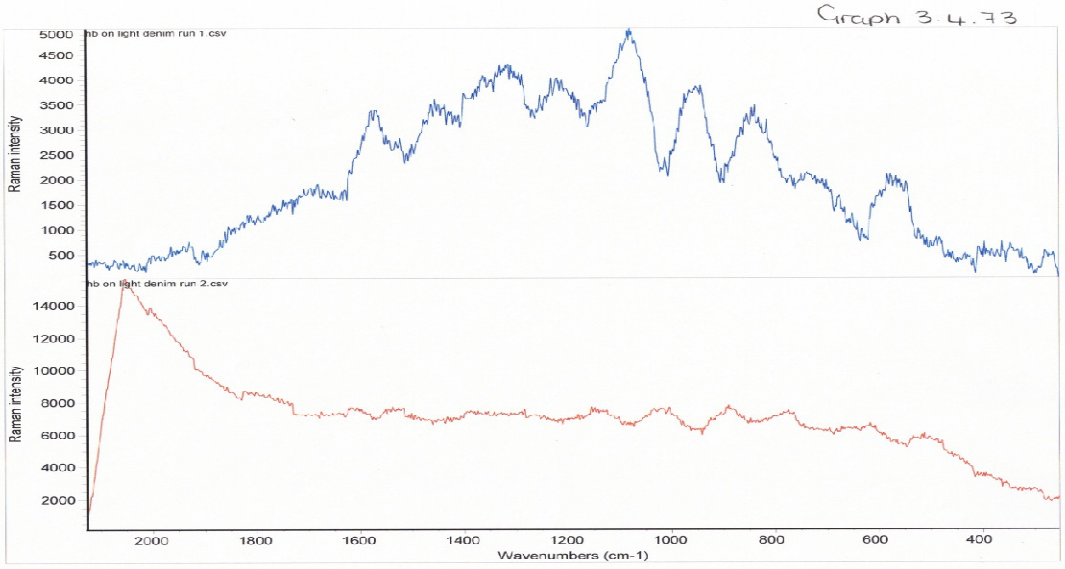
Graph 3.4.71



Graph 3.4.72



Graph 3.4.73



Graph 3.4.74